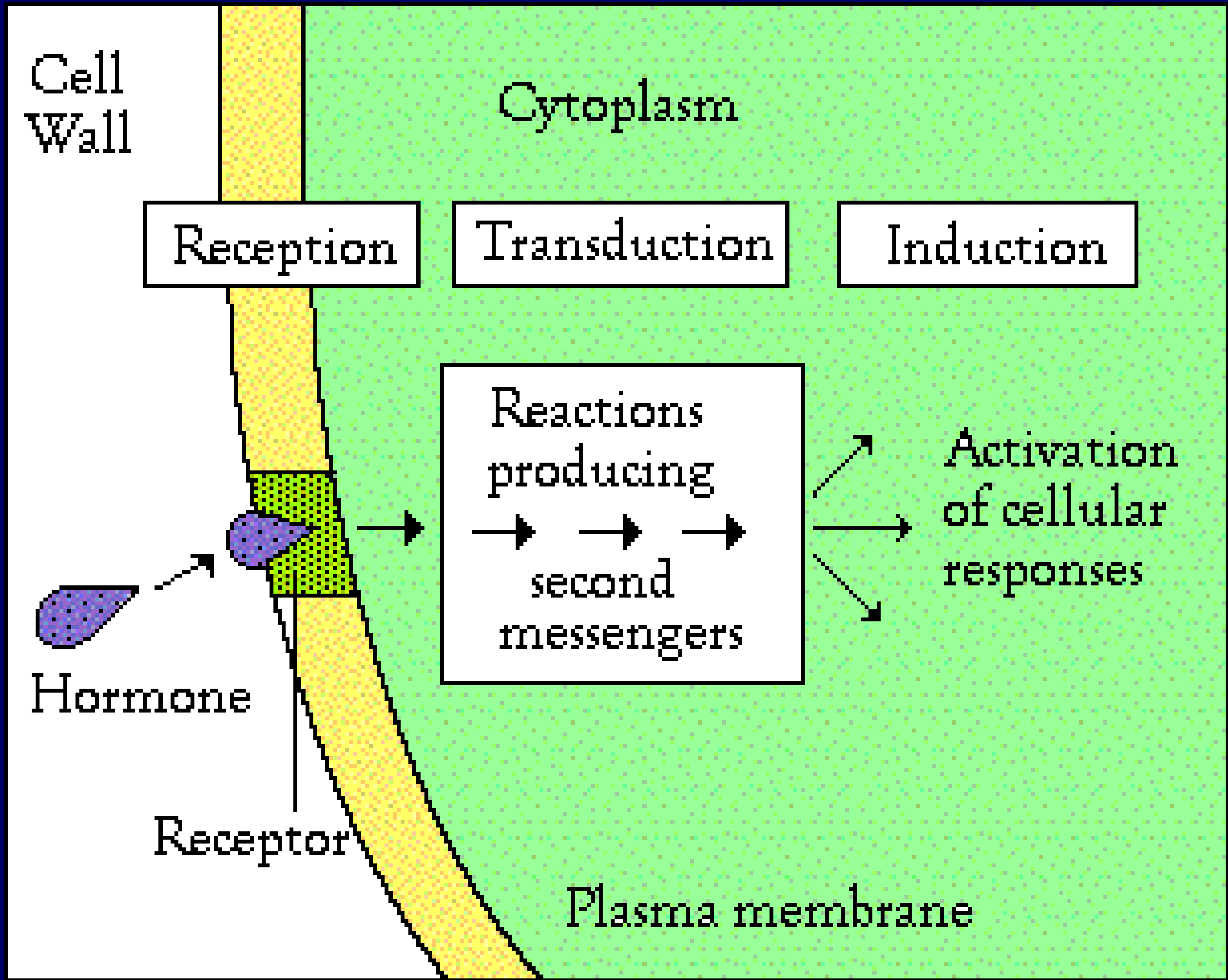
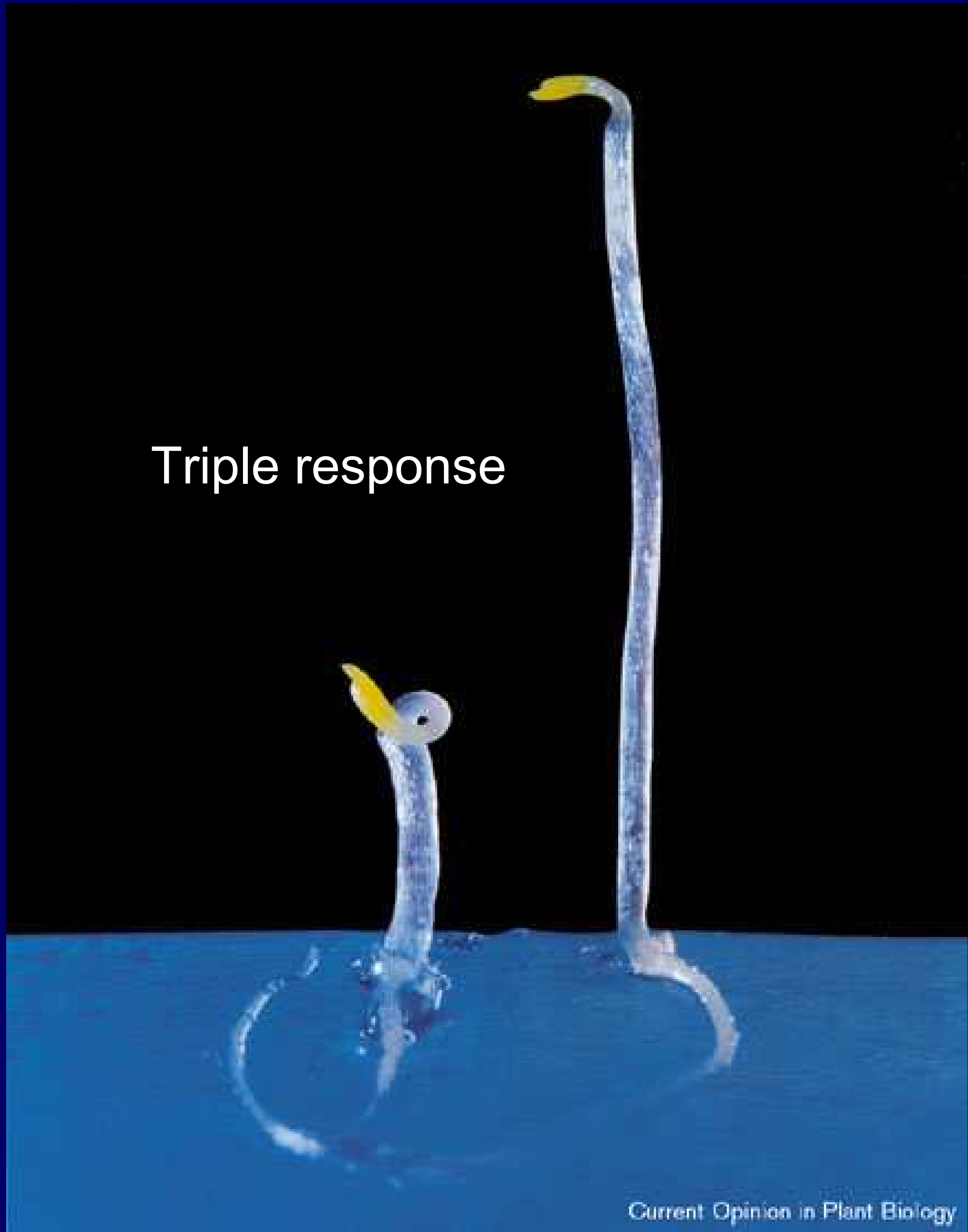
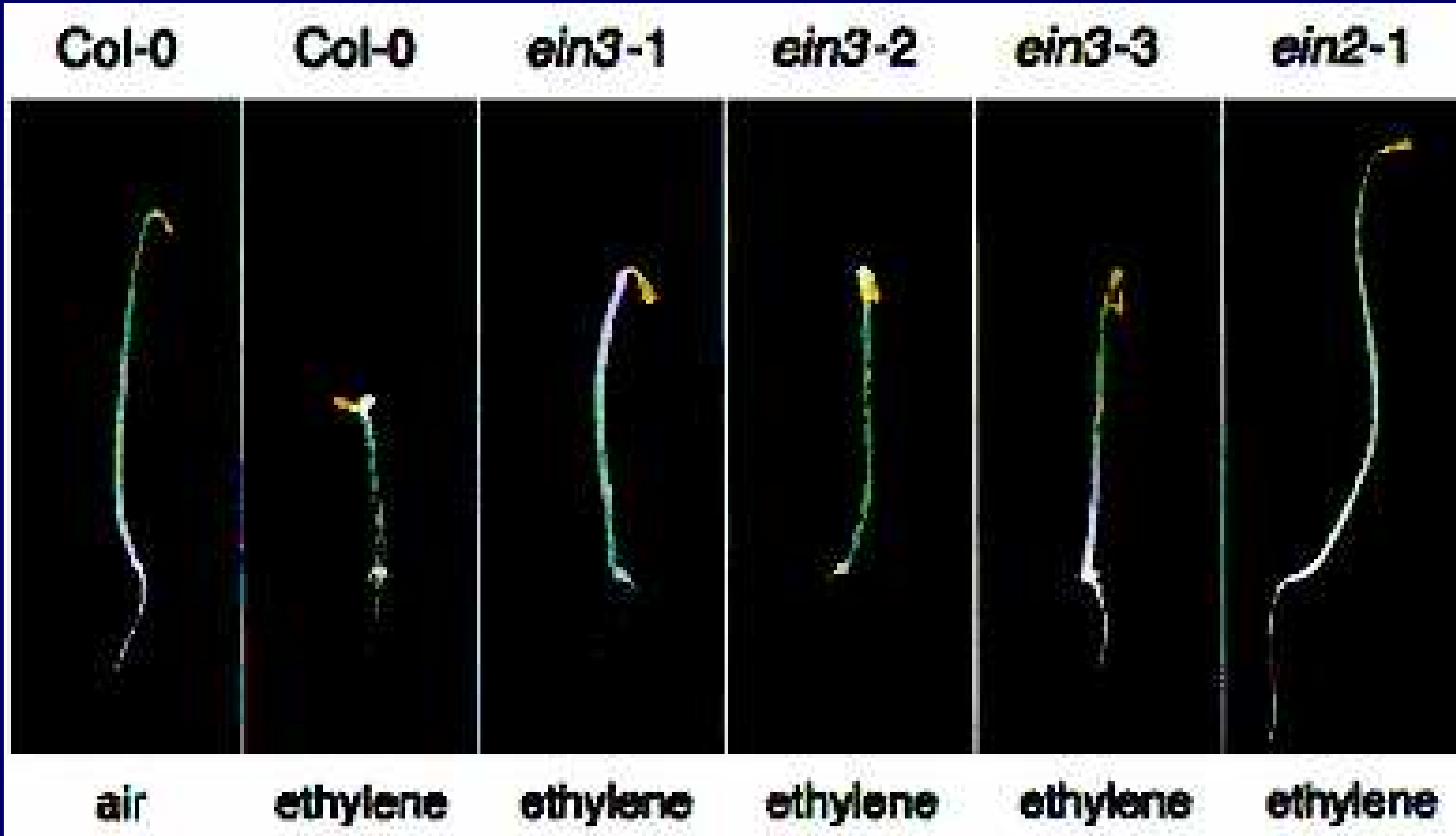
Signal Transduction



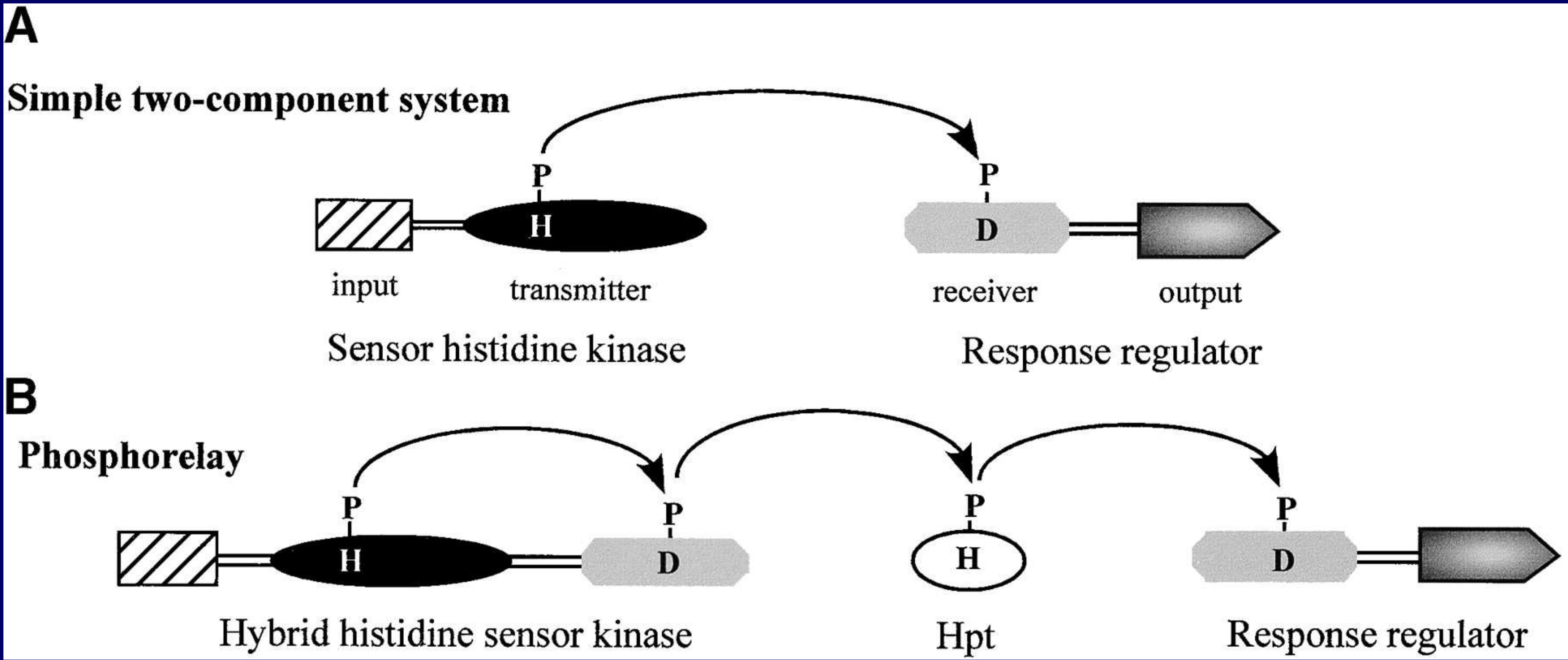
Mutant screens for ethylene pathway genes



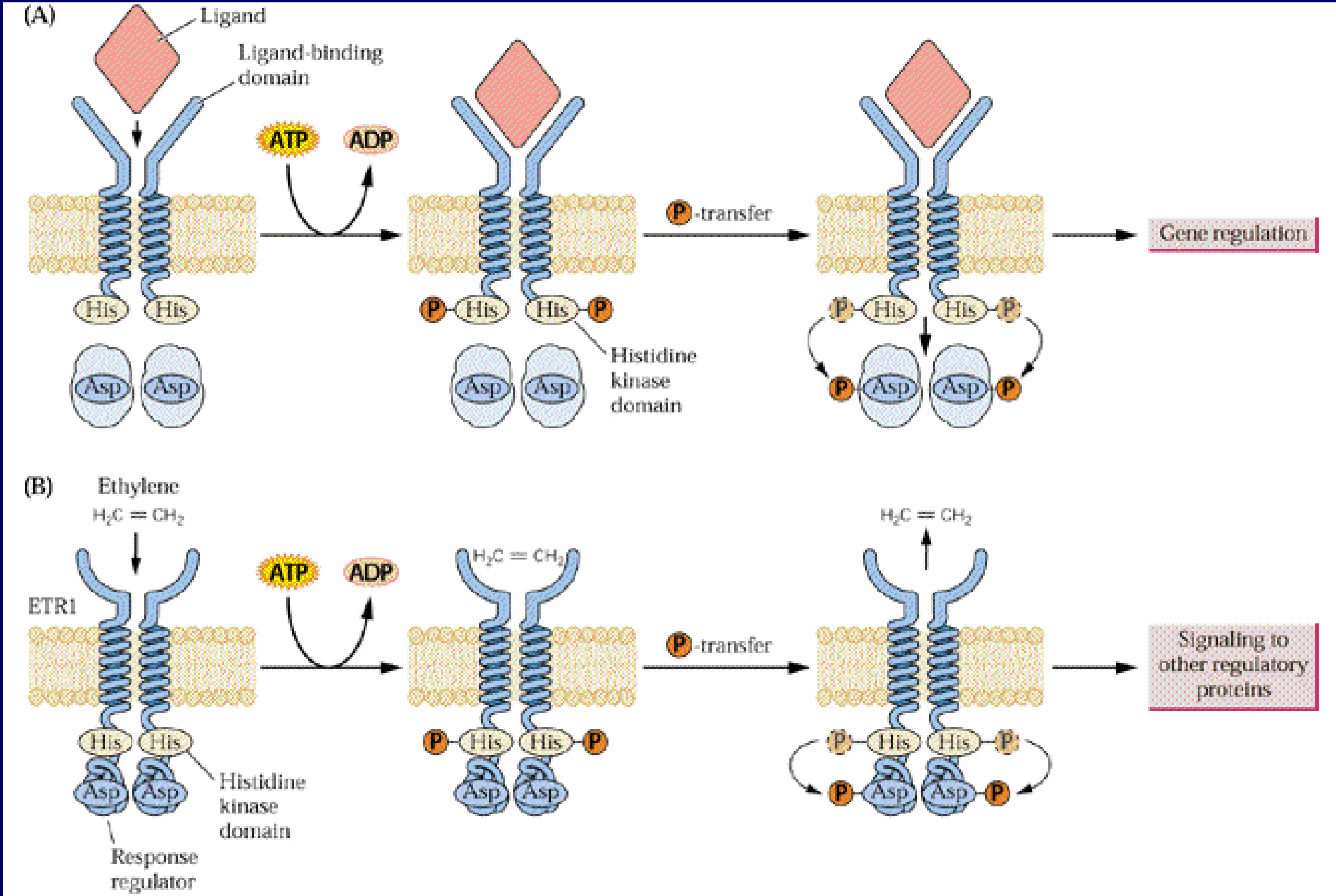
Ethylene signal transduction pathway



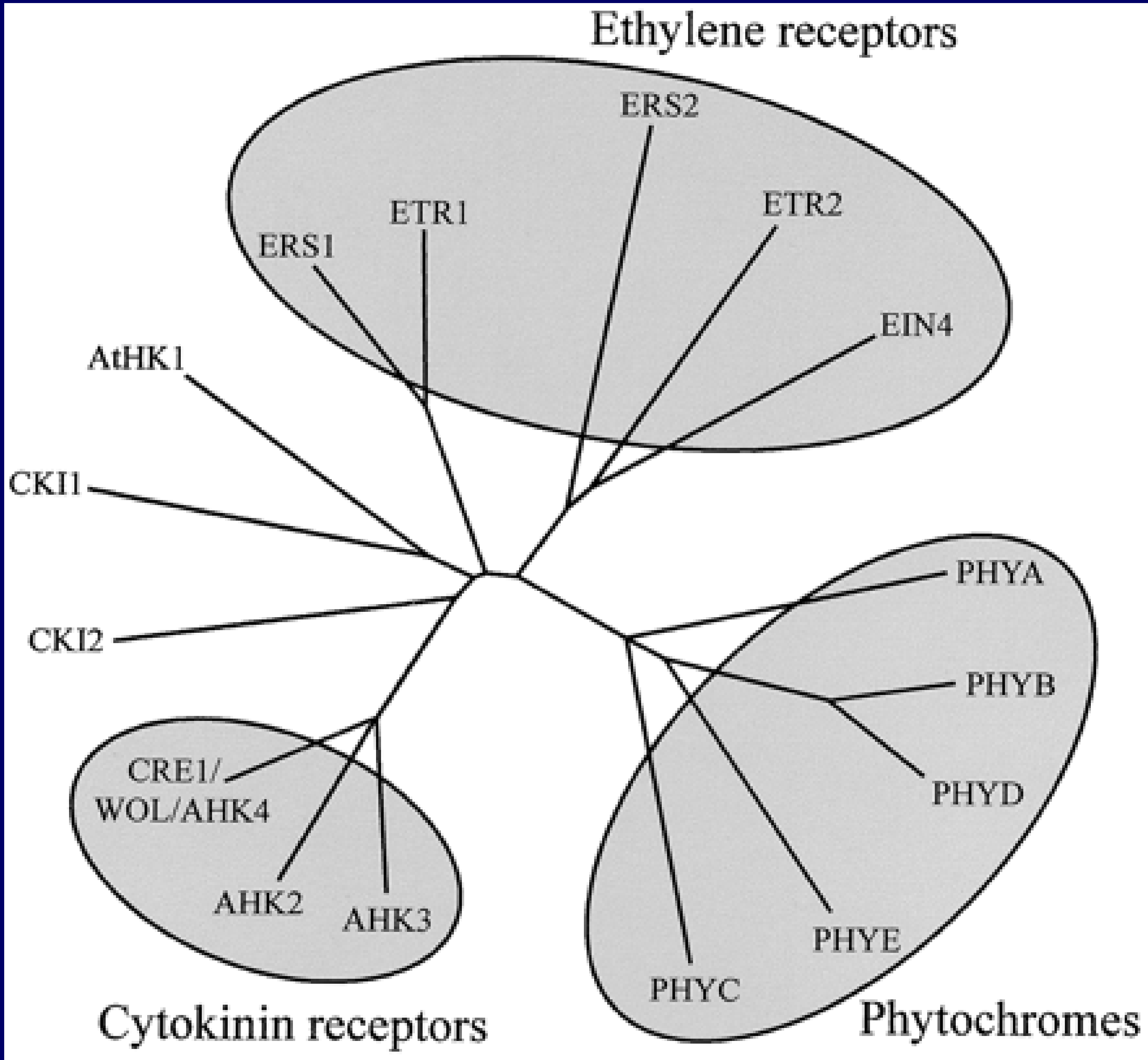
Ethylene signal transduction pathway



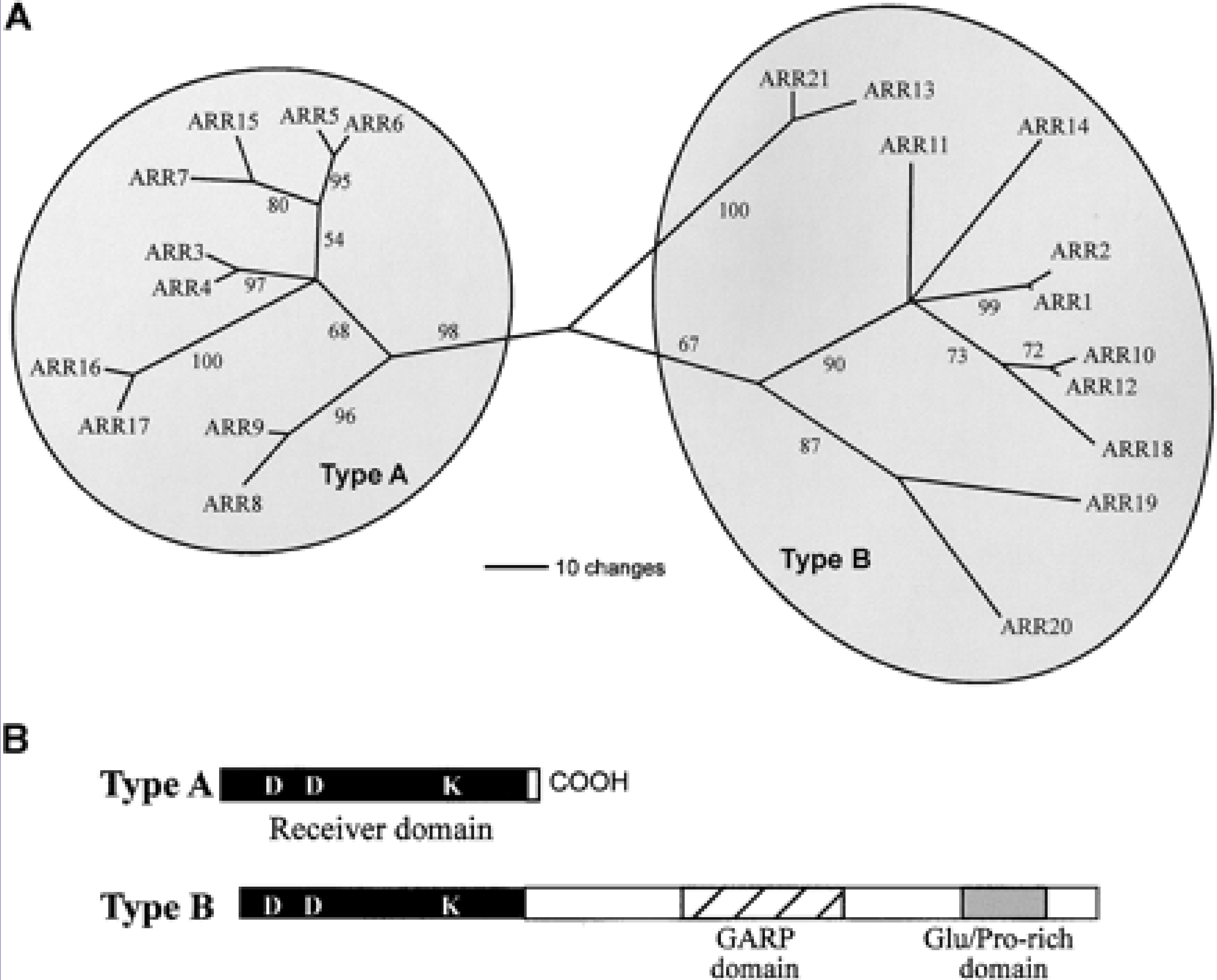
Ethylene signal transduction pathway



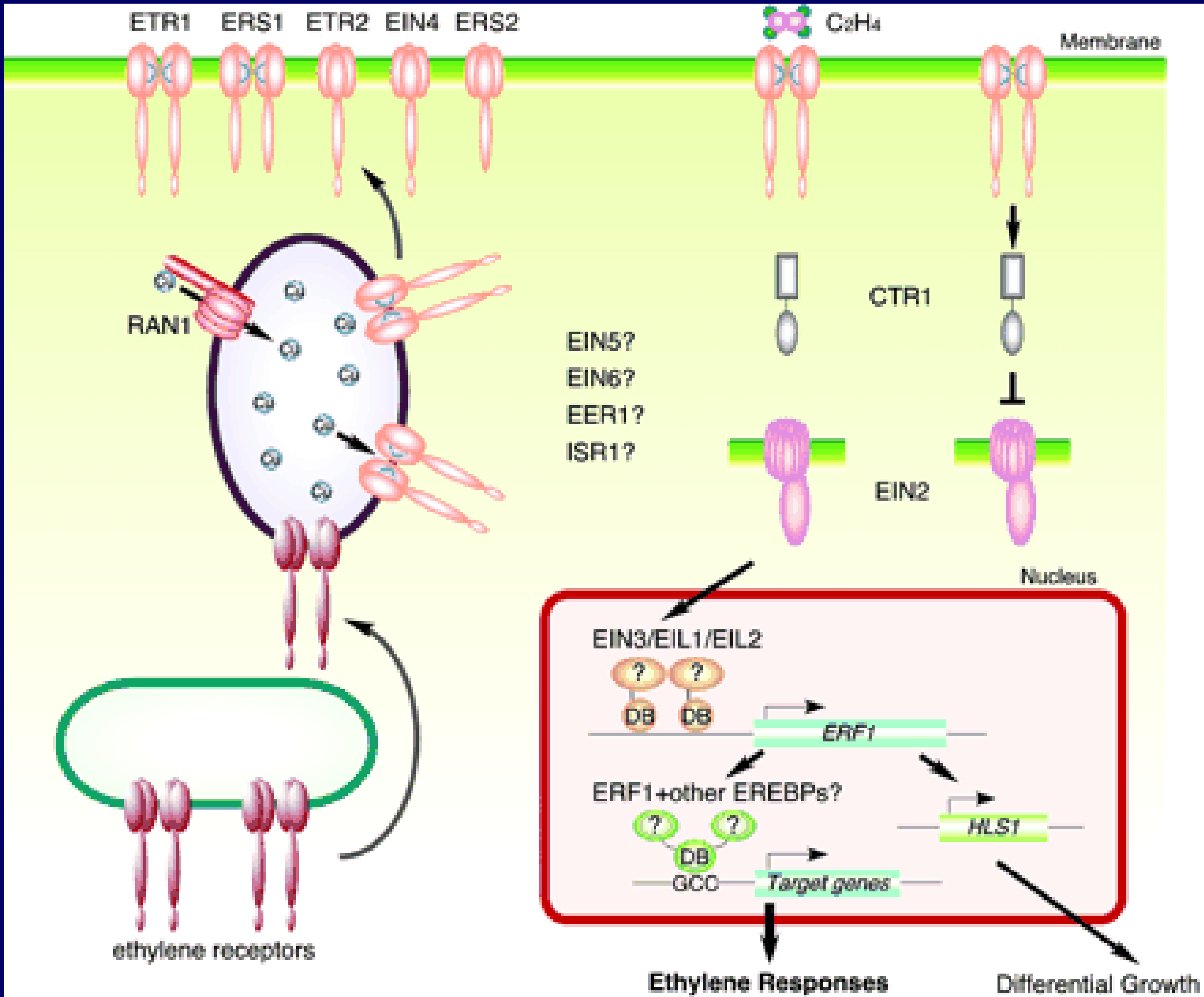
His-kinases in Arabidopsis



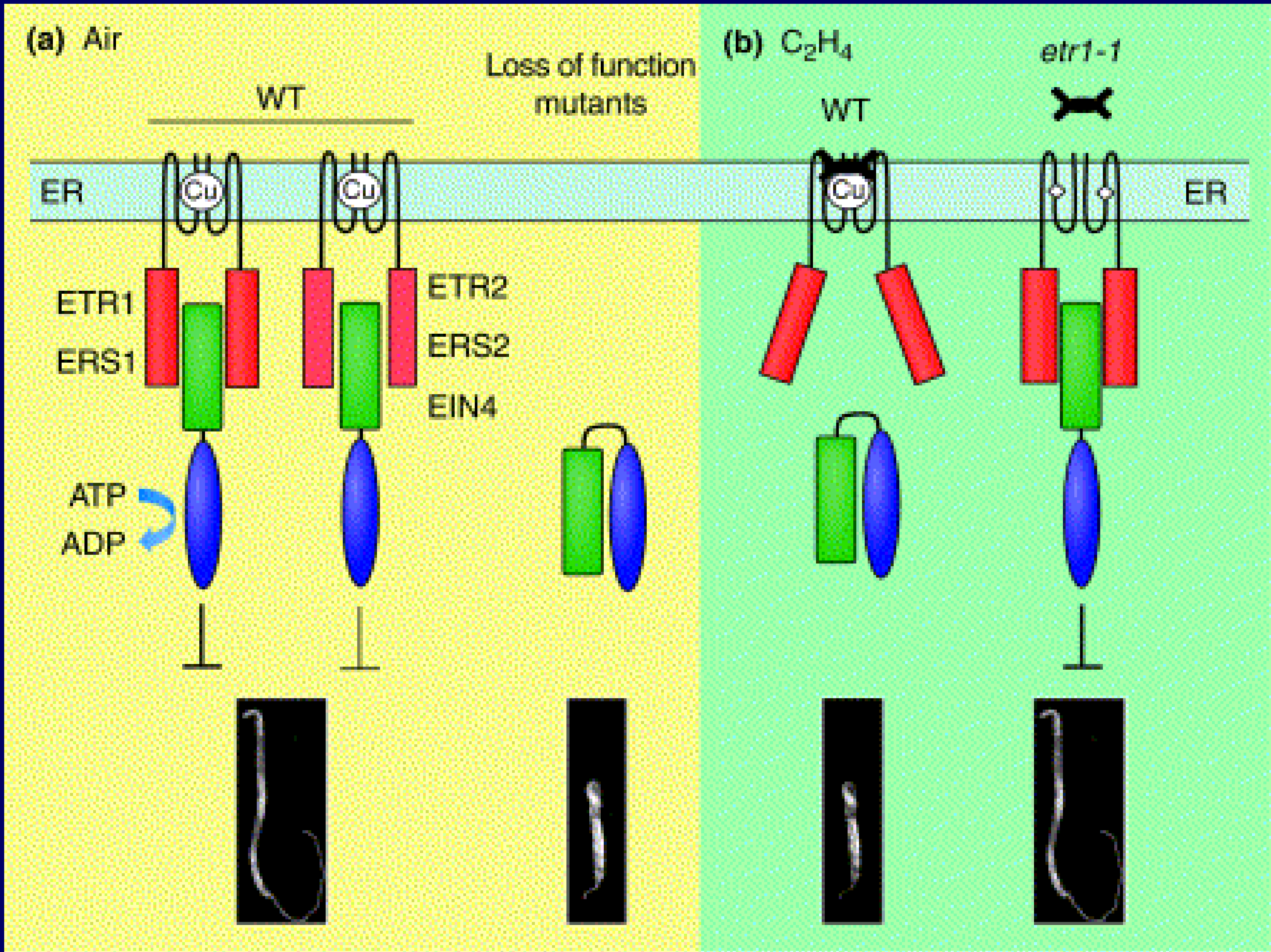
Response Regulators in Arabidopsis



Ethylene signal transduction pathway



Genetic interactions



Current Opinion in Plant Biology

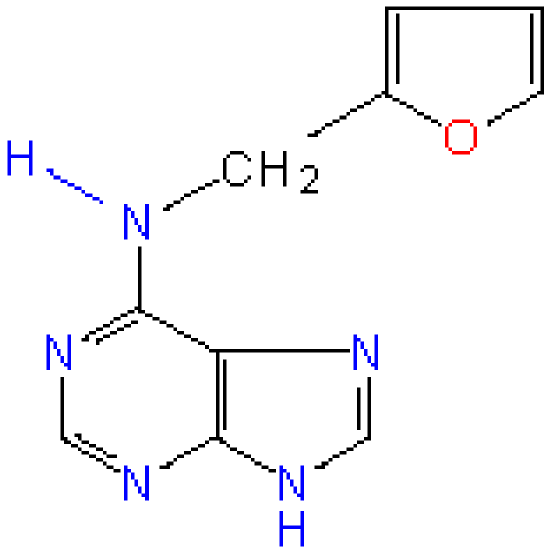
CYTOKININ – what is important?

Synthesis – *IPT* genes

Degradation – CK-oxidase

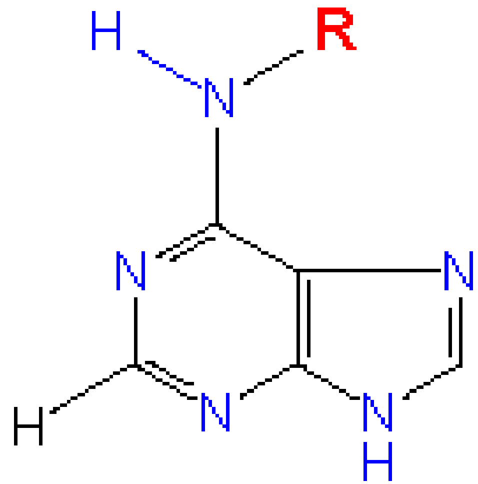
Signal transduction – forward genetics
activation tagging
CK regulated genes

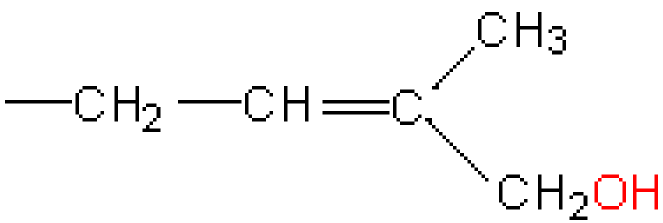
Cytokinins



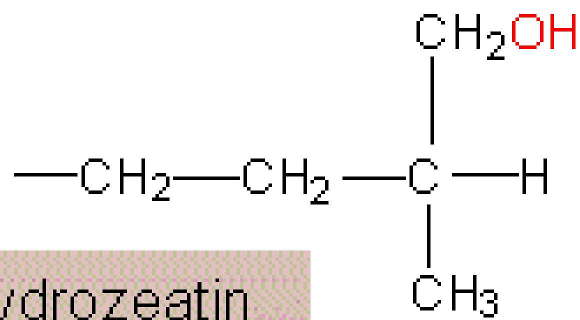
Kinetin:
6 - (2 - Furfuryl -
7 - Aminopurin)

Cytokinin
(Grundstruktur)



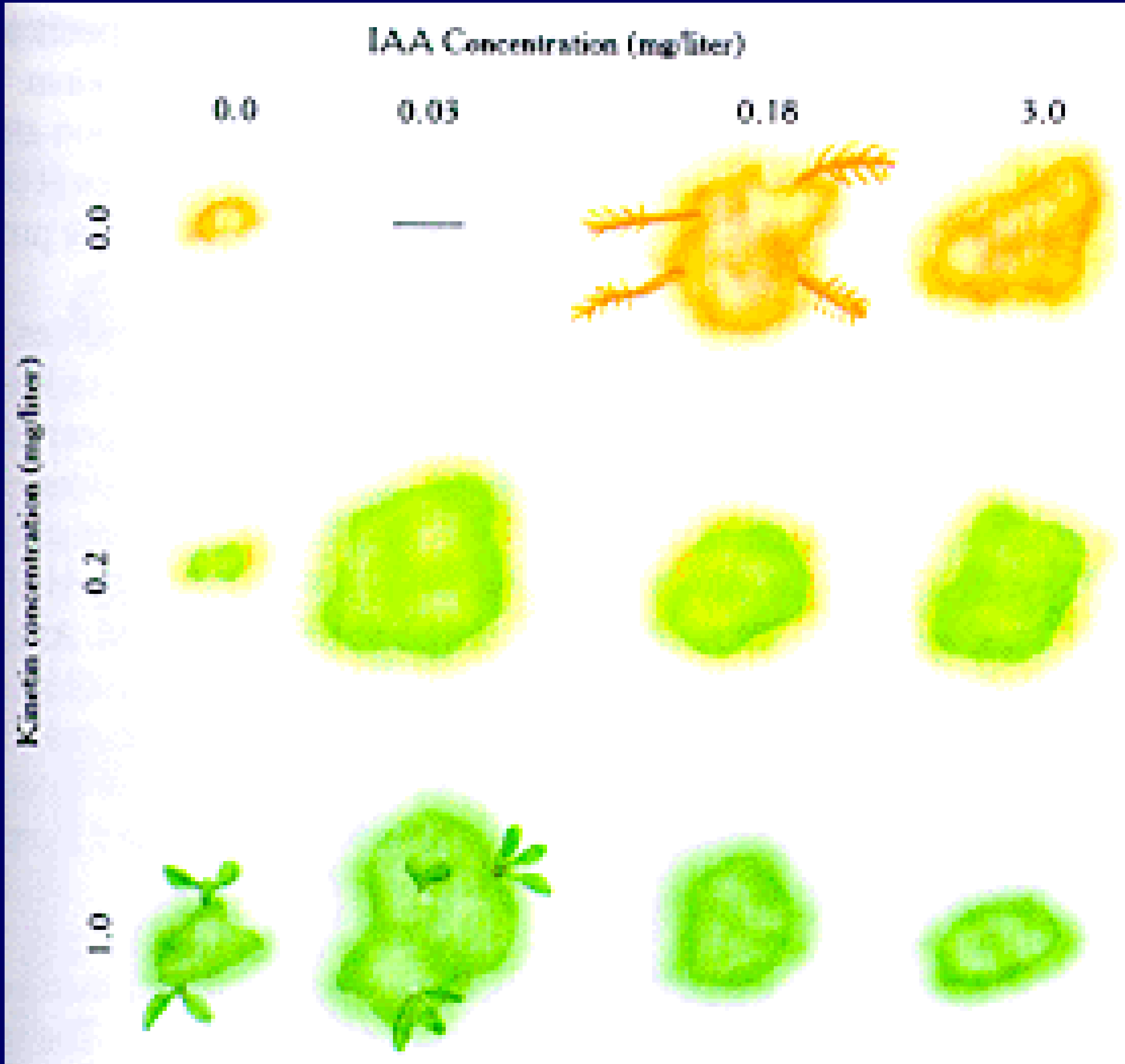


Zeatin

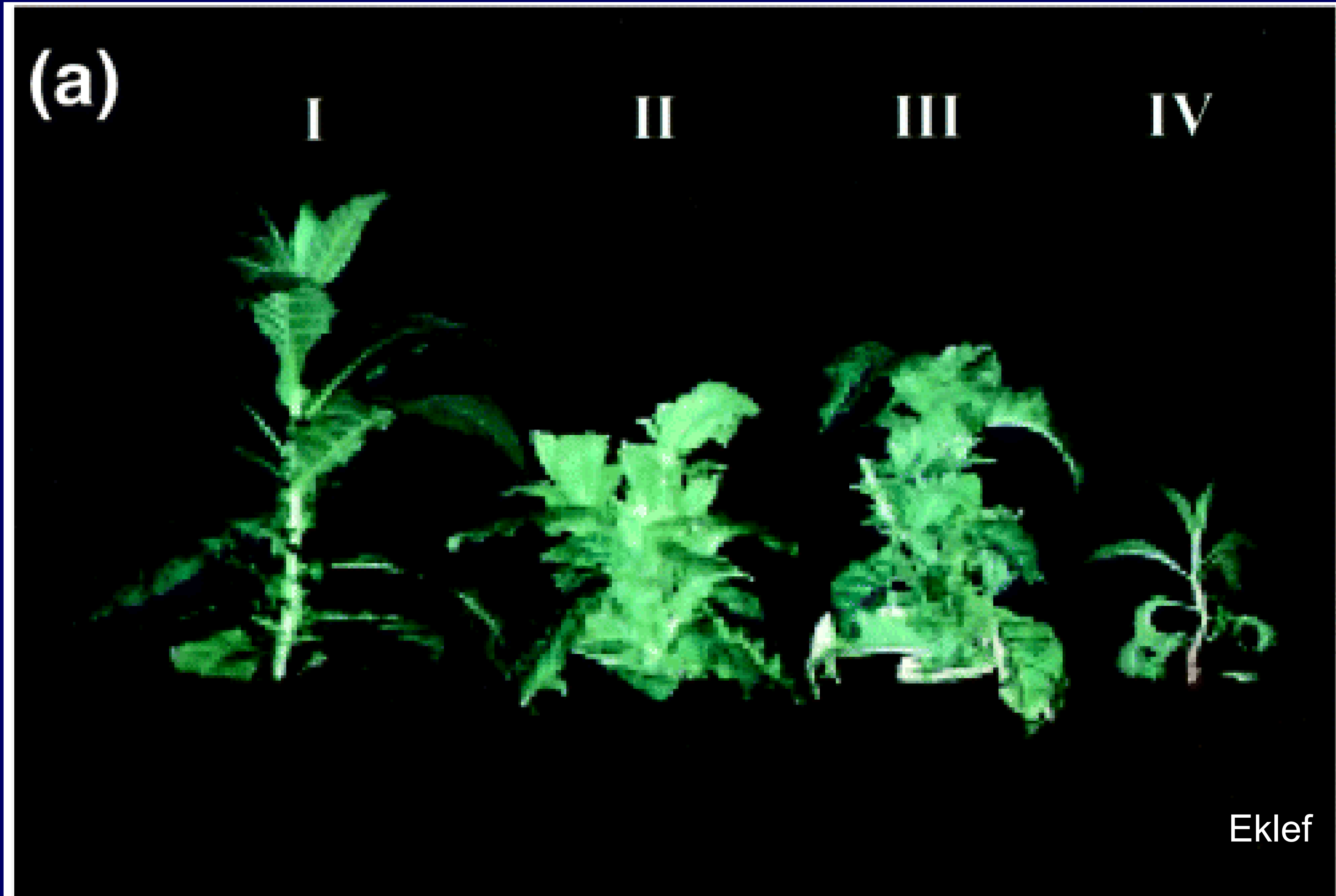


Dihydrozeatin

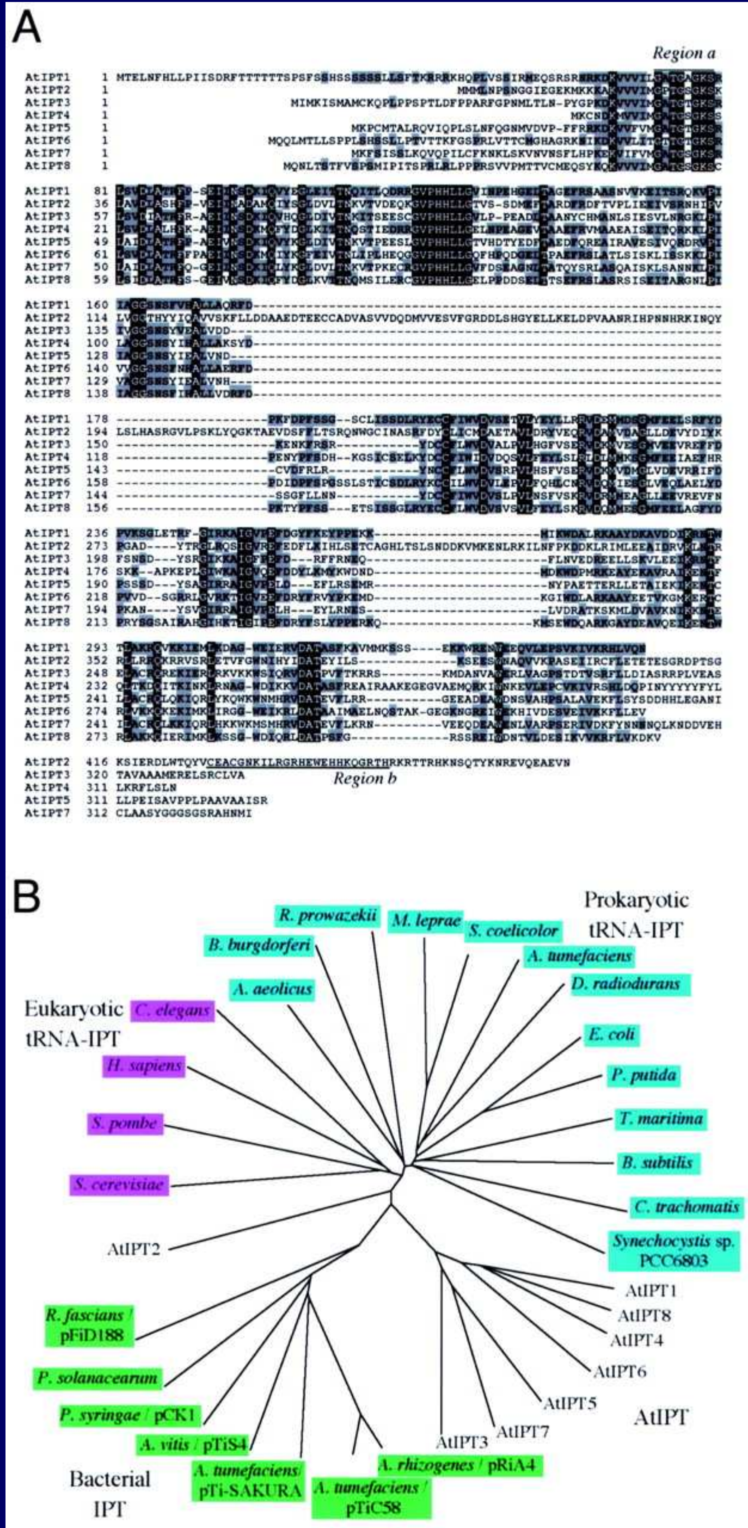
Effect of CK on regeneration

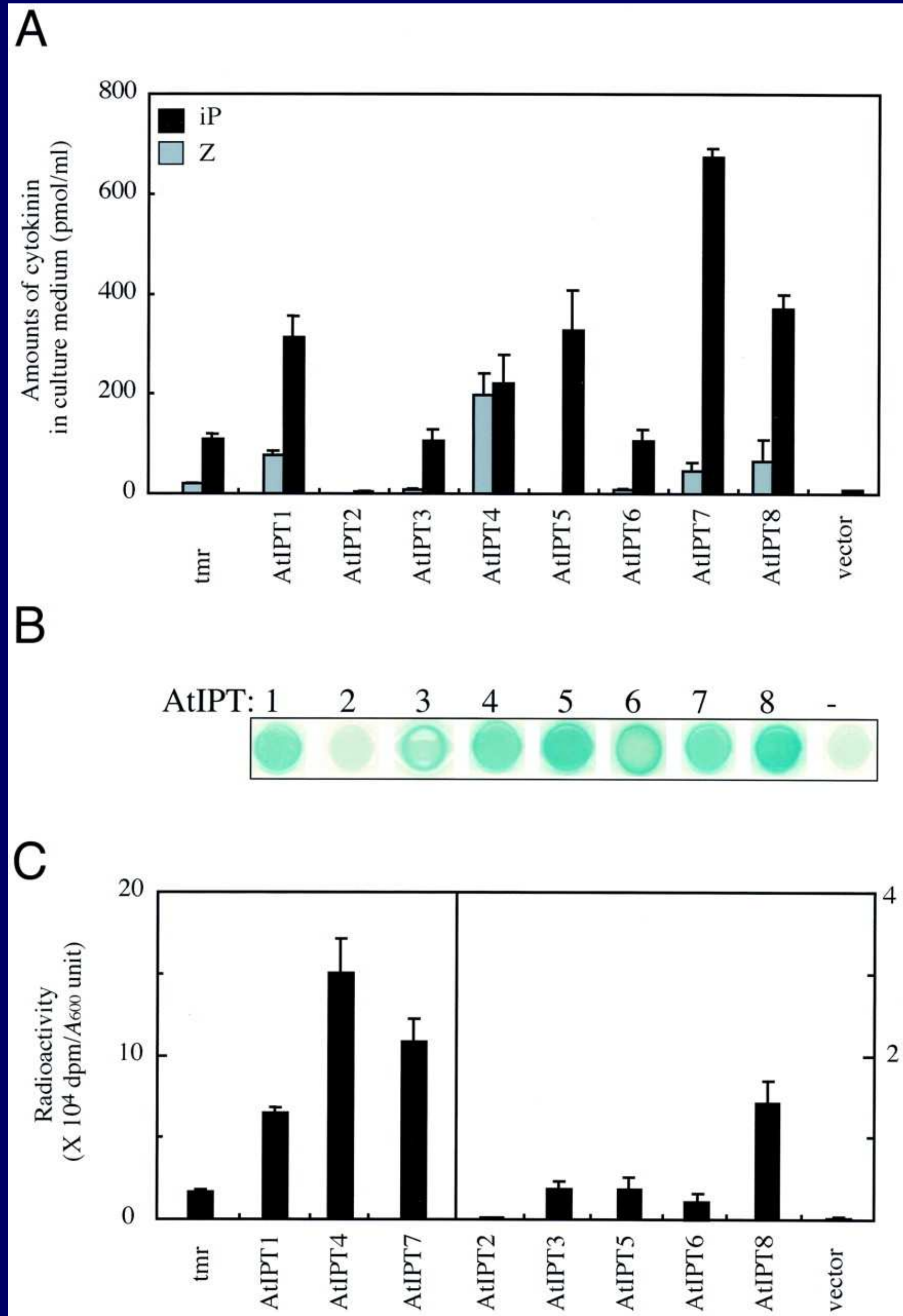


Manipulating of CK levels by overexpression of bacterial *IPT*



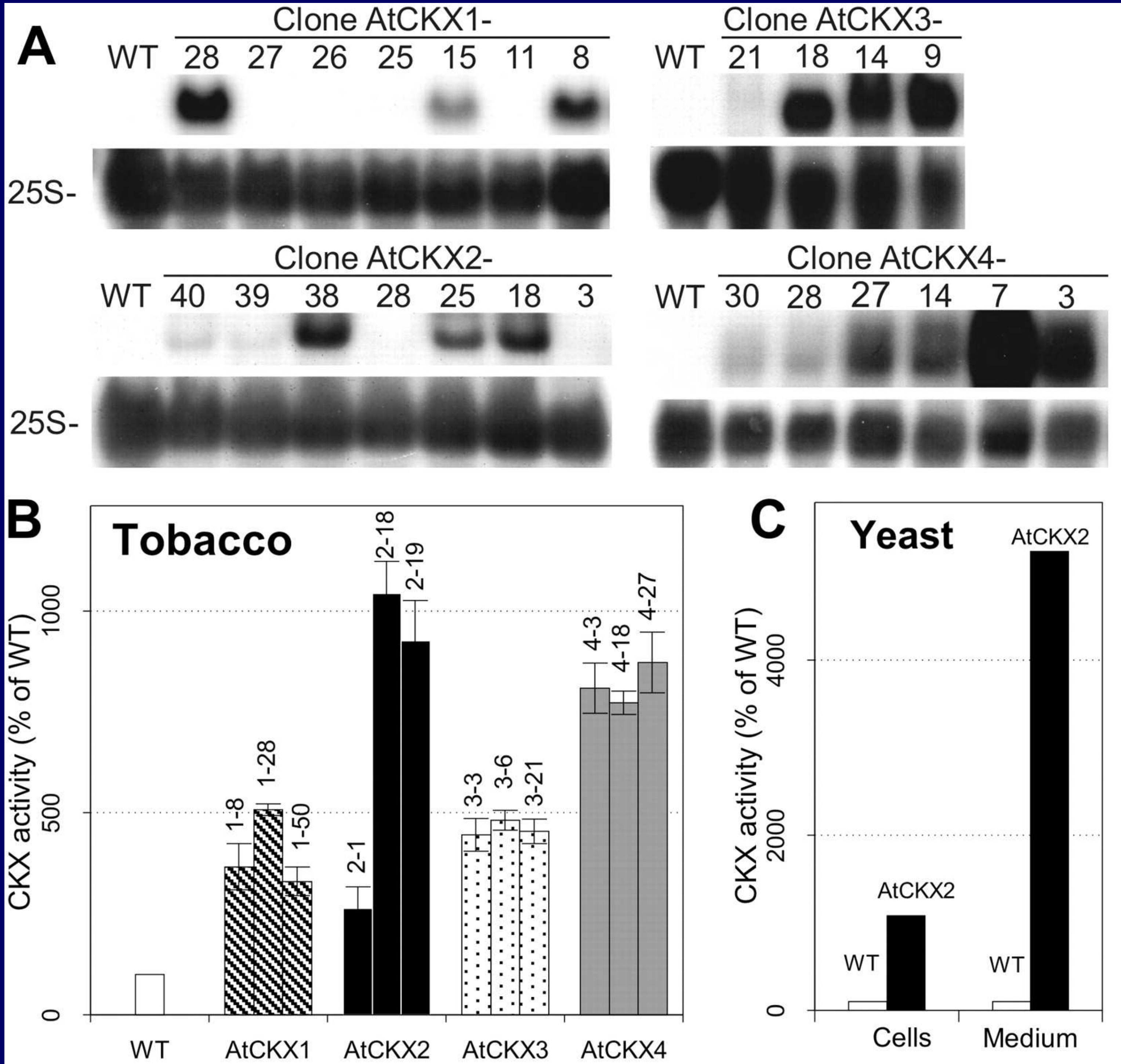
Isolation of Arabidopsis *IPTs*





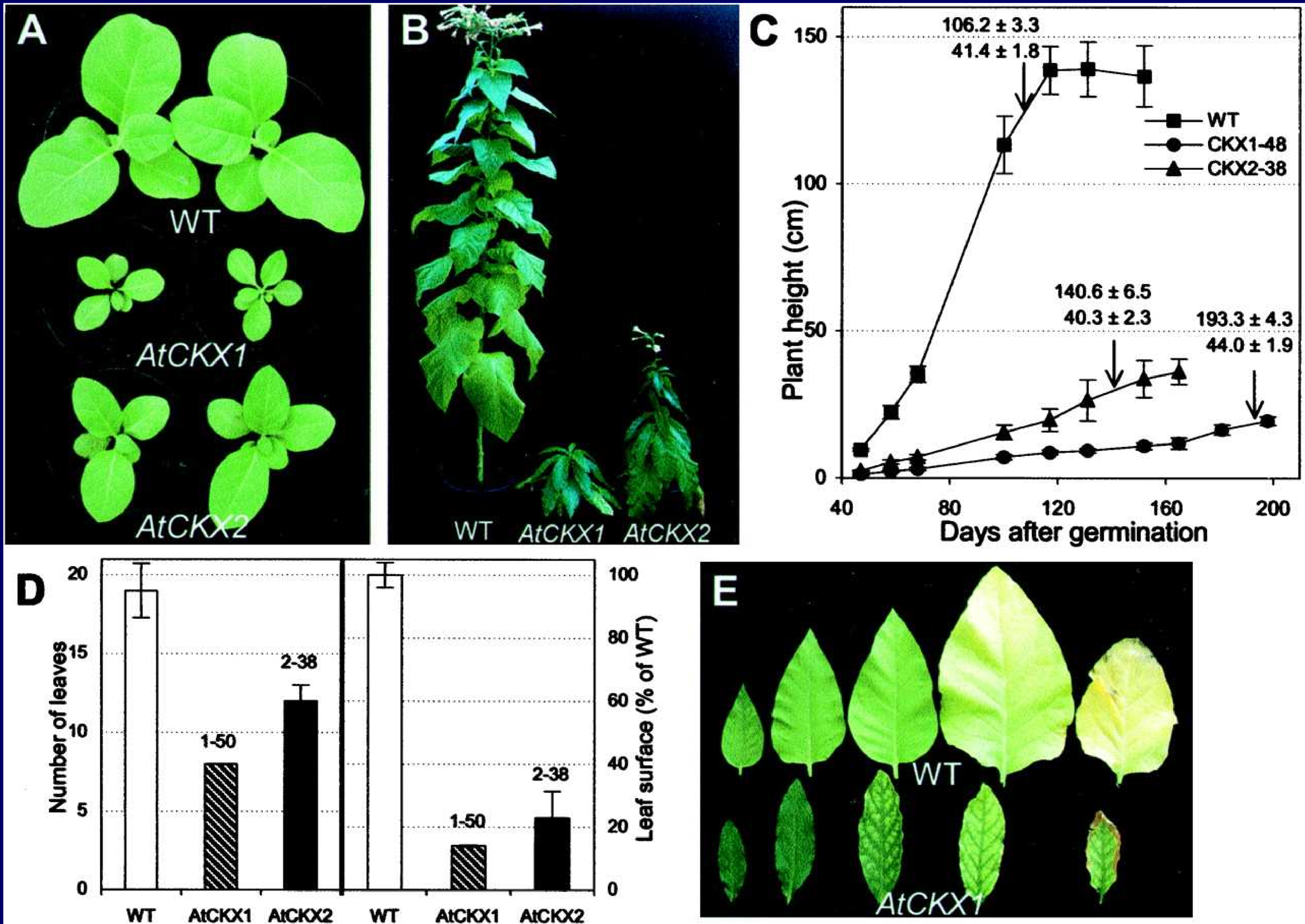
Takei

Isolation of CK-oxidase (AtCKX)

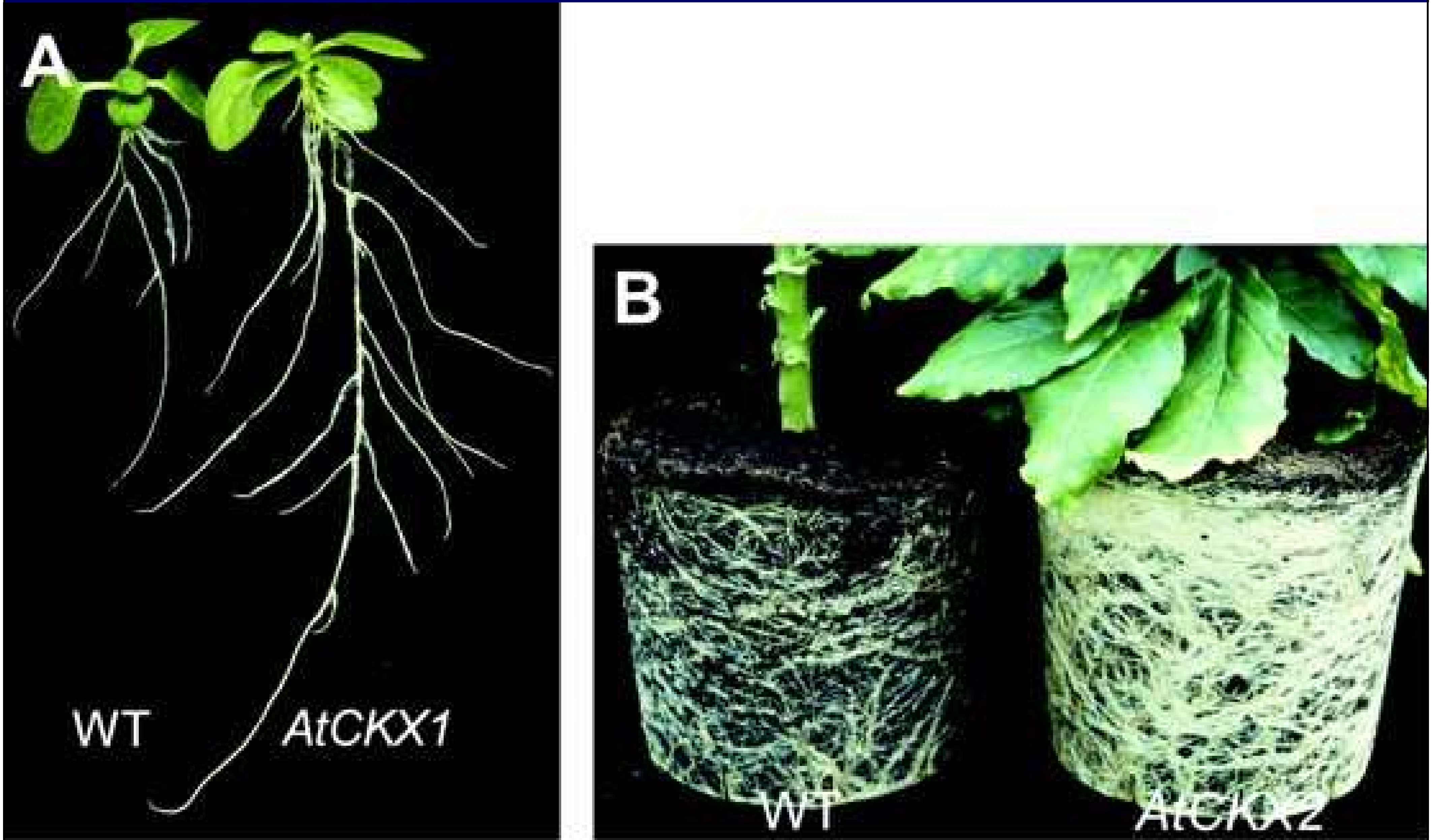


Werner

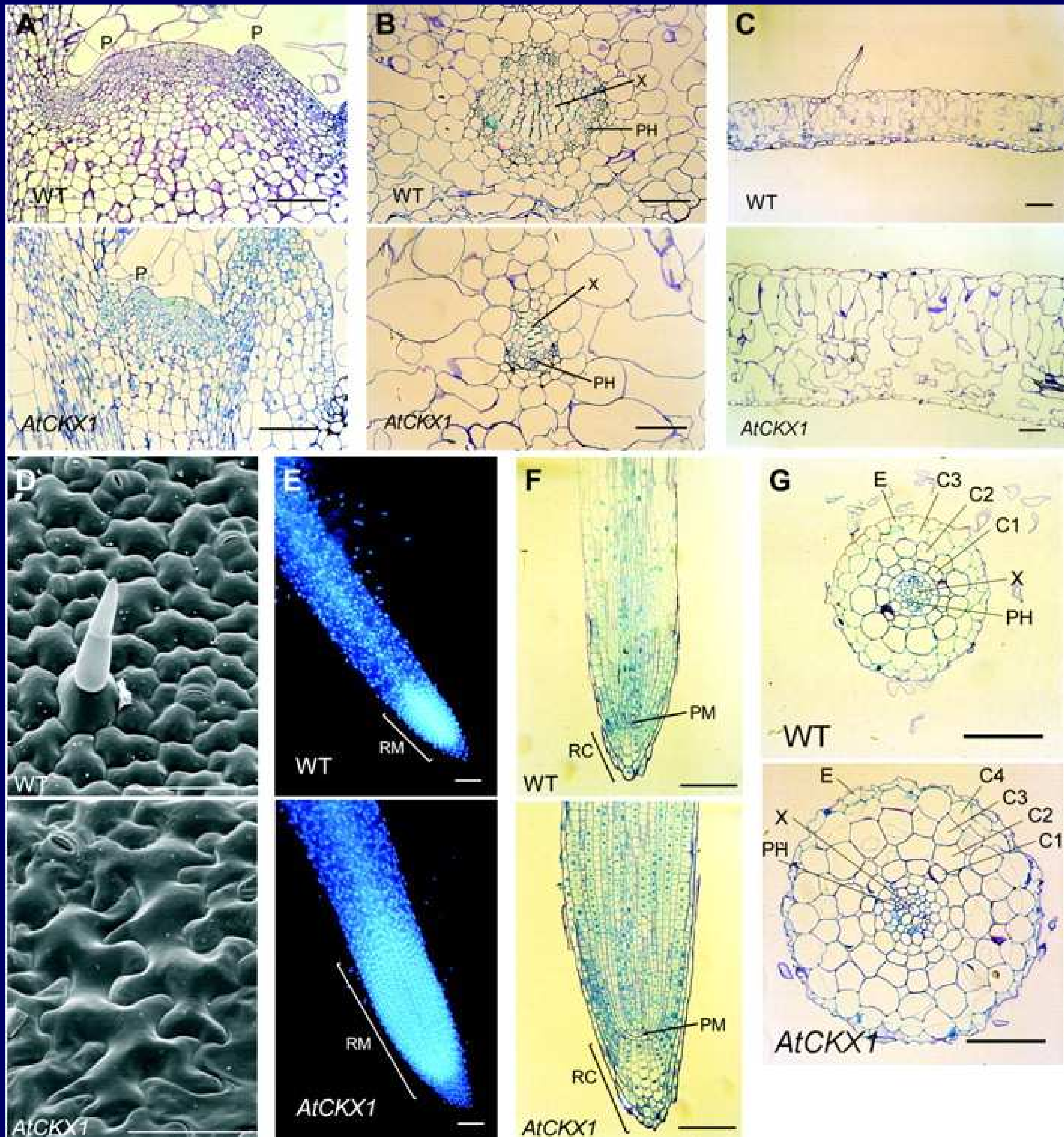
AtCKXs overexpression in tobacco



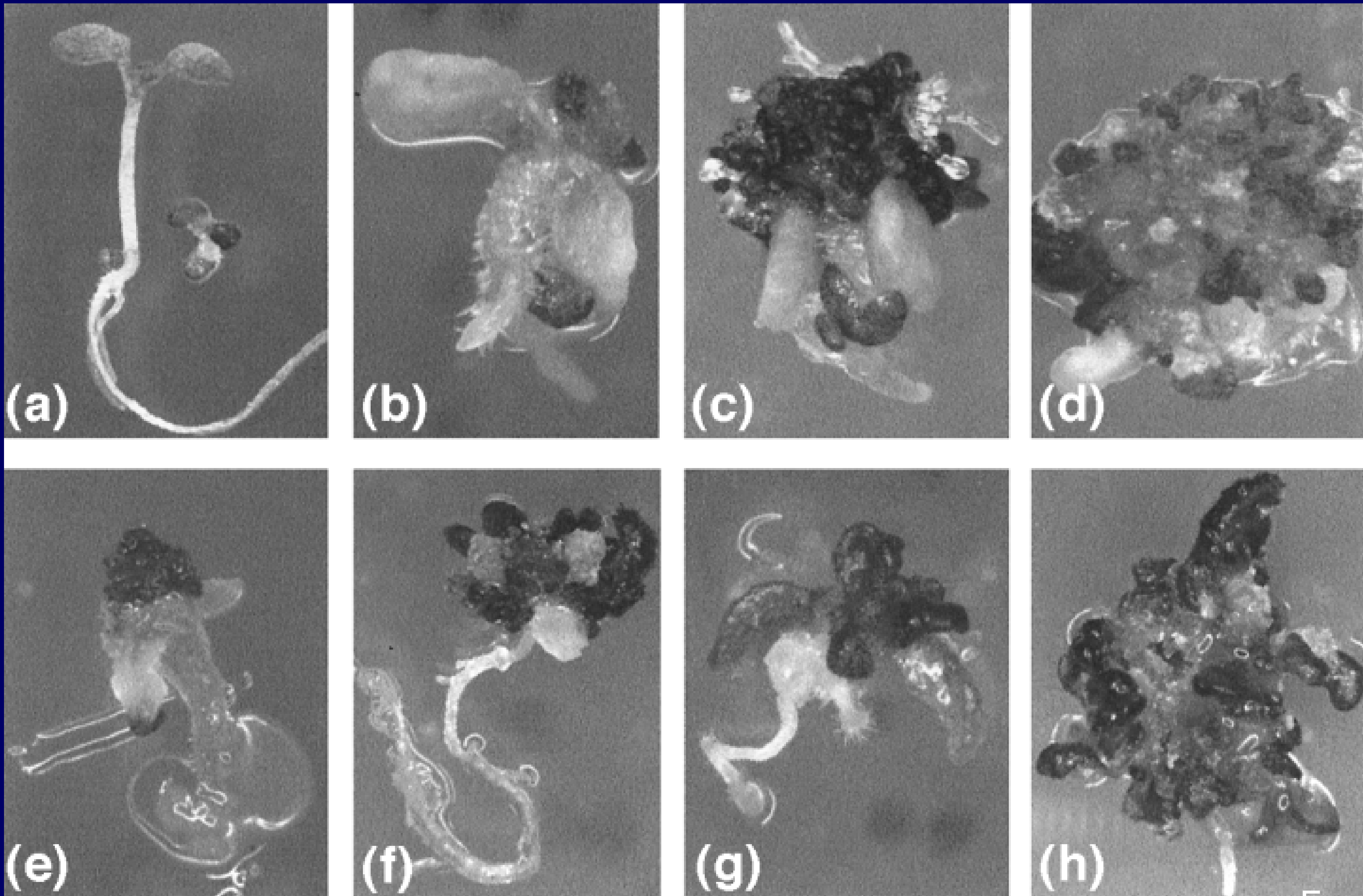
Effect of AtCKX on tobacco root



Morphology of AtCKX tobacco plants

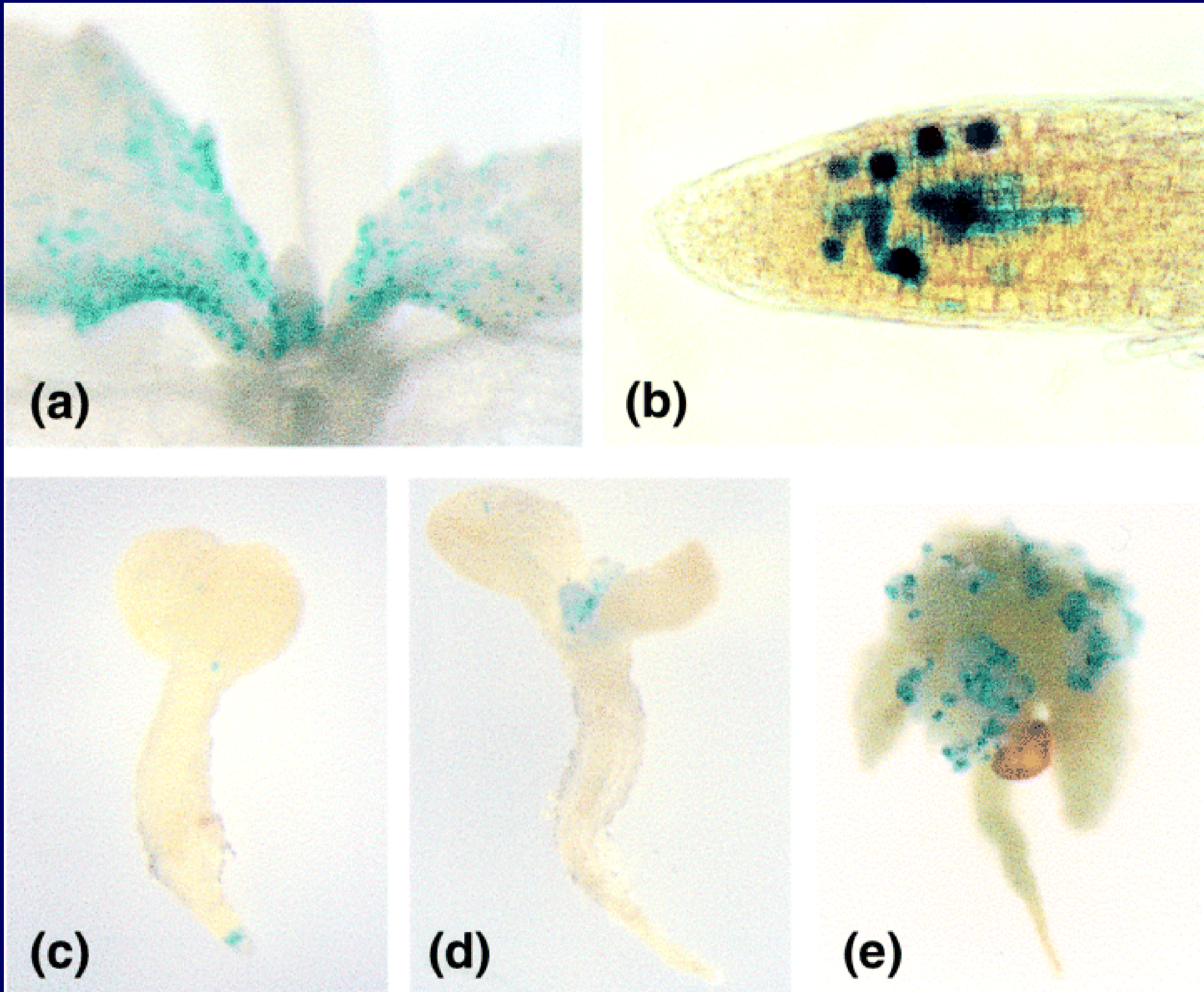


Tumor shoot development (*tsd*) and *pasticcino* (*pas*) mutants

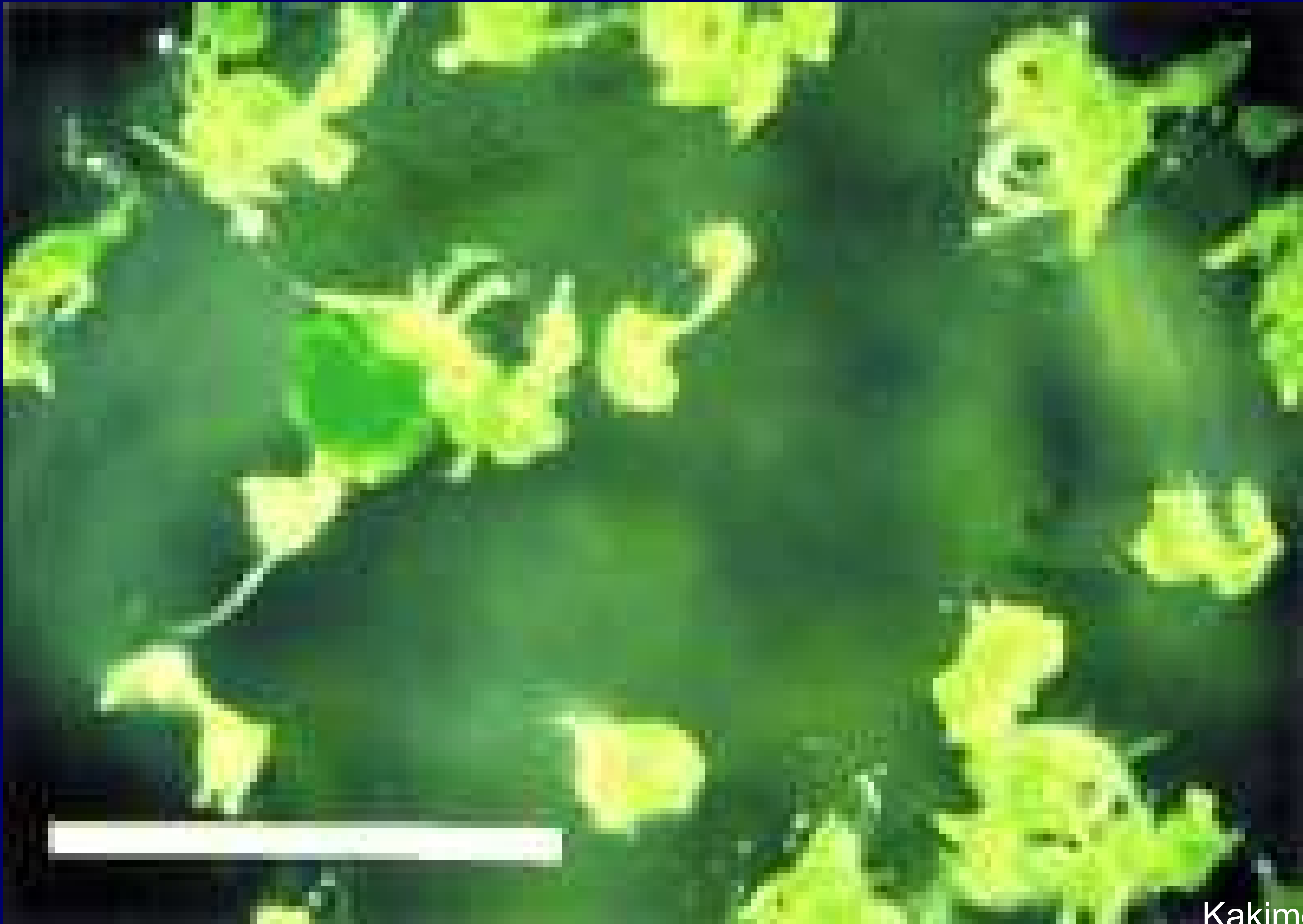


Frank

Use of *CyclinB::GUS* for cell division monitoring

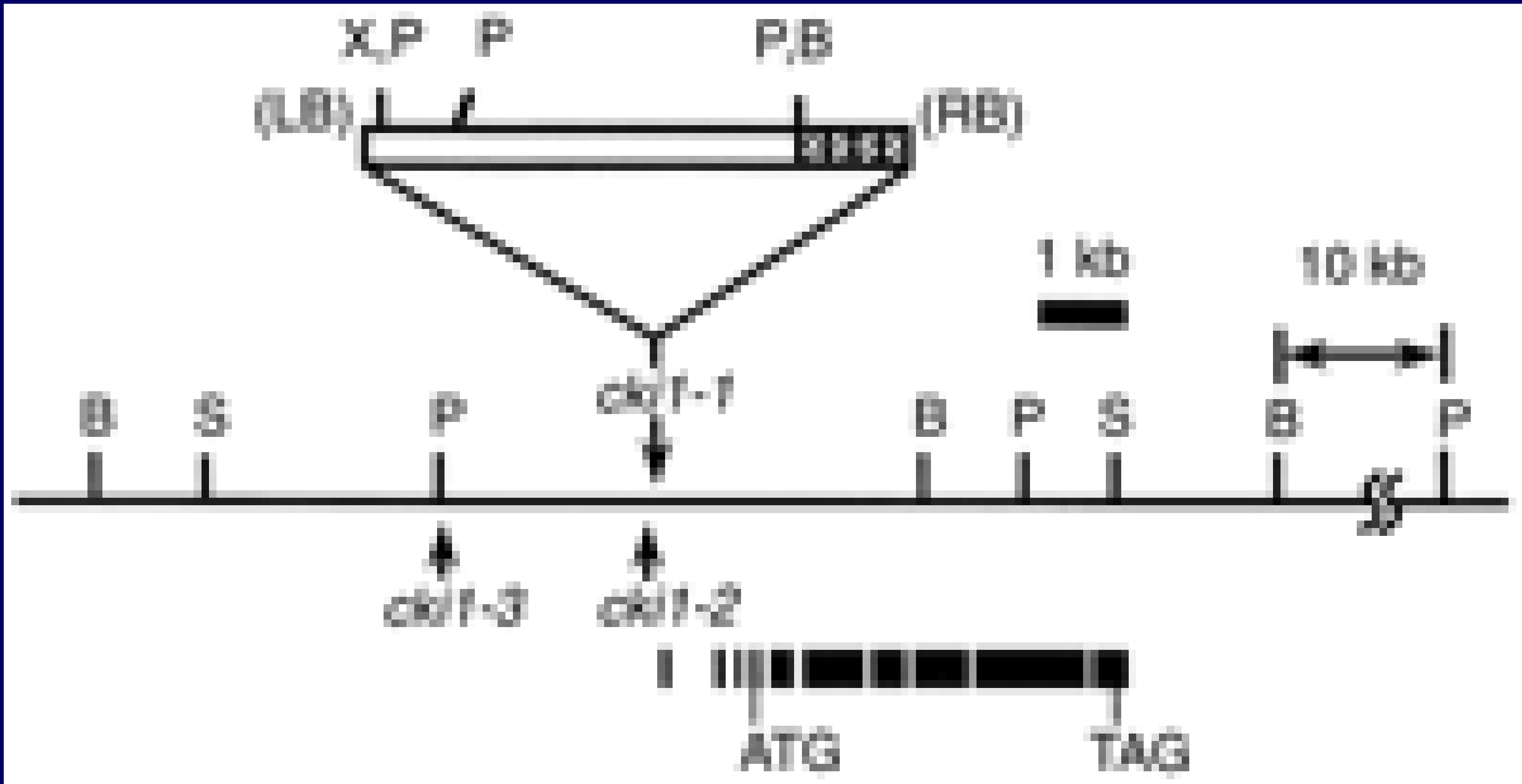


Isolation of CK independent (*cki1*) mutant



Kakimoto

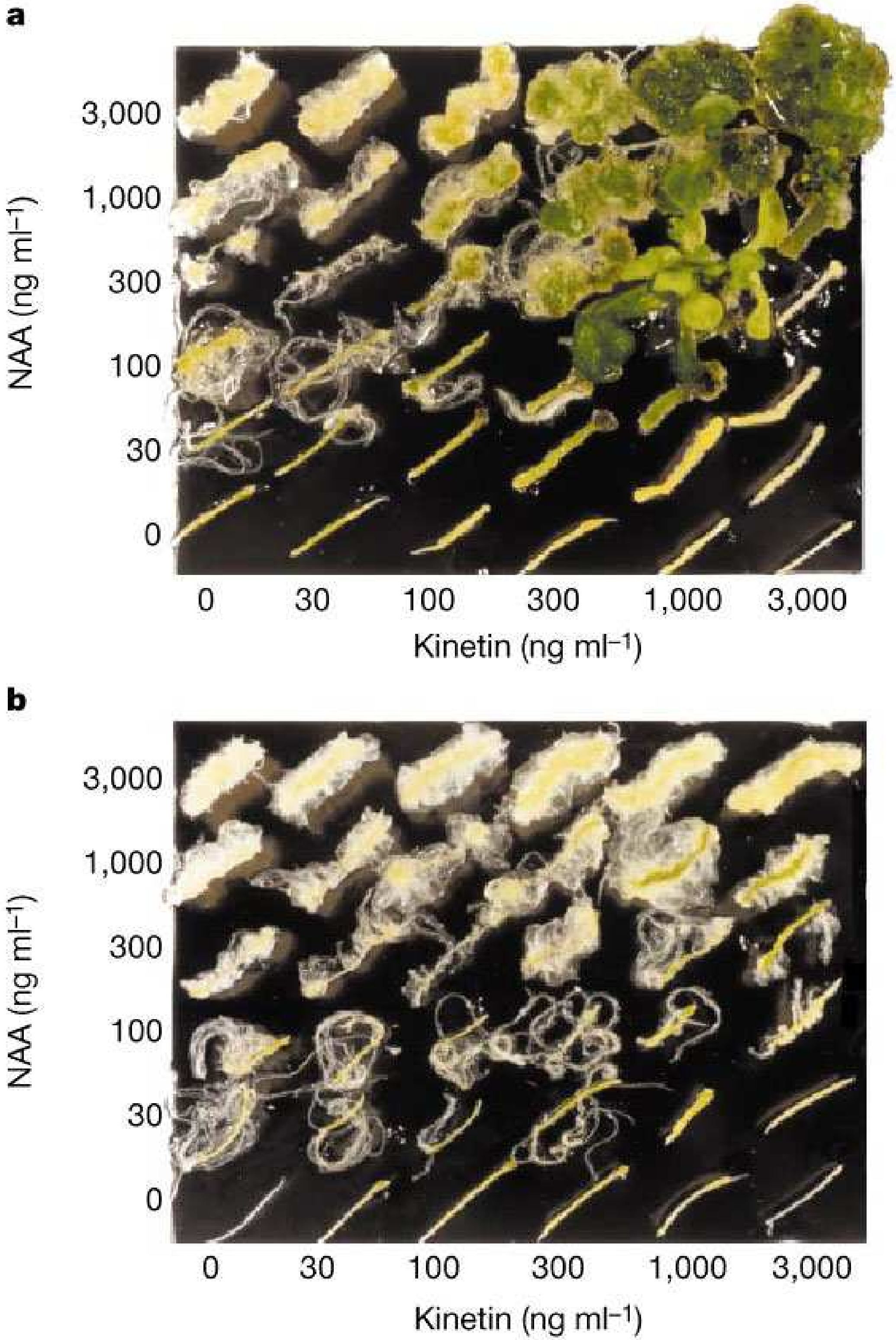
Identification of *CKI1* gene

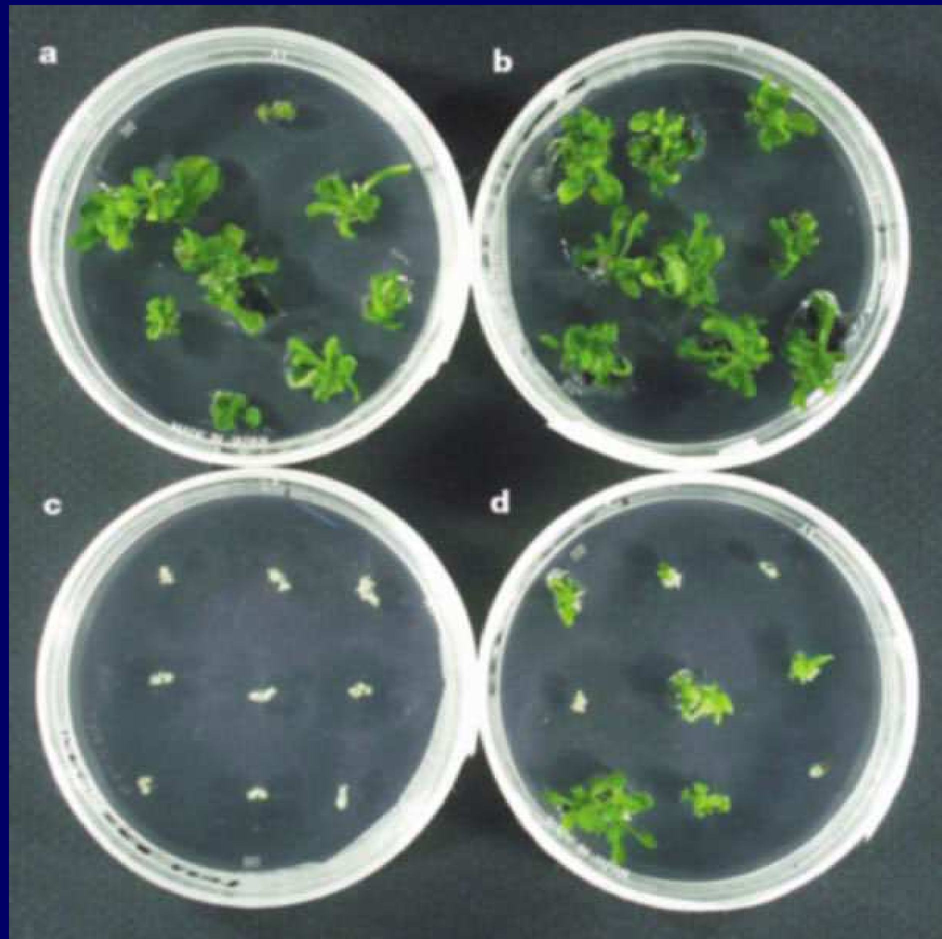


Kakimoto

Verification - *35S::CKI1* transgene

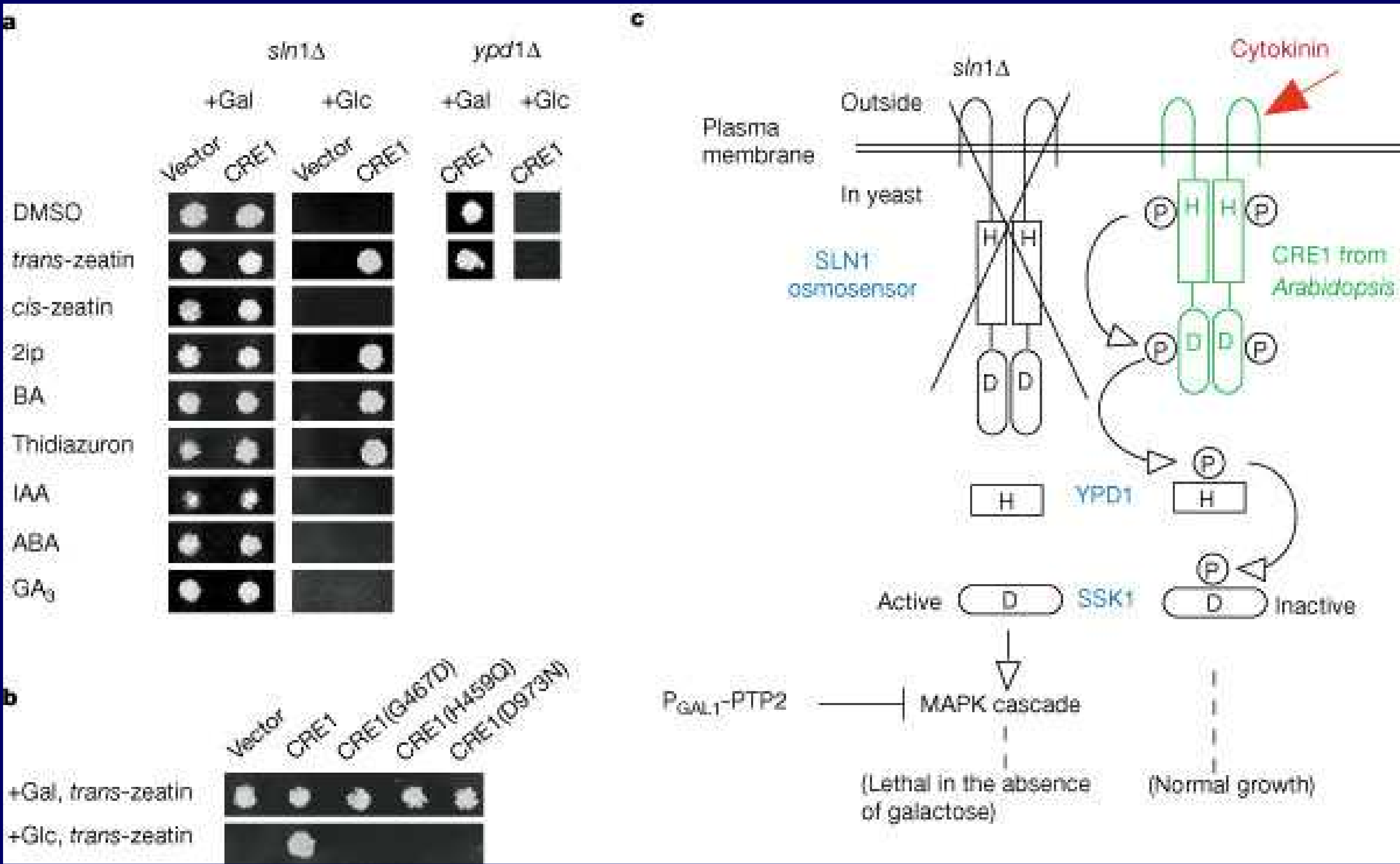
Next strike
- CK response mutant (*cre1*)



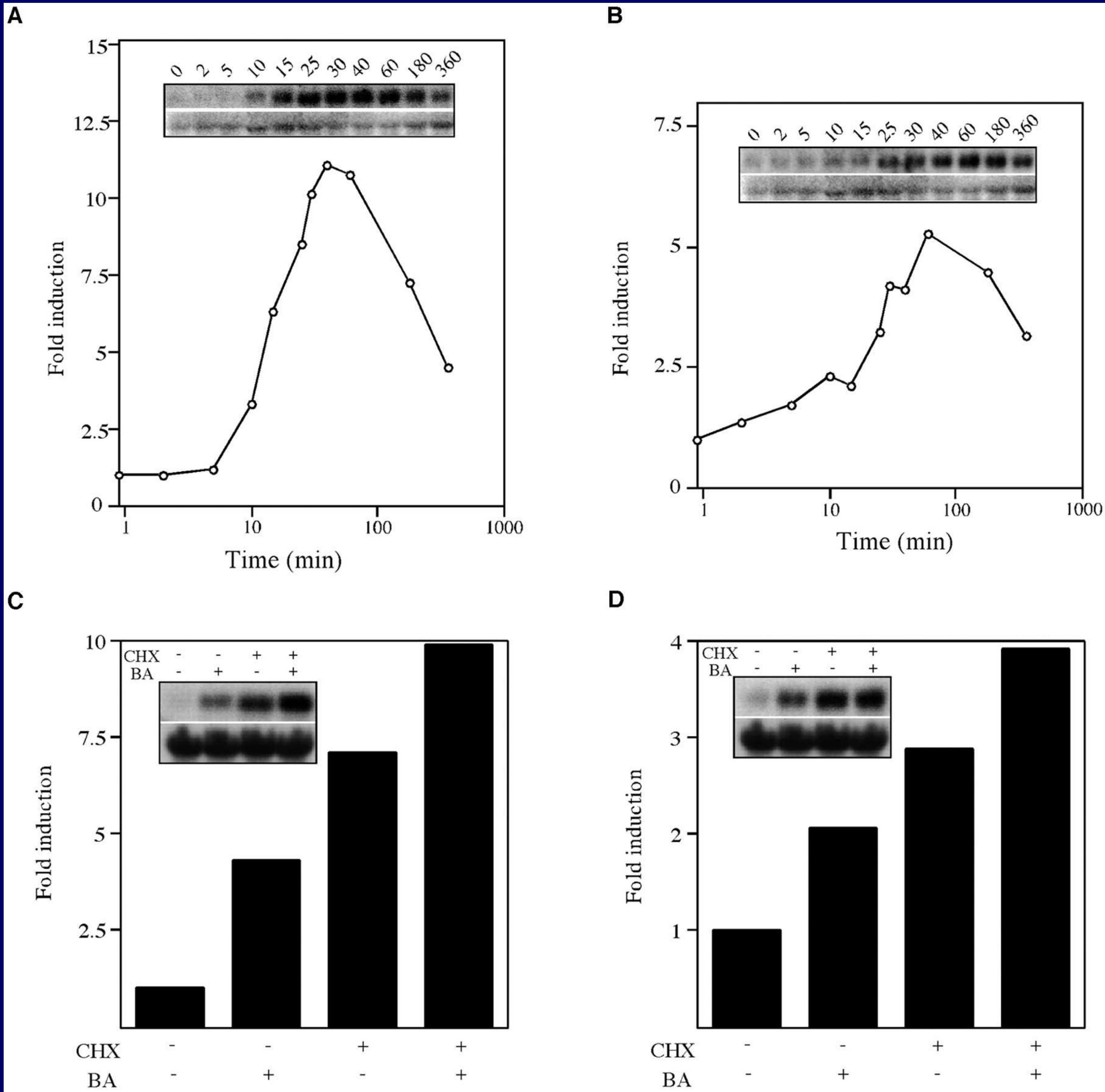


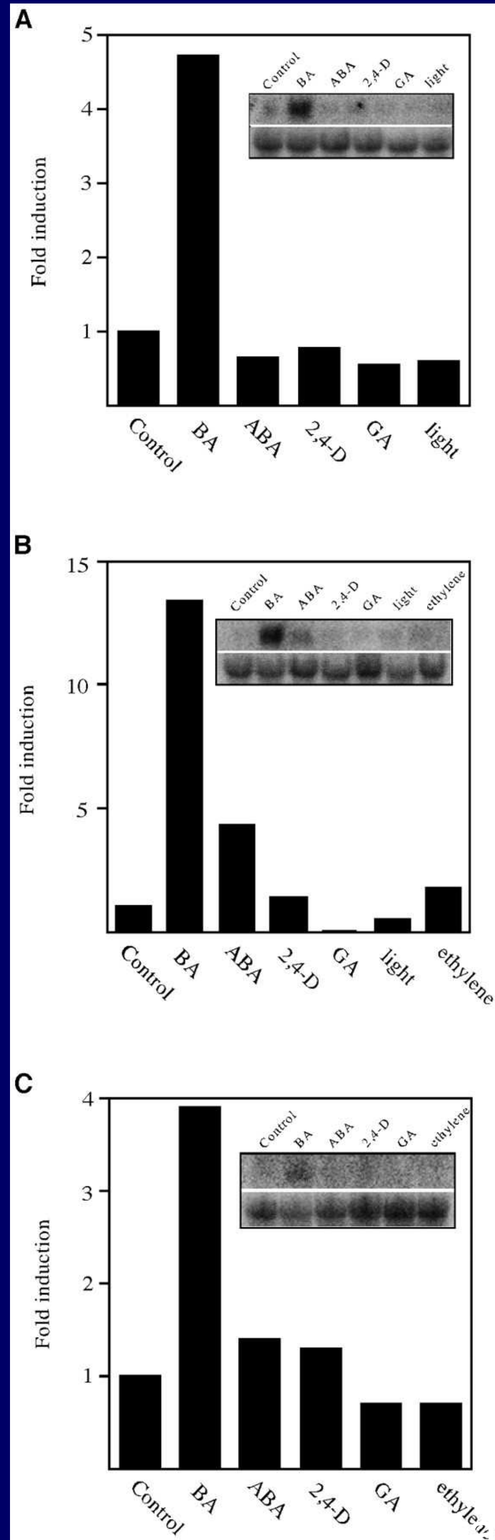
Inoue

Piece of genius - complementation



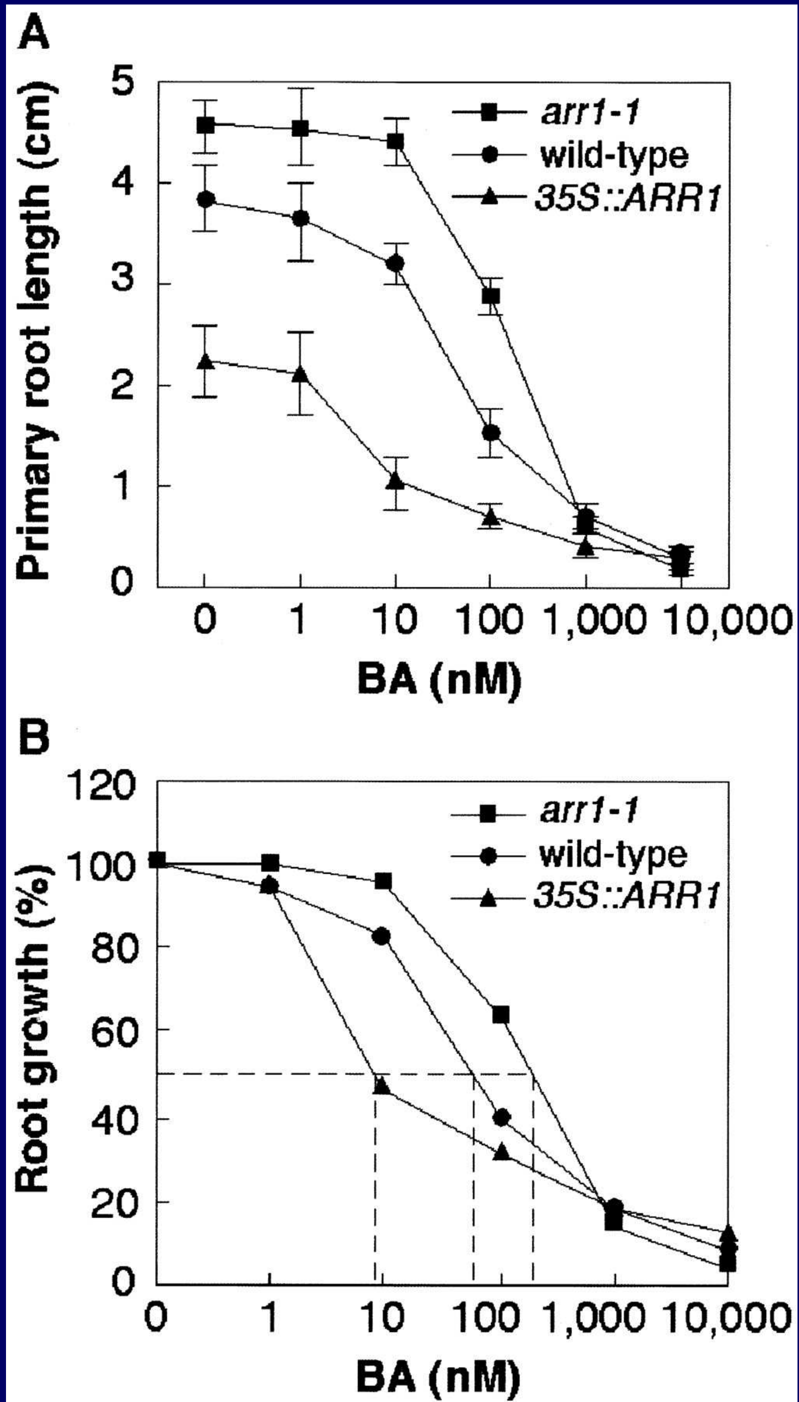
CK responsive genes - *ARRs*

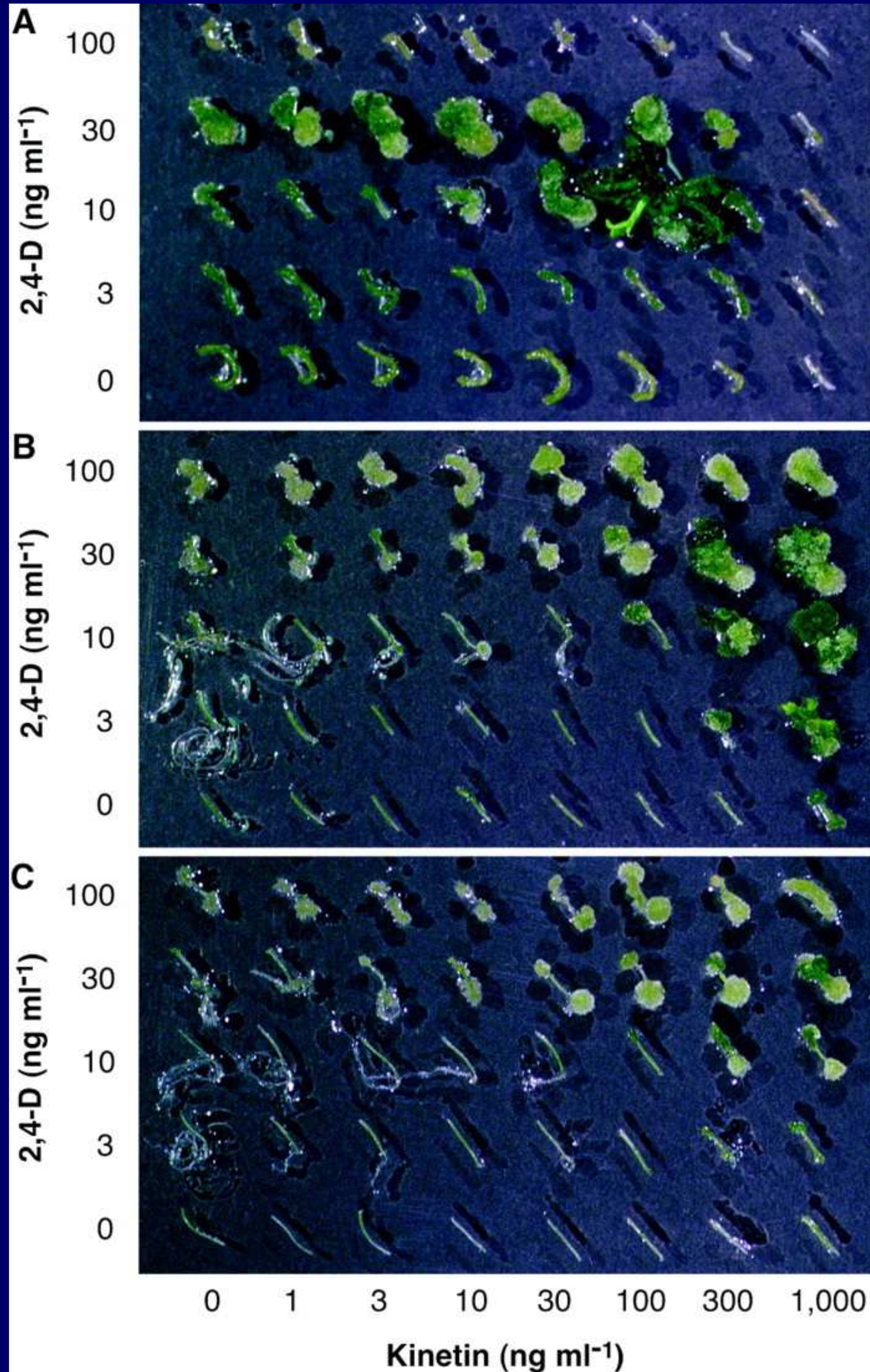




Brandstatter

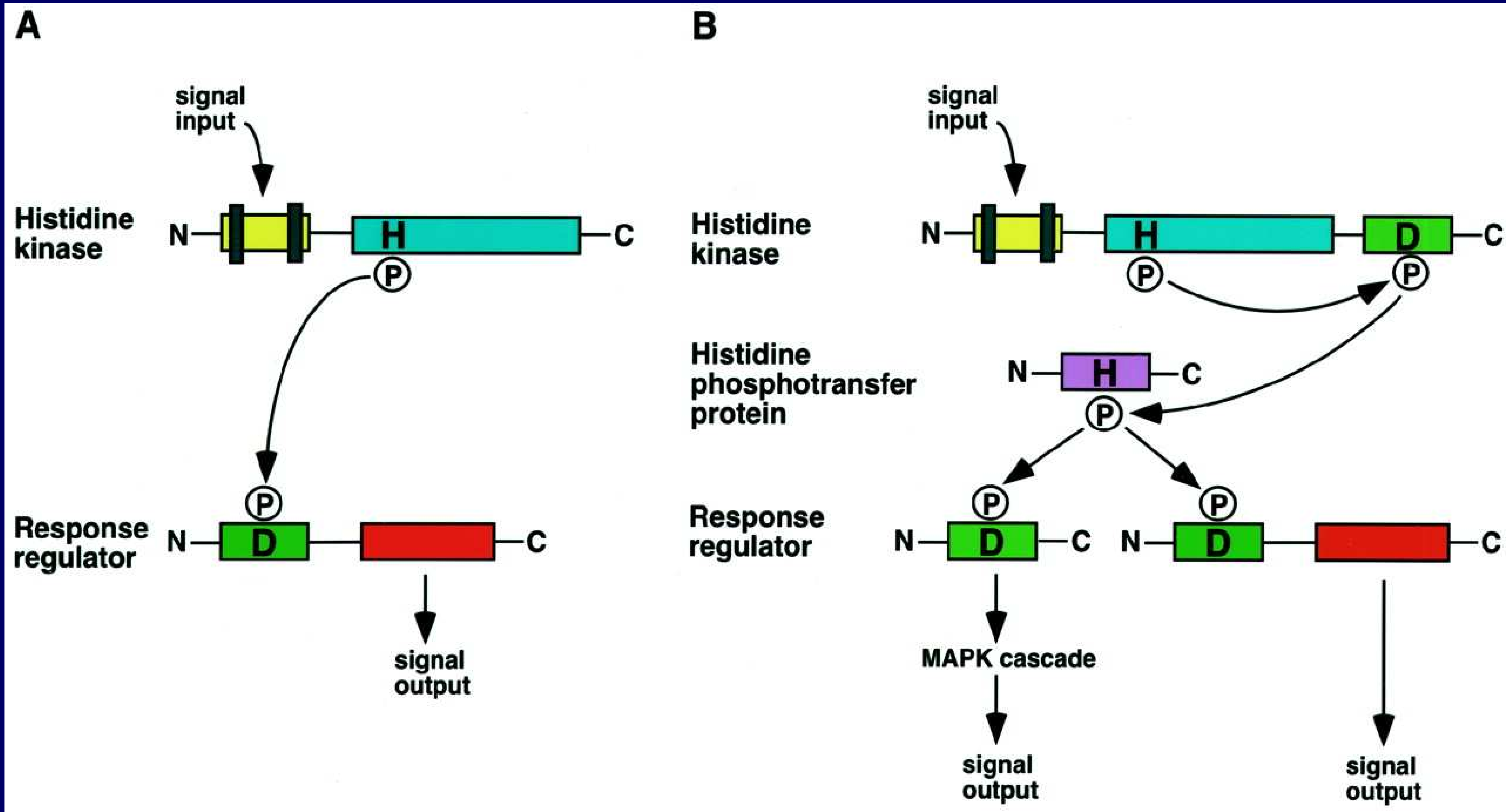
Phenotypes of *arrs*





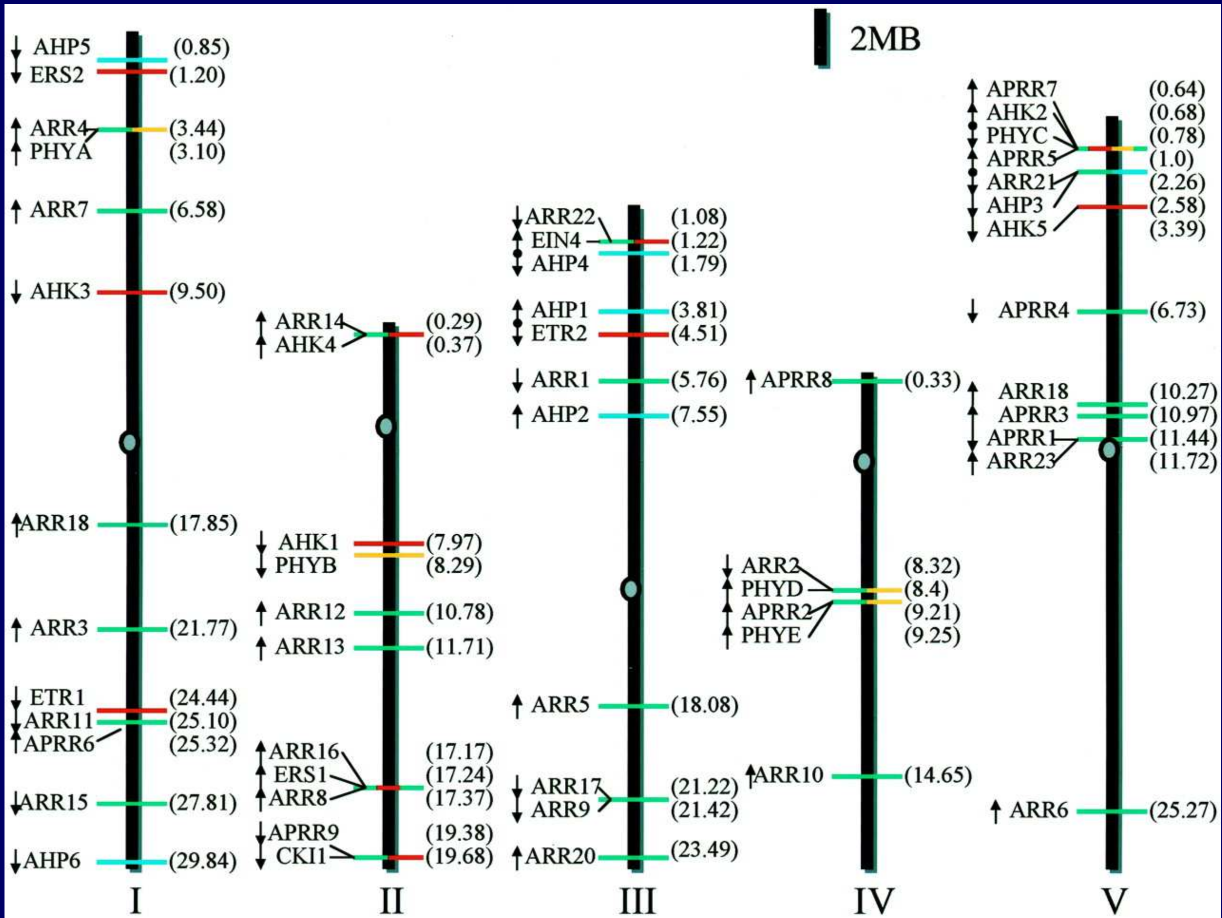
wt

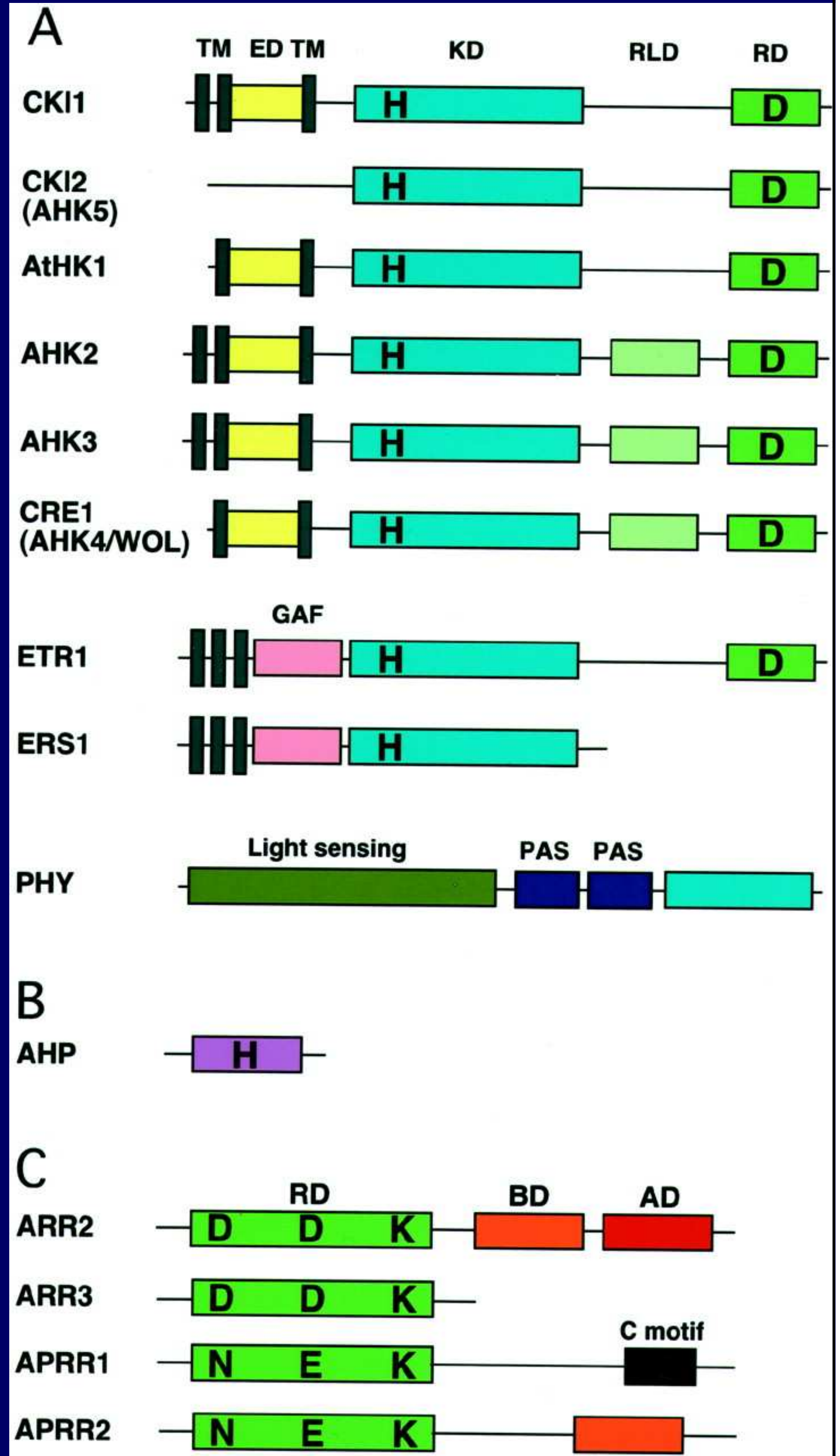
His kinase transduction pathway



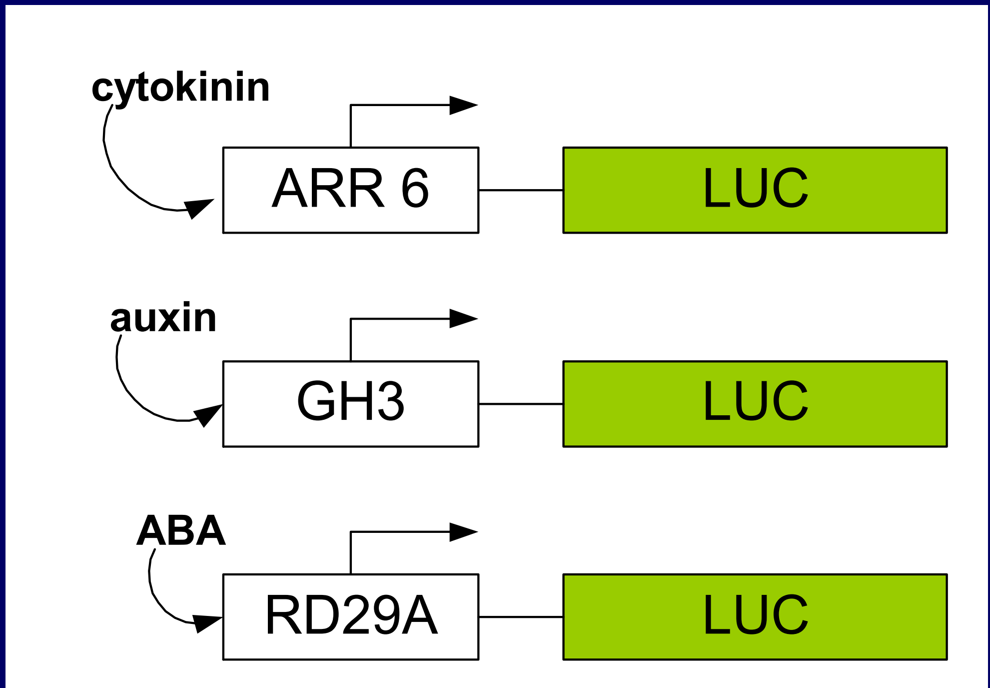
Hwang

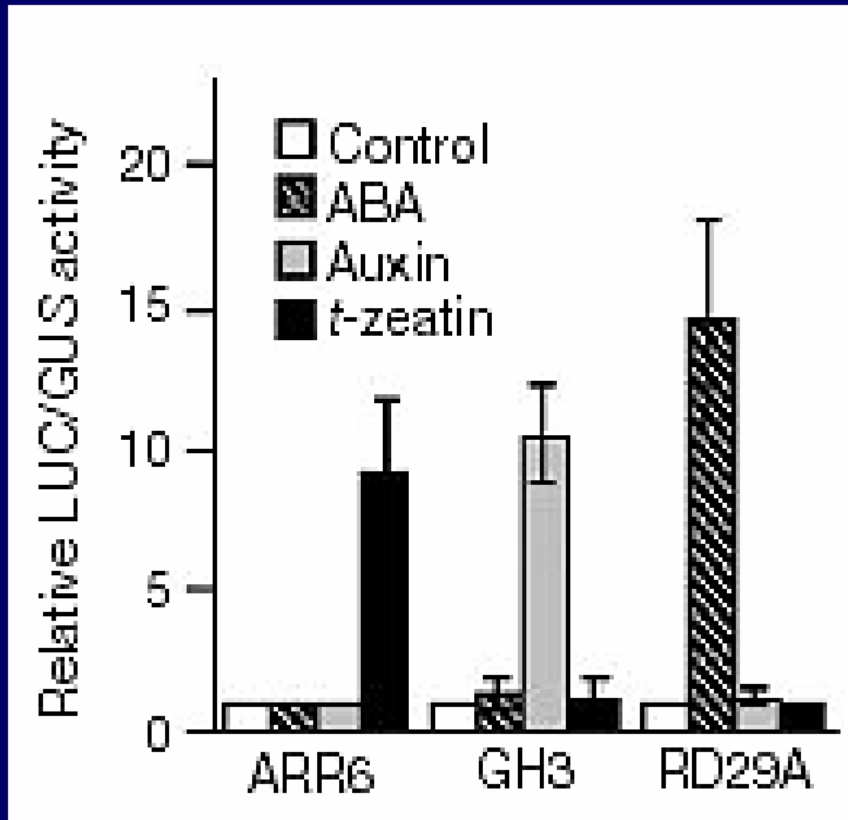
His kinase pathway components in Arabidopsis



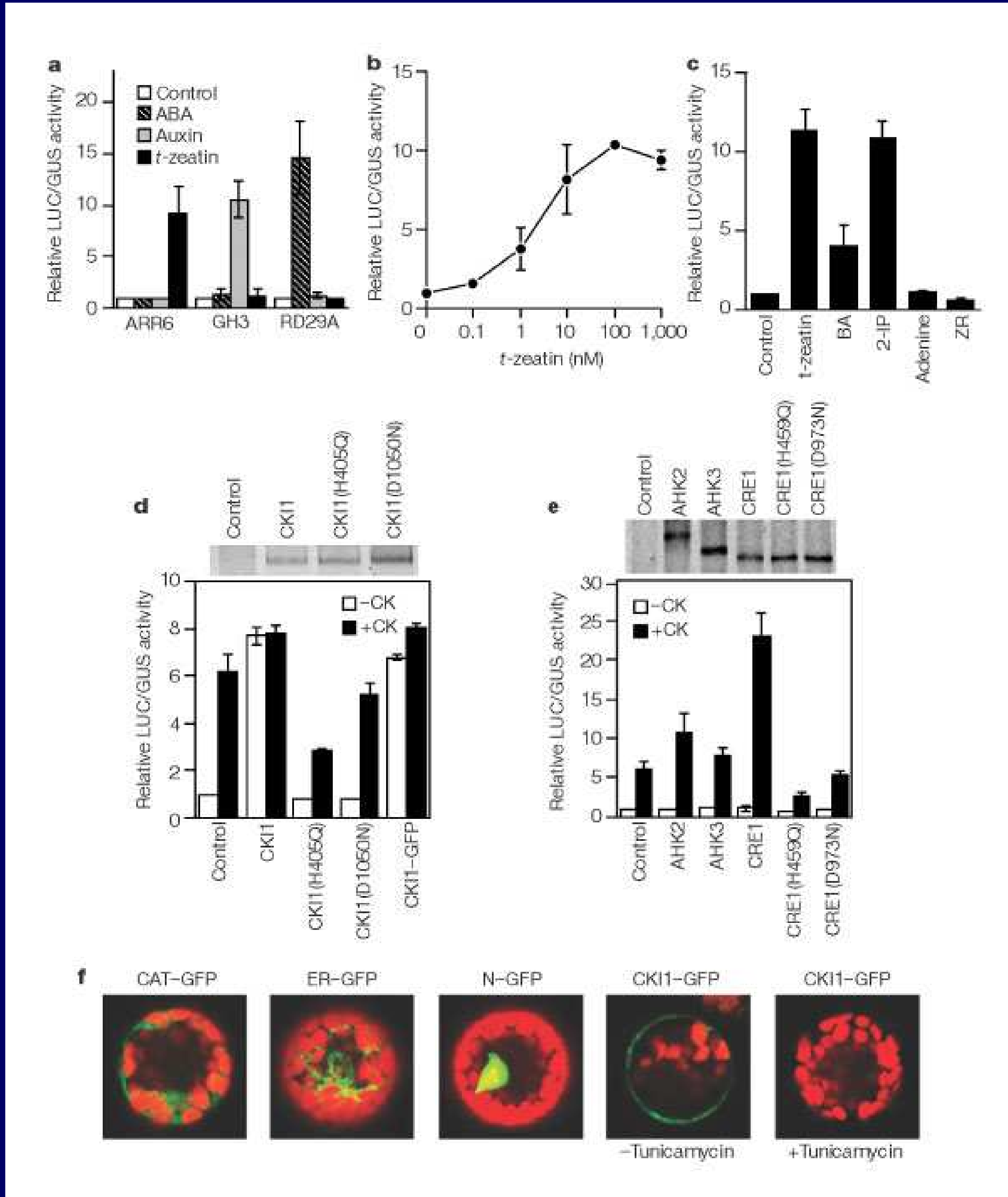


Games with protoplasts
- Reporters for different pathways



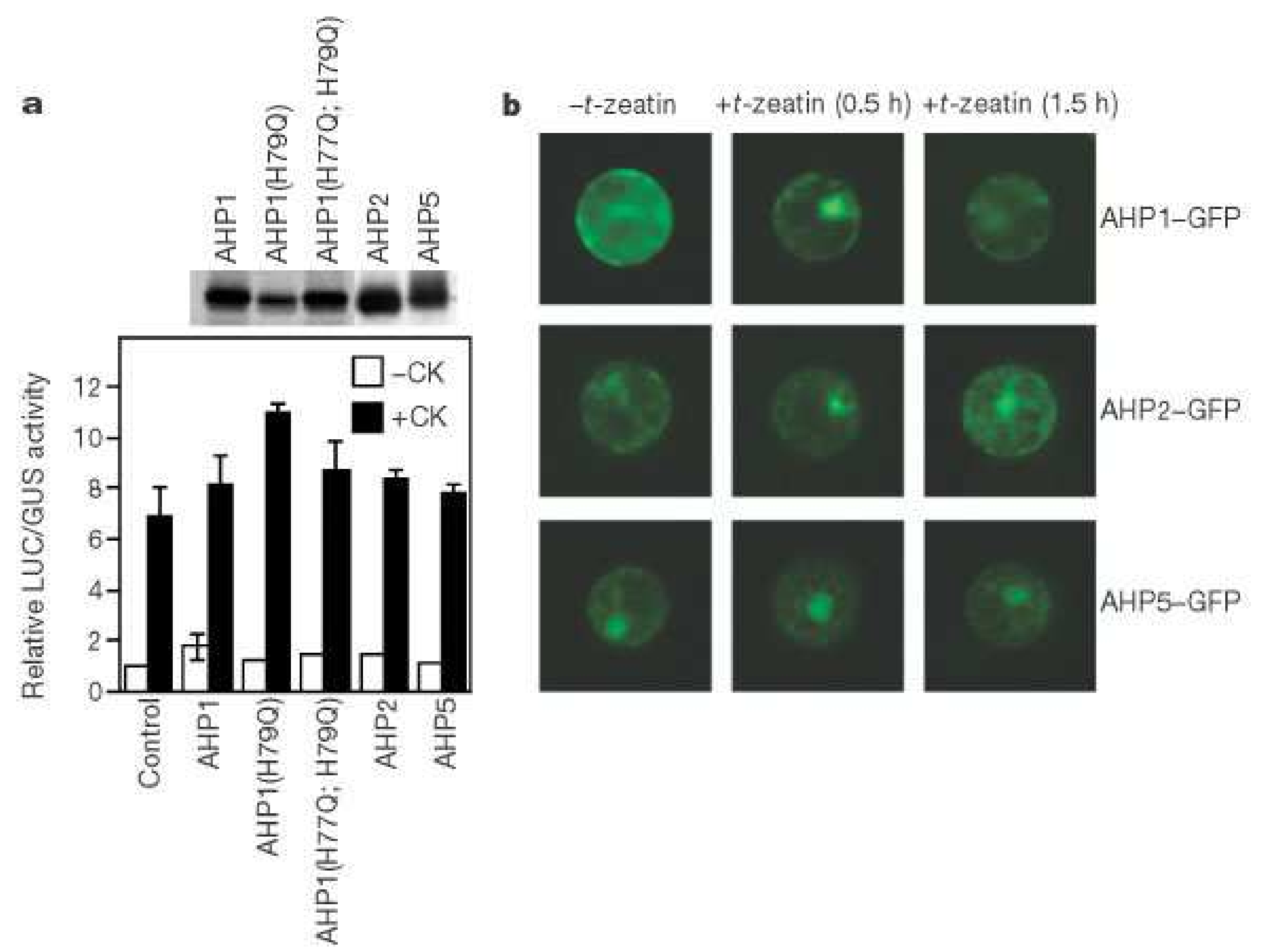


Games with protoplasts

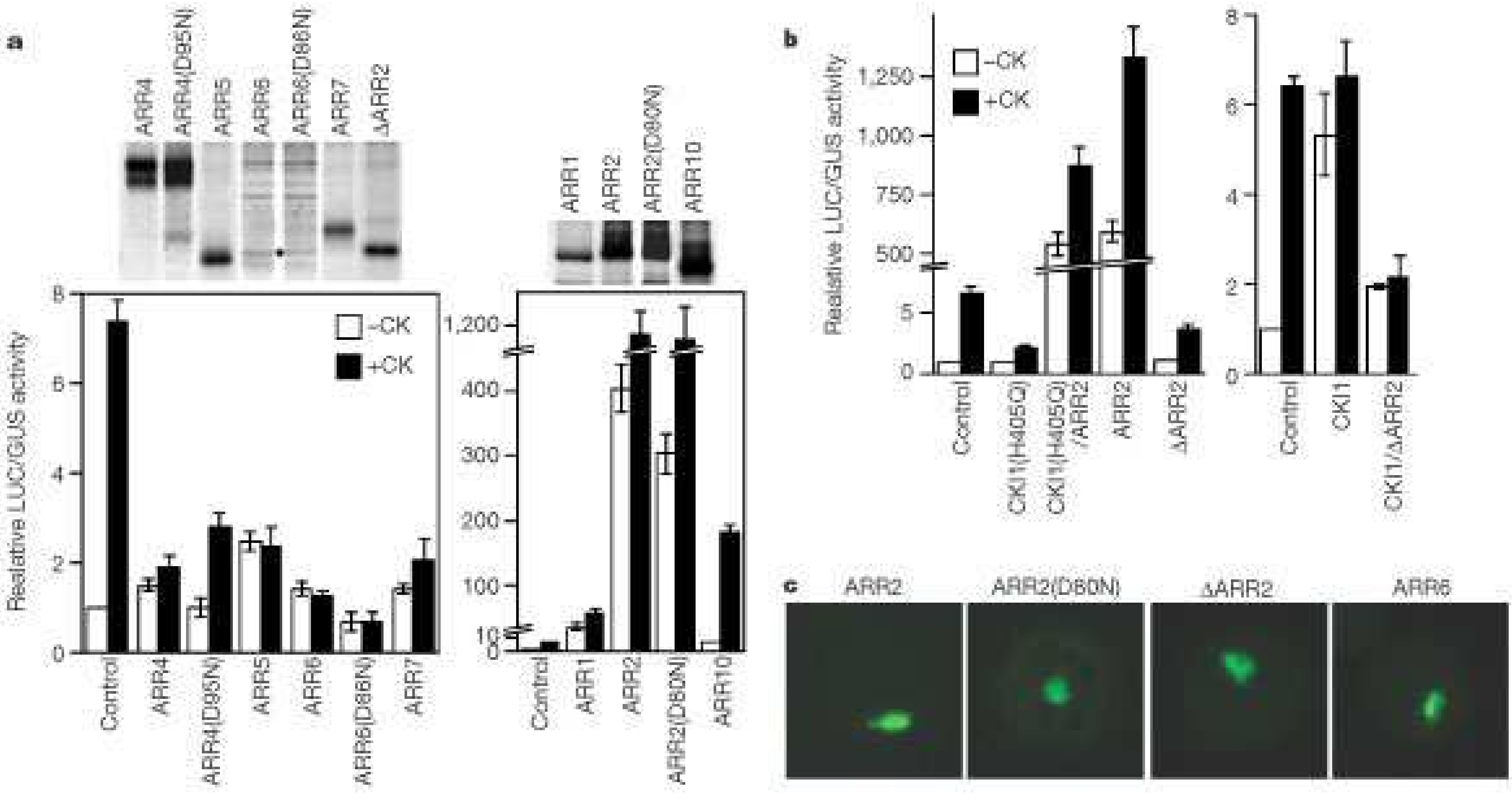


Hwang

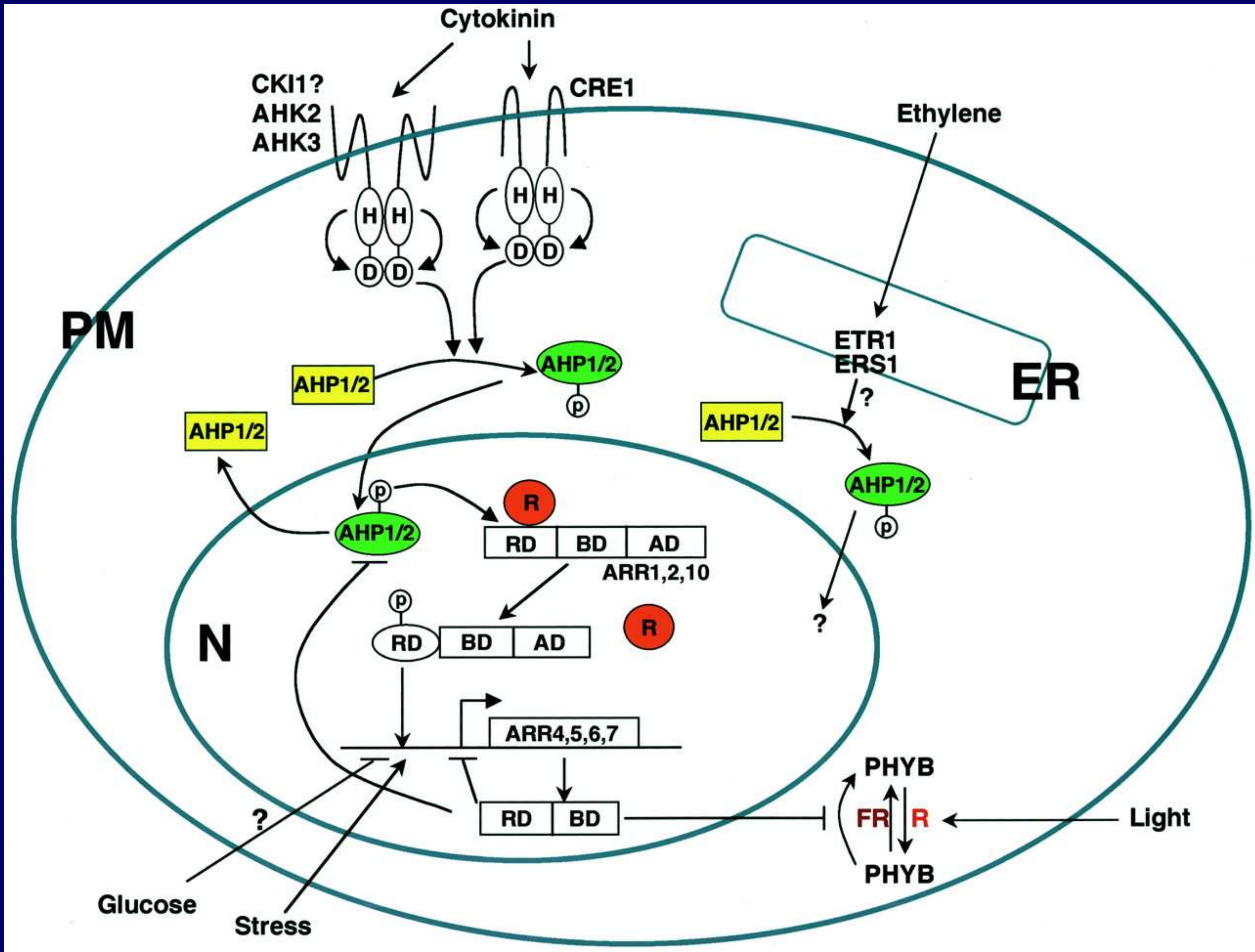
AHPs – shuttle to nucleus



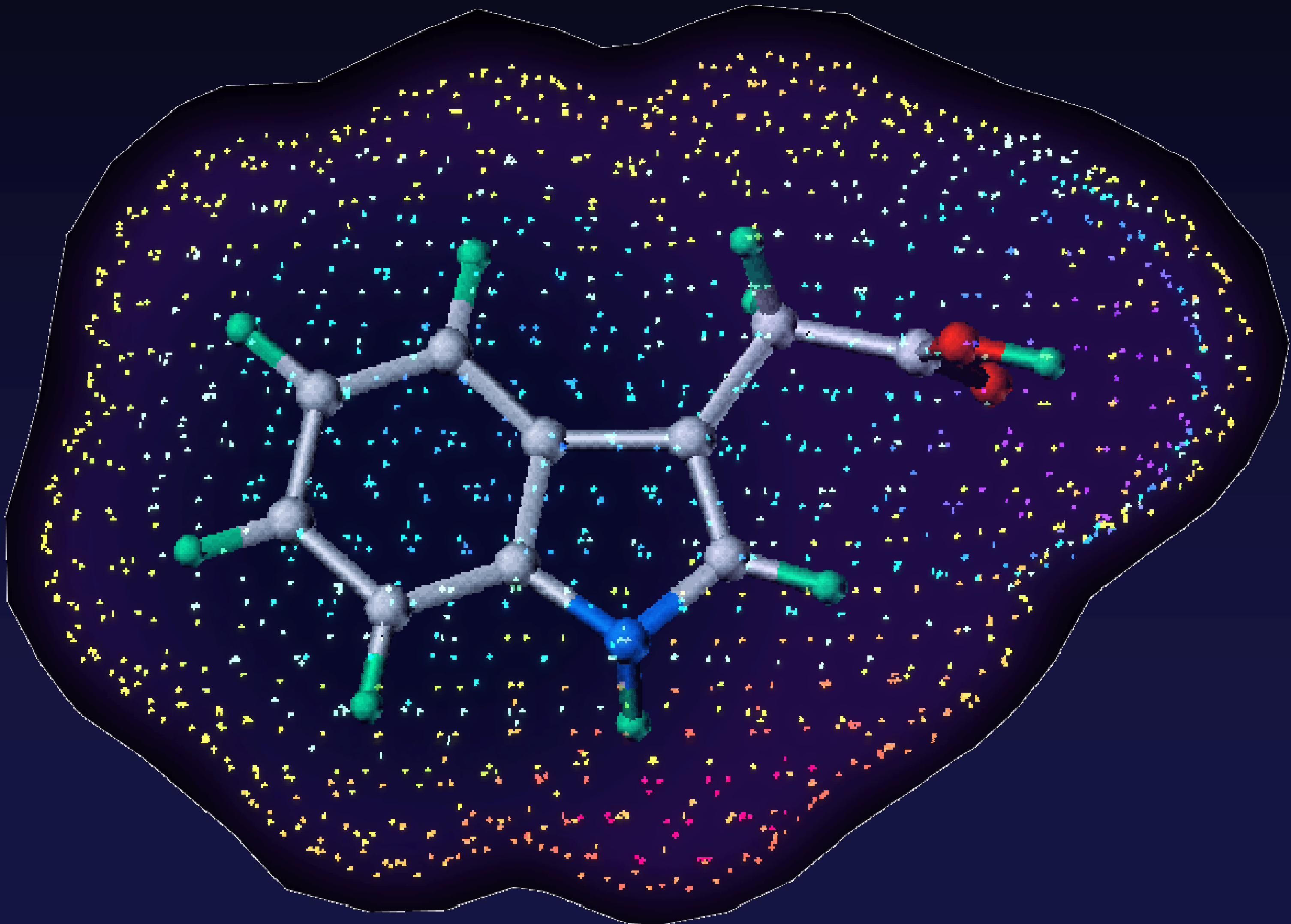
Opposite effects of two classes of ARRs on CK signalling



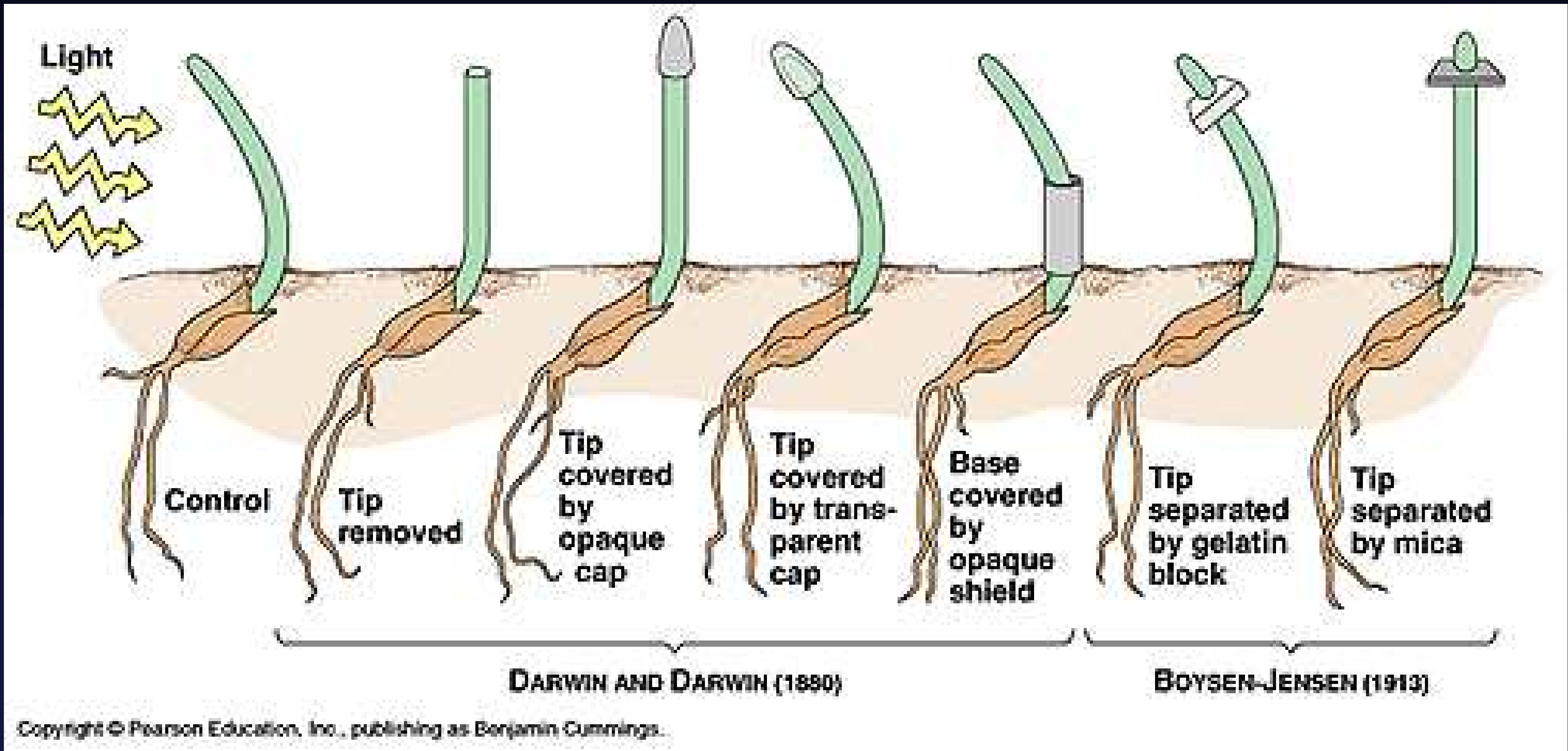
Games with protoplasts



Auxin Signaling and Transport

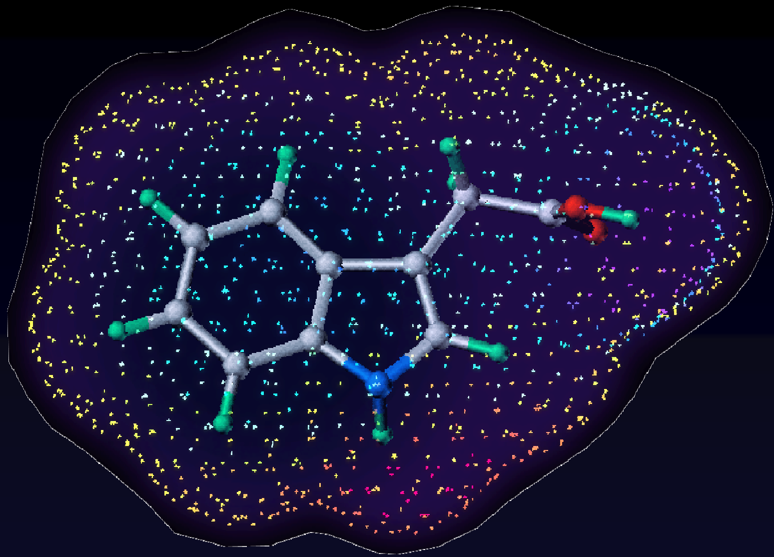


Discovery of the First Plant Signaling Molecule – Auxin and its Transport

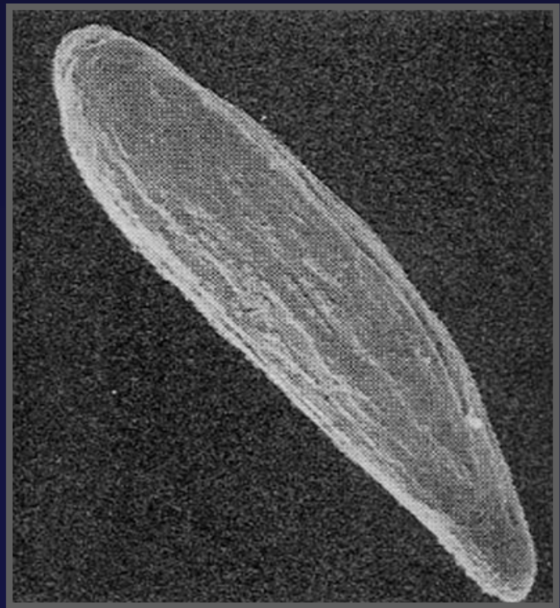


AUXIN

mediates

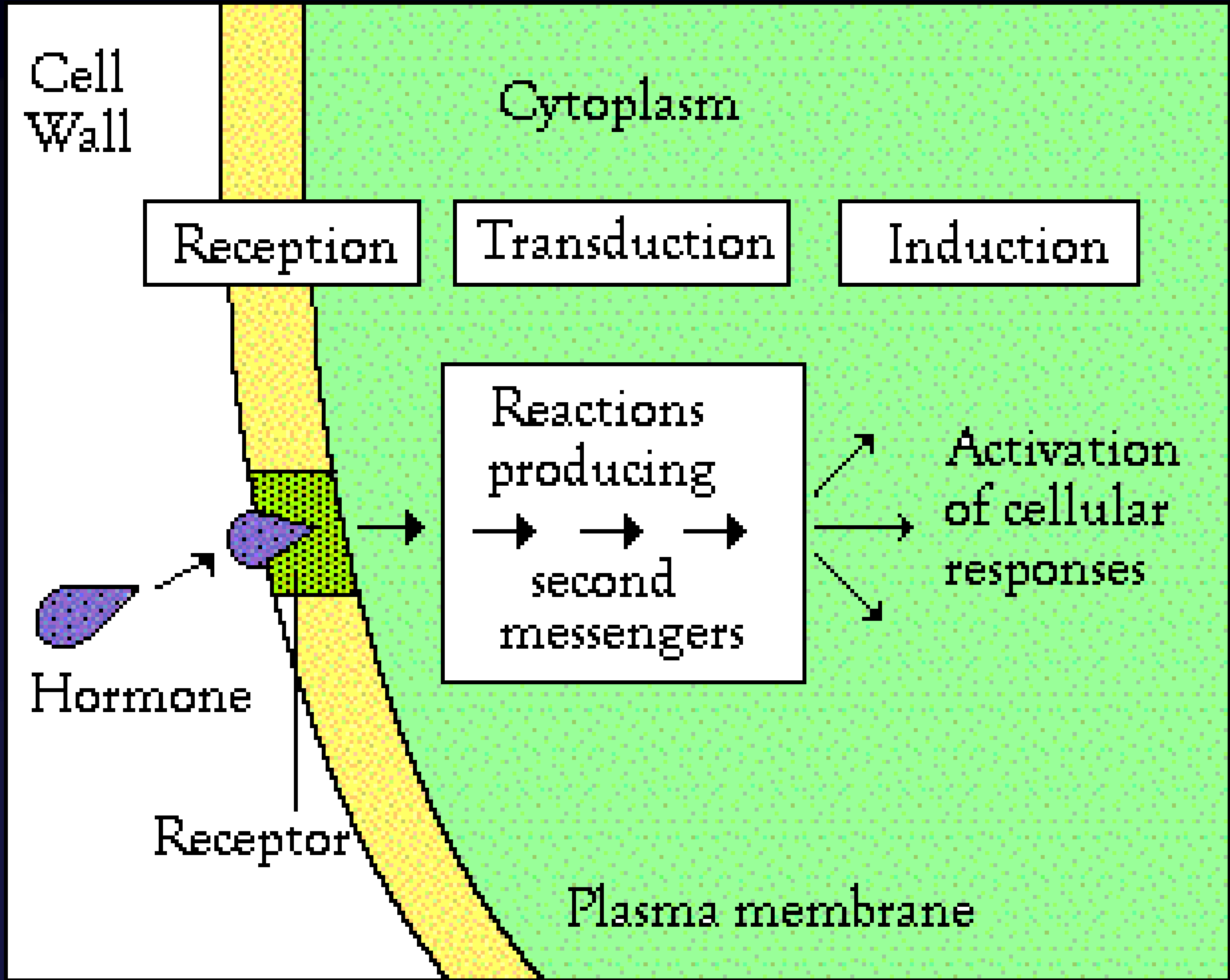


embryos



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

Signal Transduction



Biochemical Approach to Identify Auxin Receptor

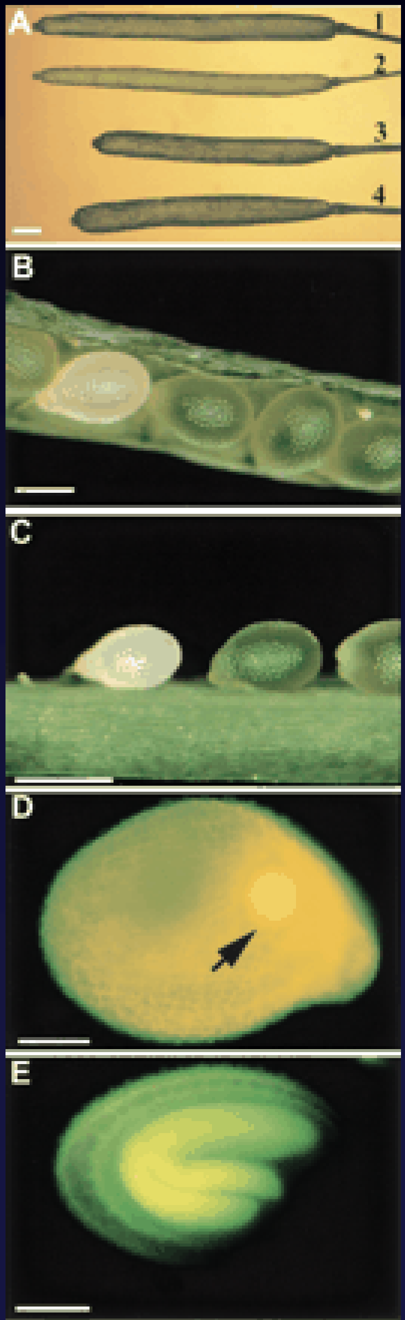
Isolation of auxin binding proteins

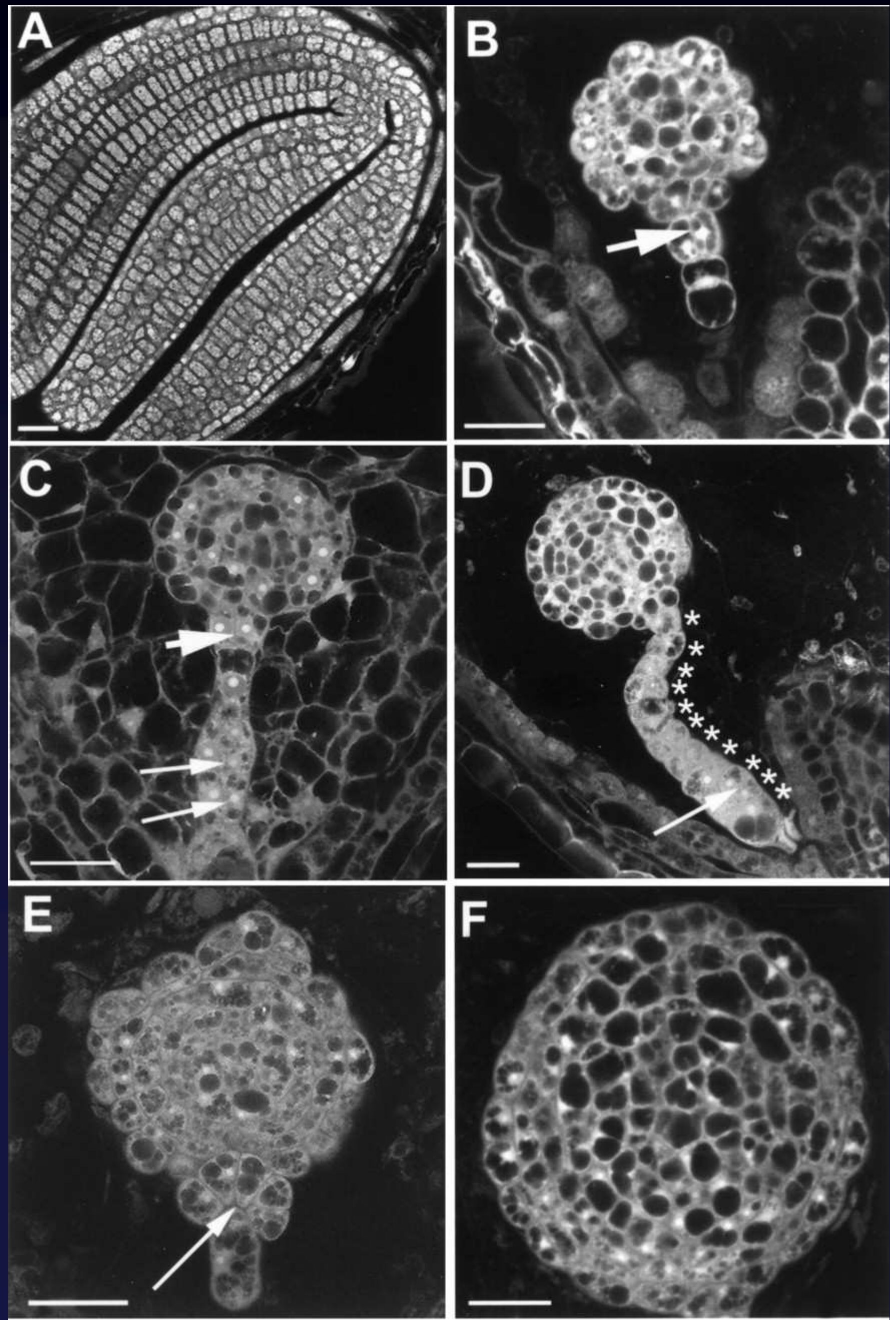
- Azidolabeling
- Affinity chromatography

Protein sequencing, cDNA screening, gene identification

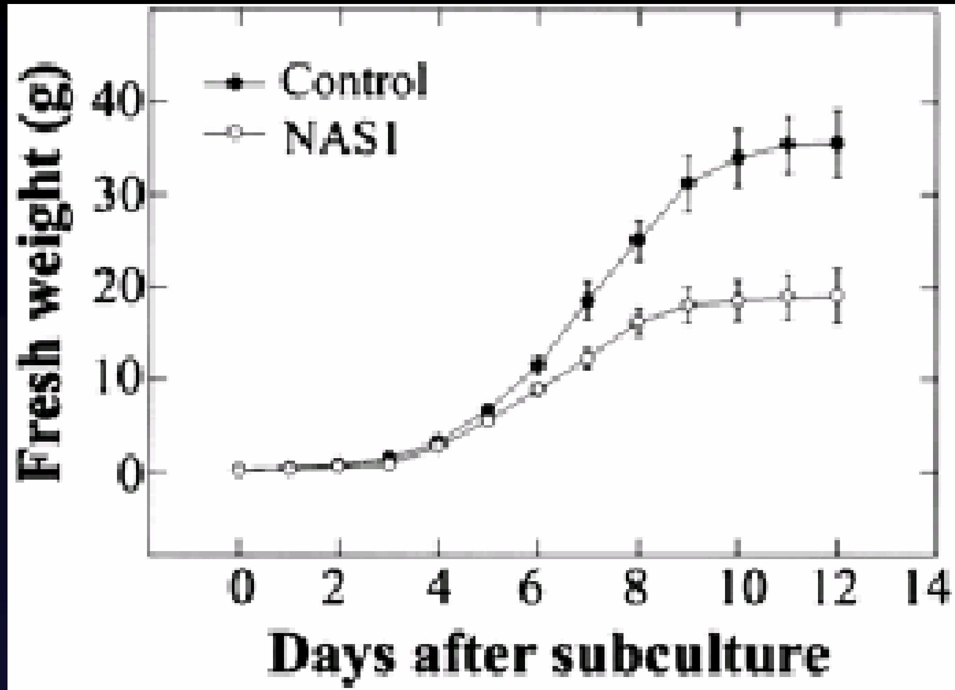
=> Auxin Binding Protein (ABP1)

Reverse Genetic – Embryo Lethal *abp1* Mutant

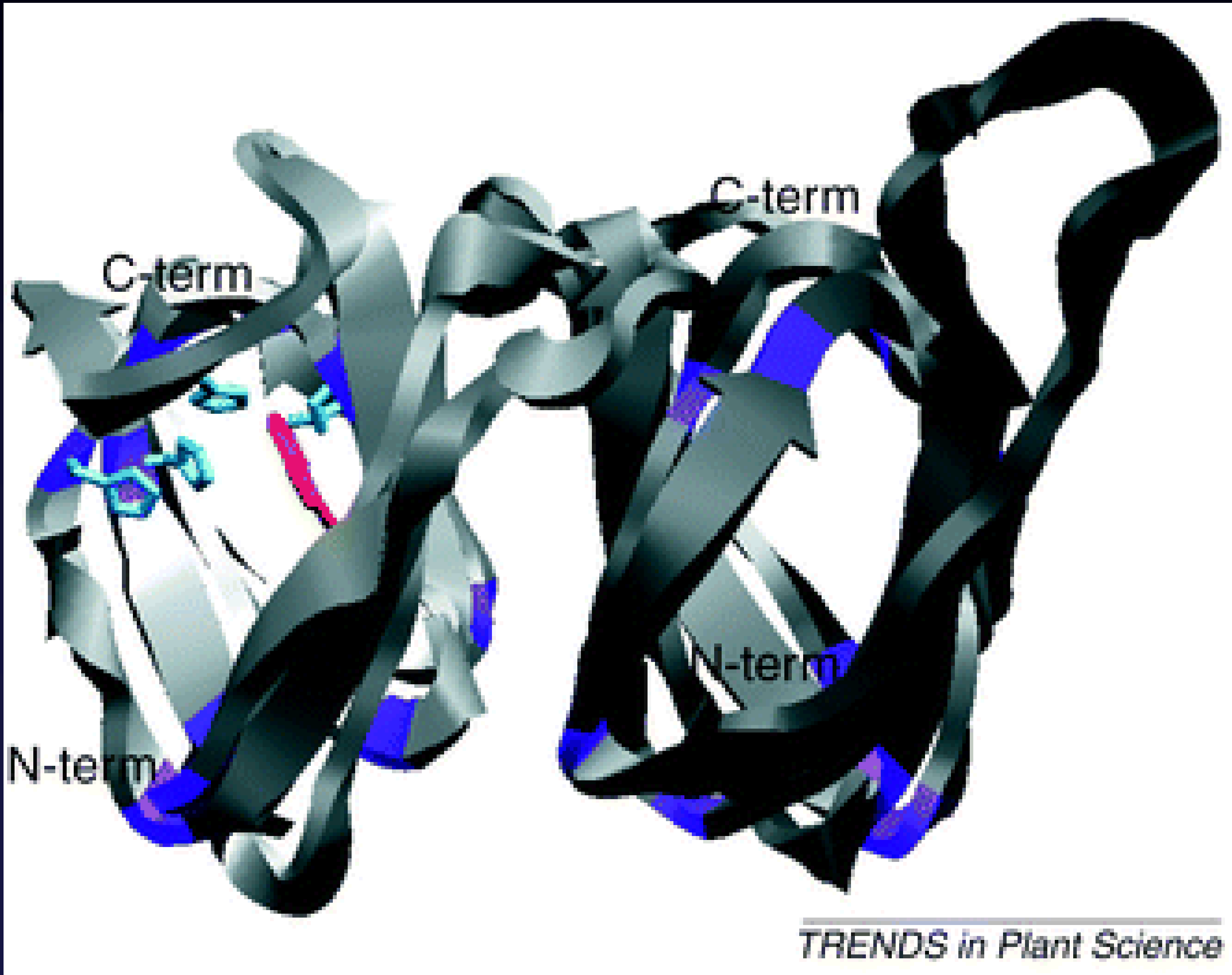




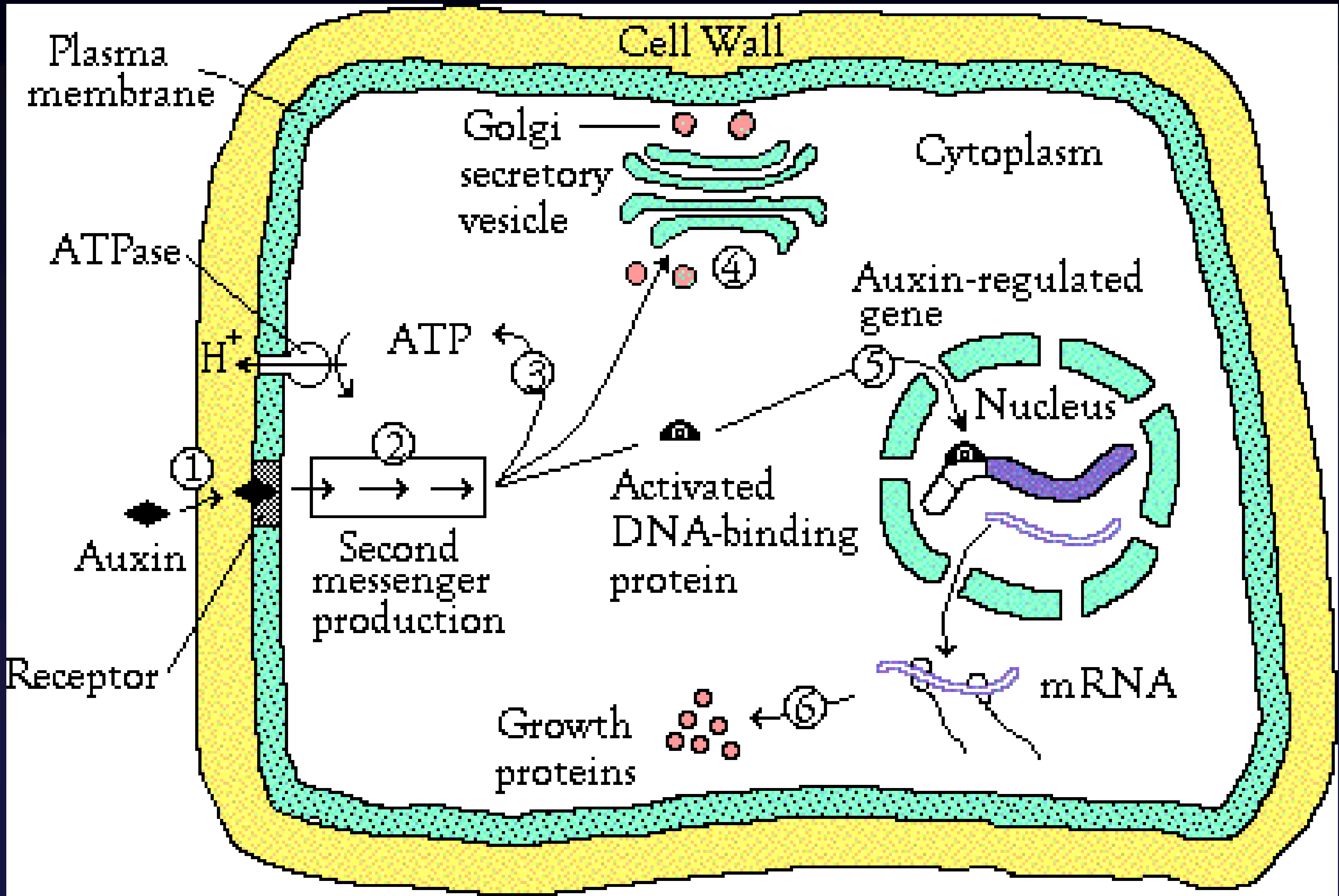
ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation



ABP1 – Structure



Optimistic Model for ABP1 Action



Genetic Approach to Identify Auxin Receptor

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

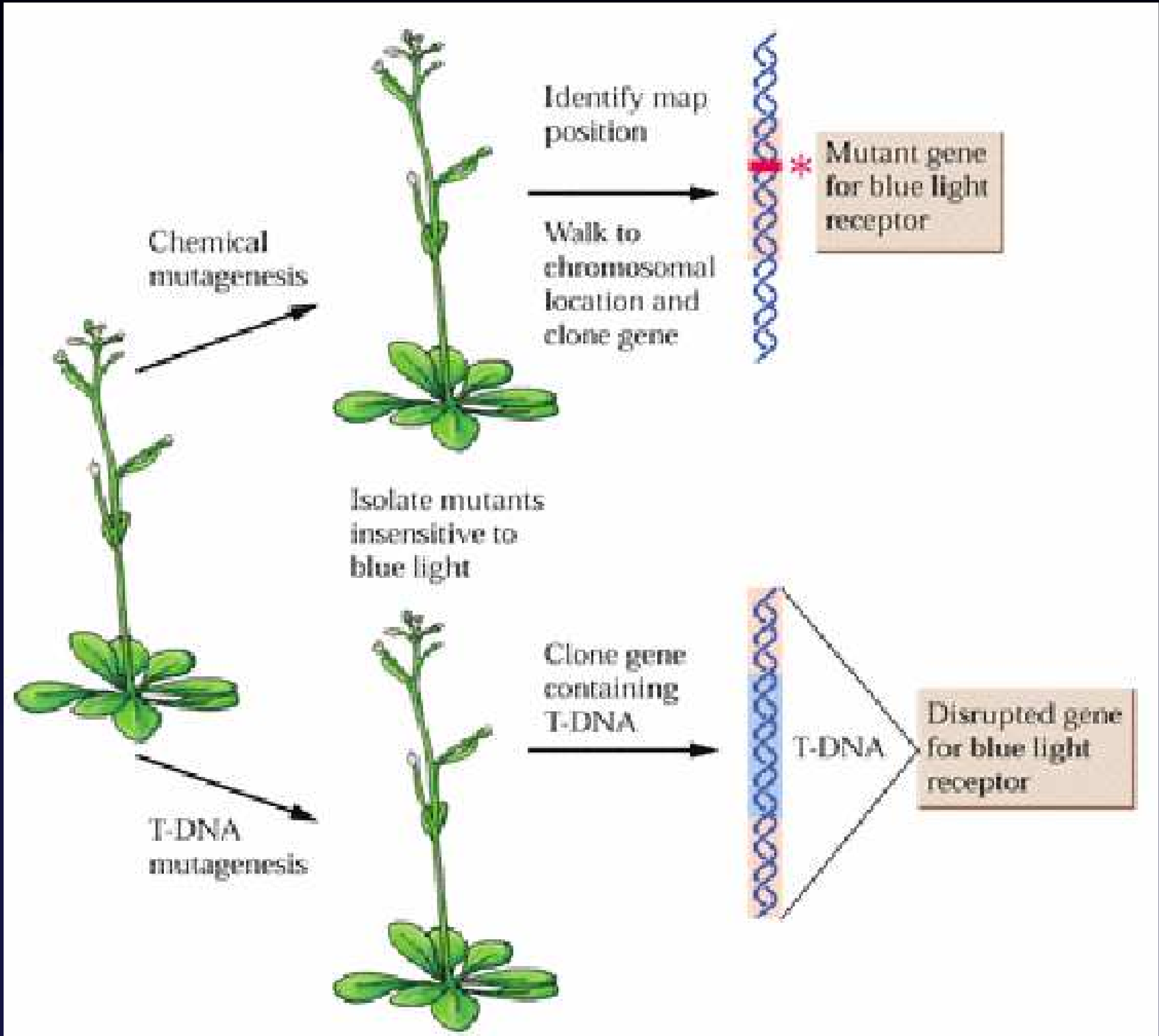
- Transport inhibitor response (tir):
tir1 – tir7

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

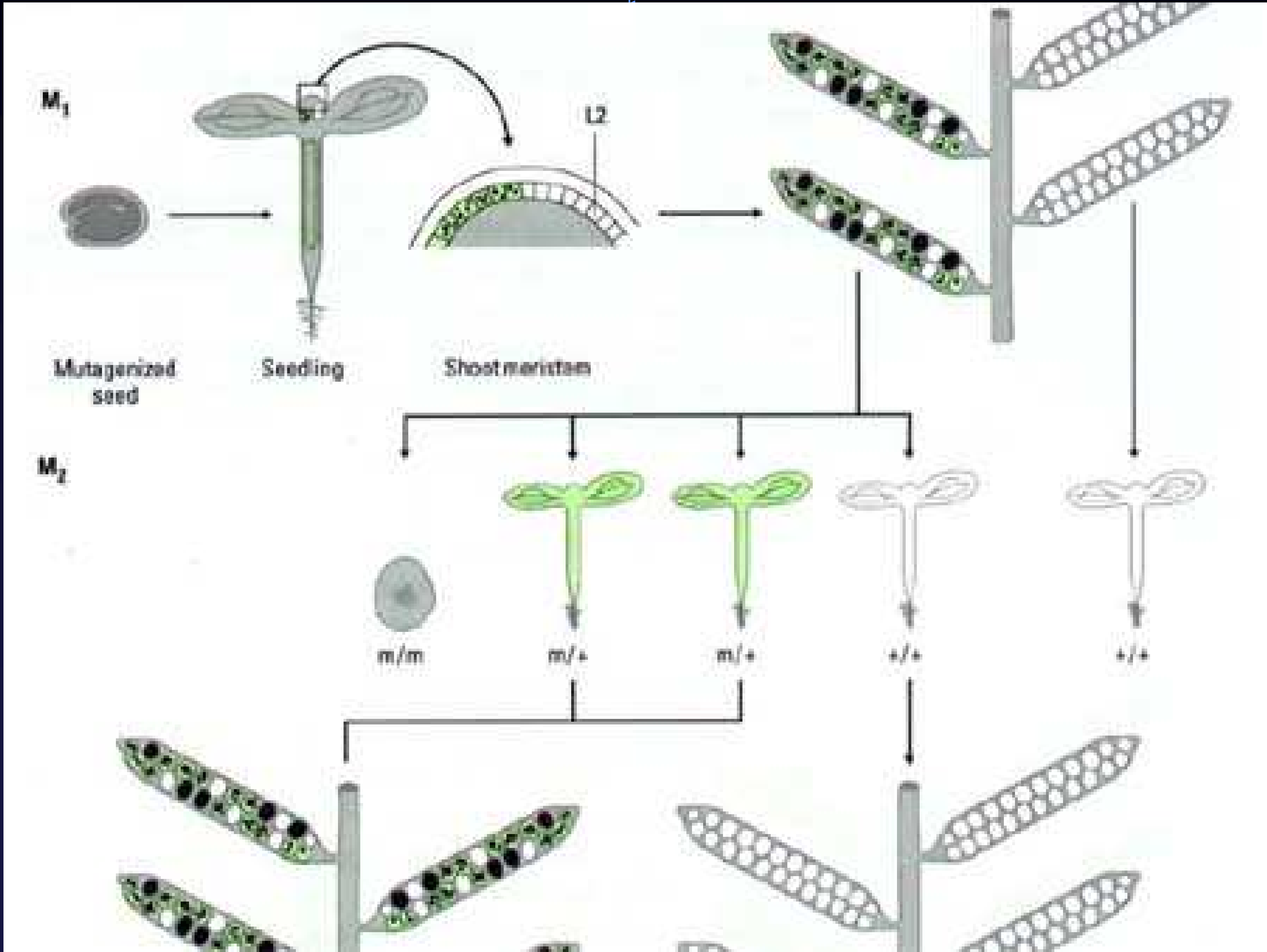
=> Role of regulated protein degradation and transcriptional regulation in auxin signaling

None of the identified gene looks like a receptor

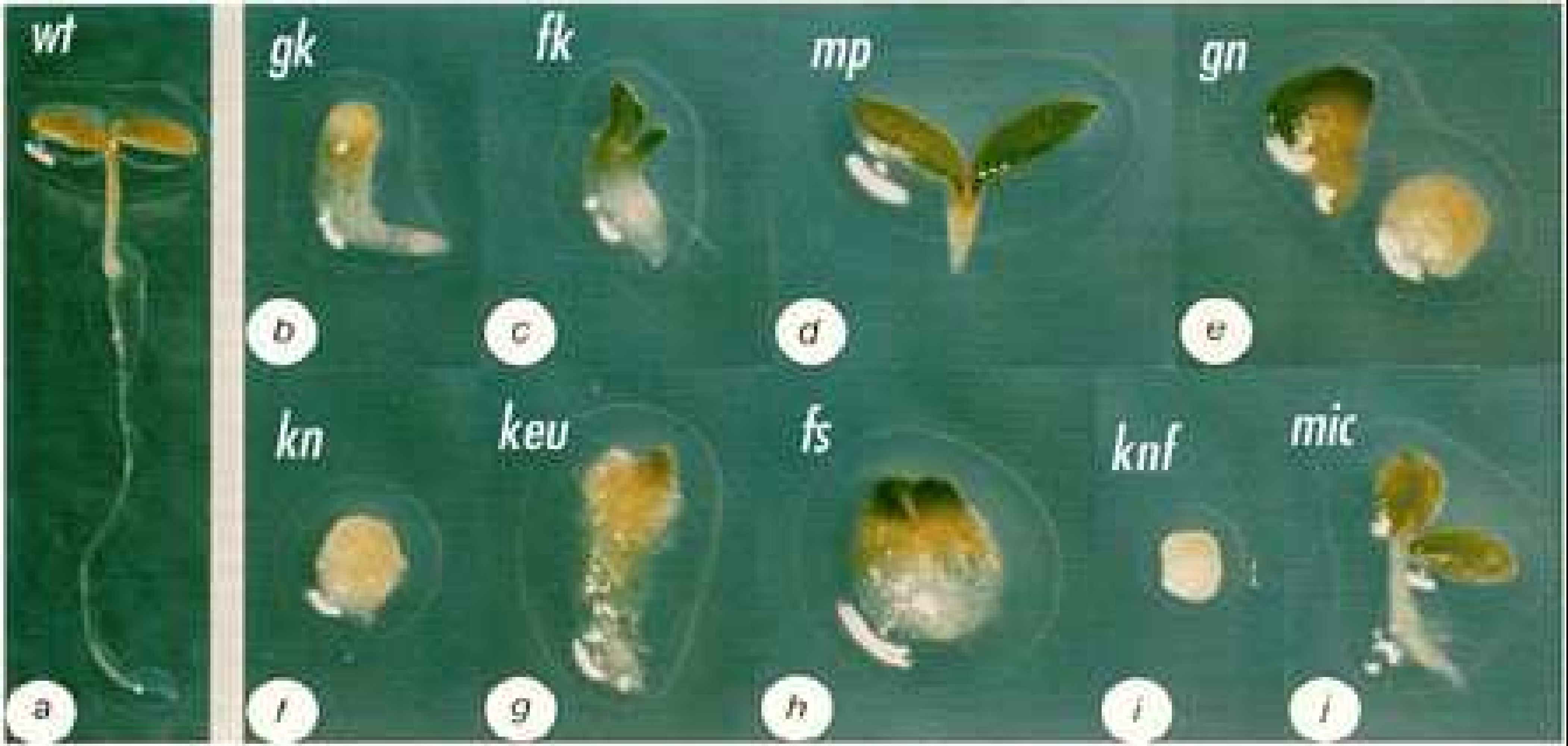
Forward genetics



EMS Mutagenesis



Mutant Screen at Seedling Level



Molecular Biology Approach to Elucidate Auxin Signaling

Does auxin regulate gene expression?

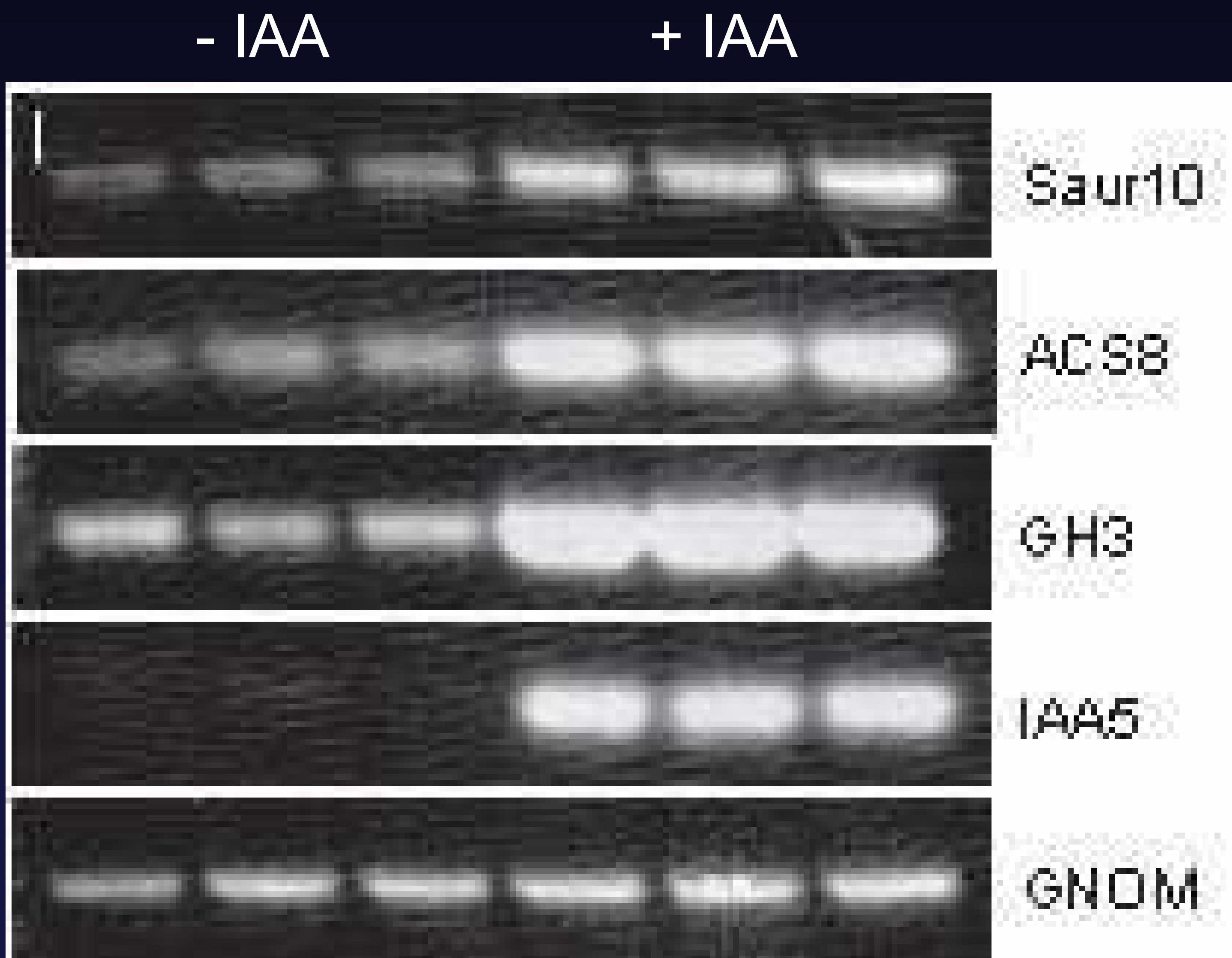
- Rapidly upregulated mRNAs
  (*GH3*, *SAUR*, *AUX/IAA* genes)

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

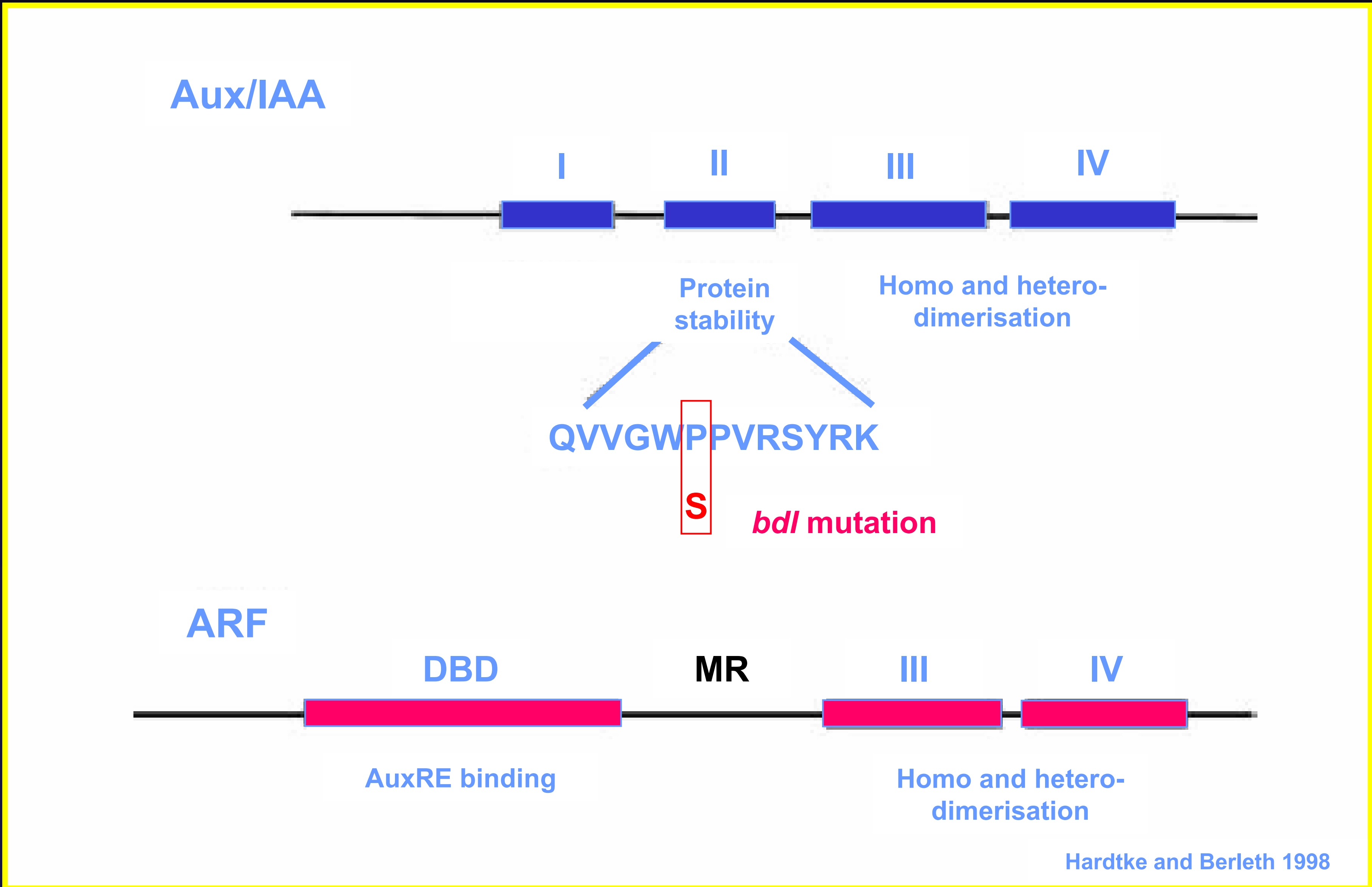
- Two hybrid => AUX/IAAs interact with ARFs

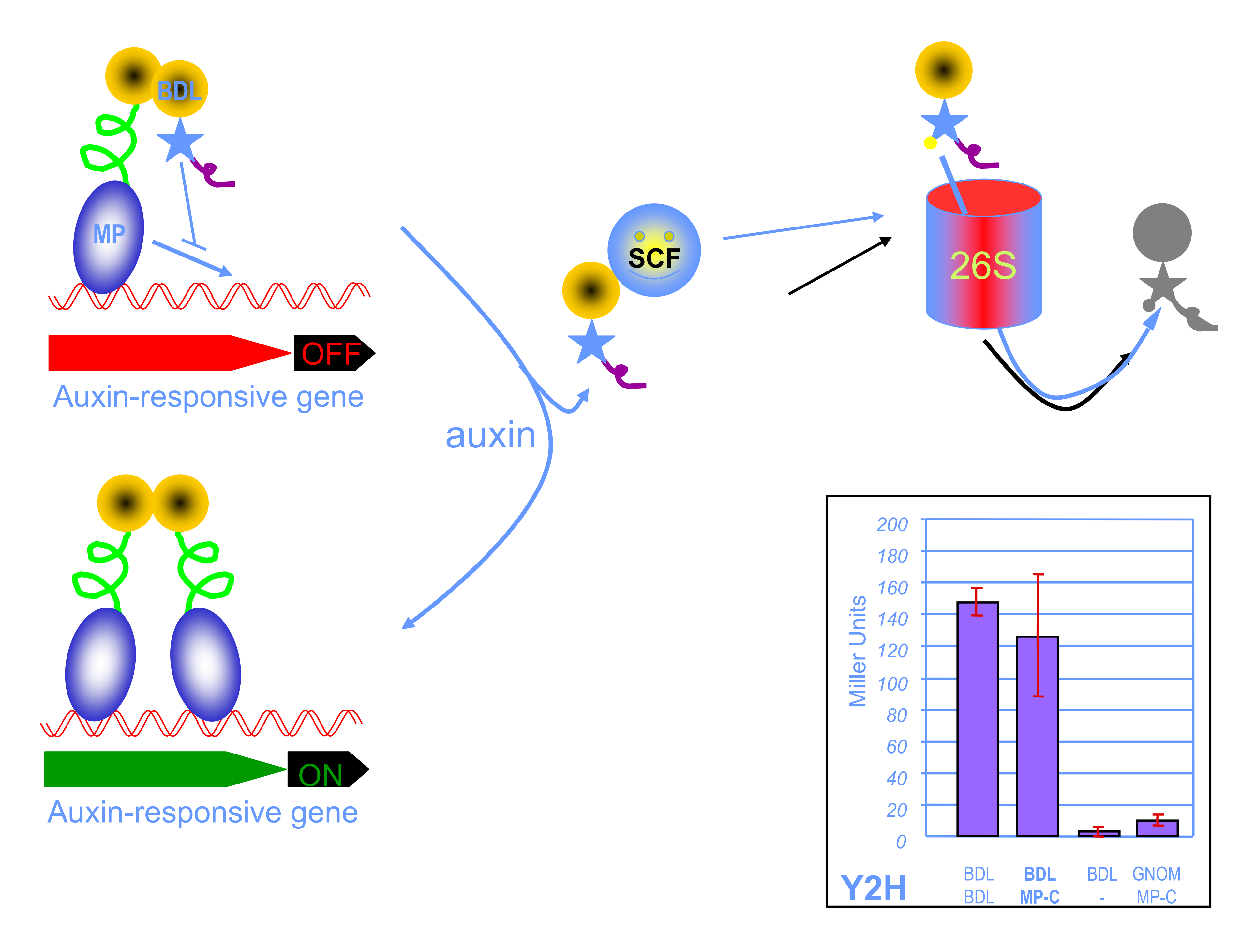
Molecular Biology Approach to Elucidate Auxin Signaling

RT-PCR

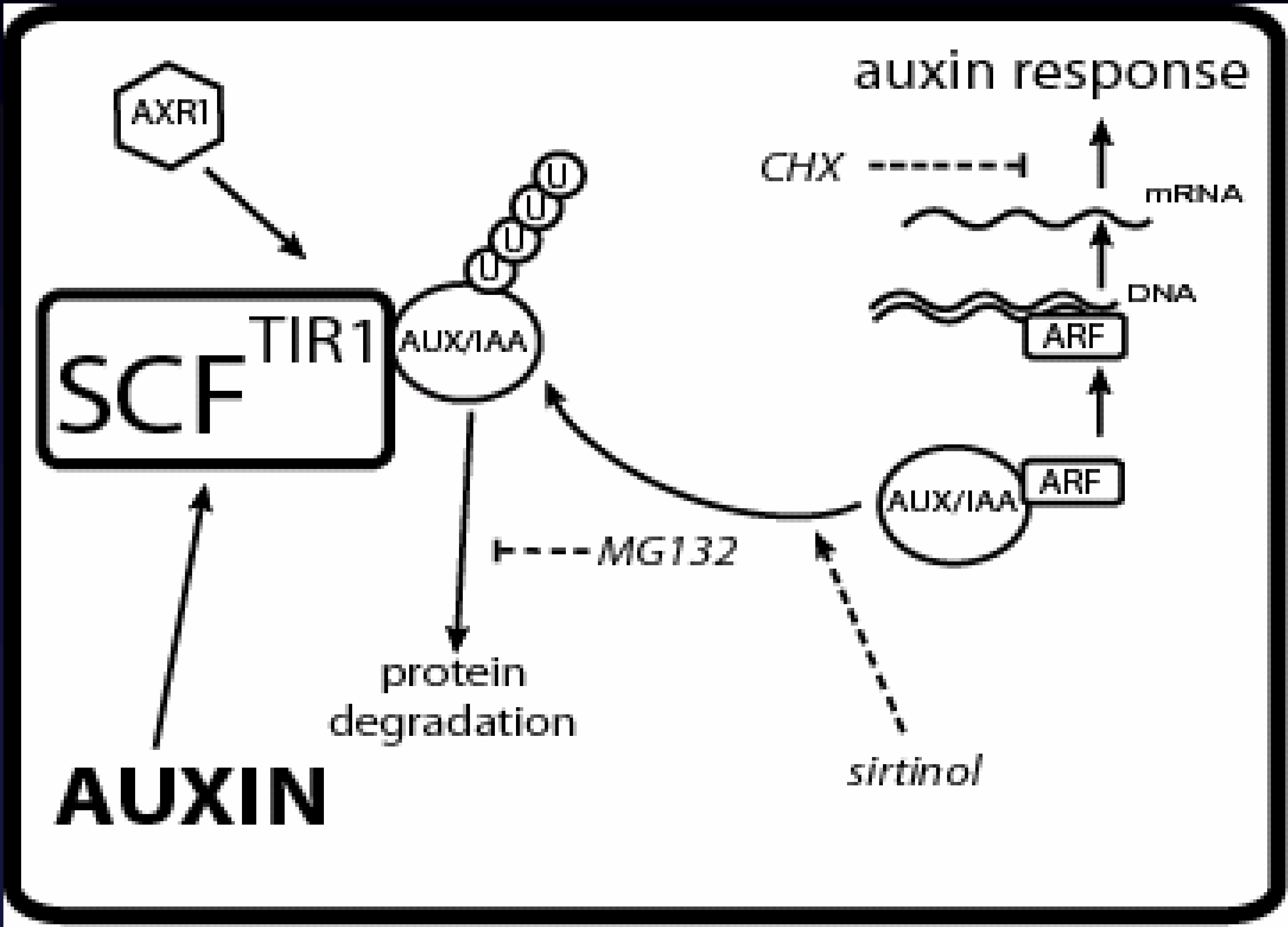


Some ARFs are **Activators**, whereas Aux/IAA **Repressors** of Auxin Response




BDL
MP
OFF
Auxin-responsive gene
auxin
SCF
26S
ON
Auxin-responsive gene
Miller Units
200
180
160
140
120
100
80
60
40
20
0
Y2H
BDL BDL
BDL MP-C
BDL -
GNOM MP-C

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*)

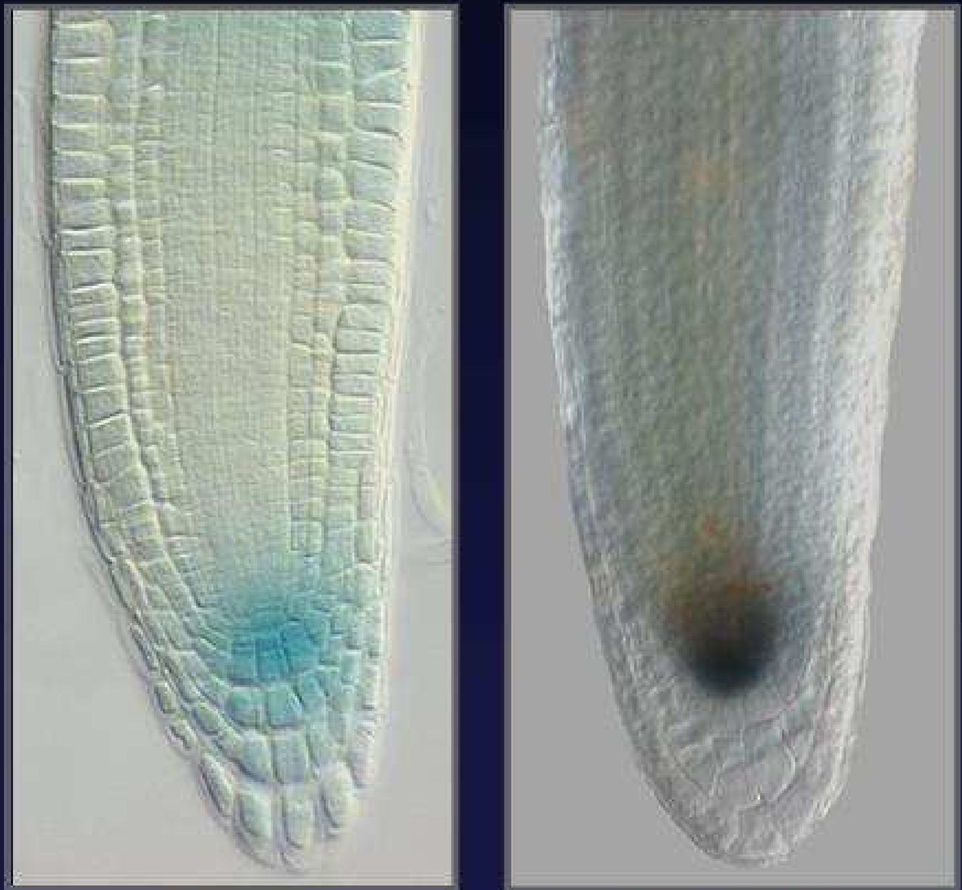
Molecular approach – auxin regulates expression
ARE in promotors of auxin regulated genes
ARF transcription factors binds to ARE
AUX/IAA proteins repress ARF and are
degraded upon auxin signal

DR5 Auxin Response Reporter

9x inv. *5´ CCTTT* TGTCTC *3´*

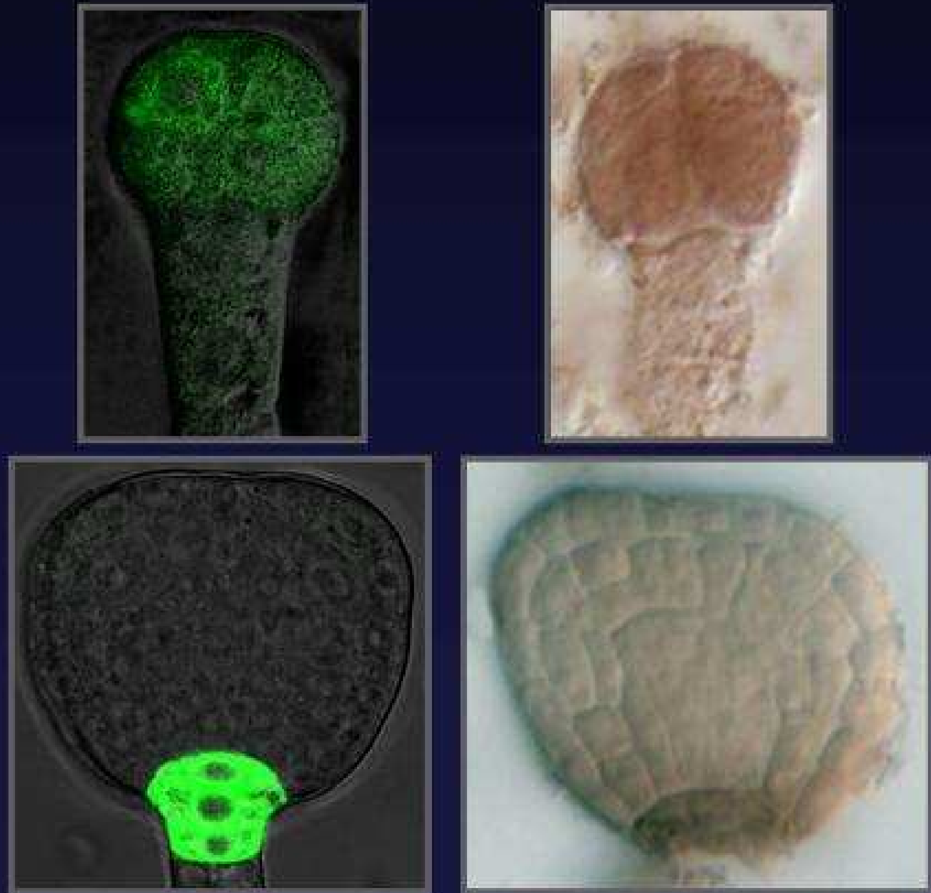
DR5rev	35S min	GUS/GFP	35S pA

Root



DR5 anti-IAA

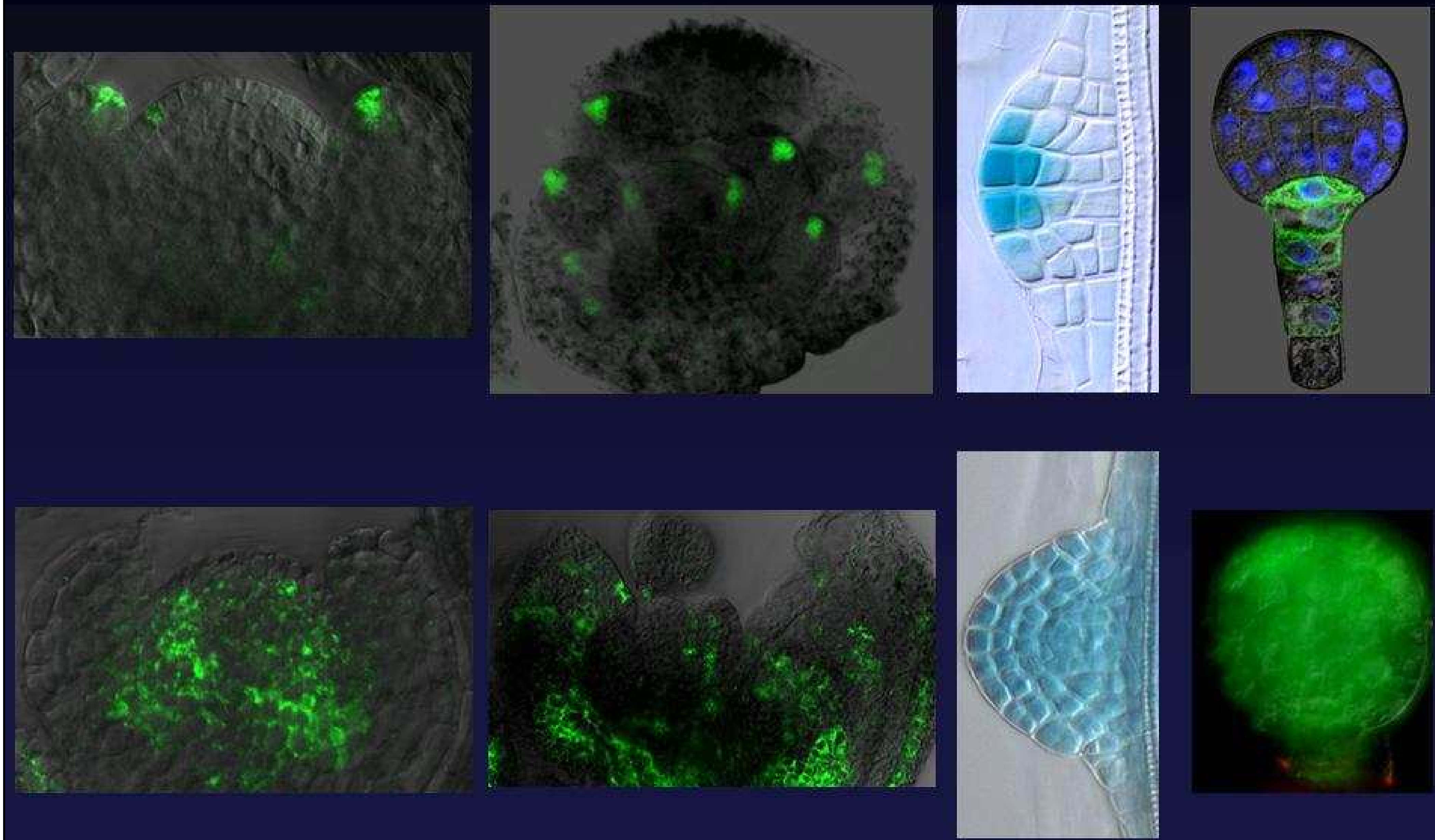
Embryos



DR5 anti-IAA

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

Local Auxin Gradients Require Active Polar Auxin Transport



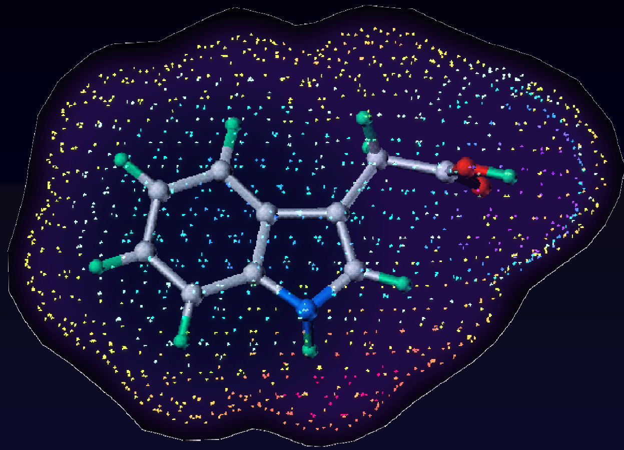
Auxin Transport

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

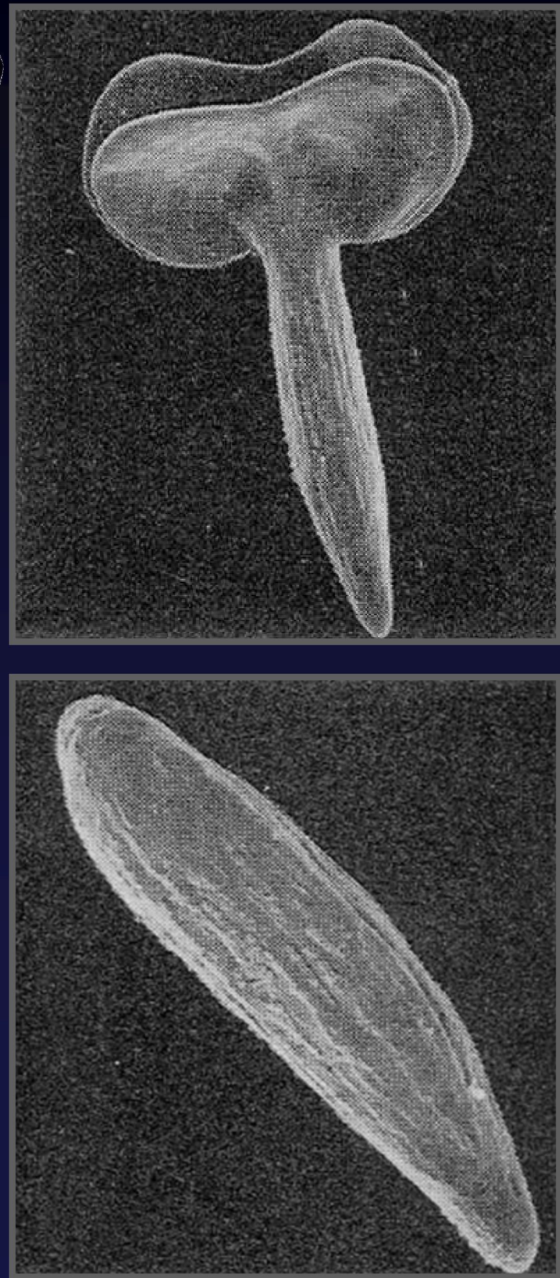
Role of GNOM dependent vesicle trafficking

PIN proteins cycling and its role

AUXIN TRANSPORT
mediates

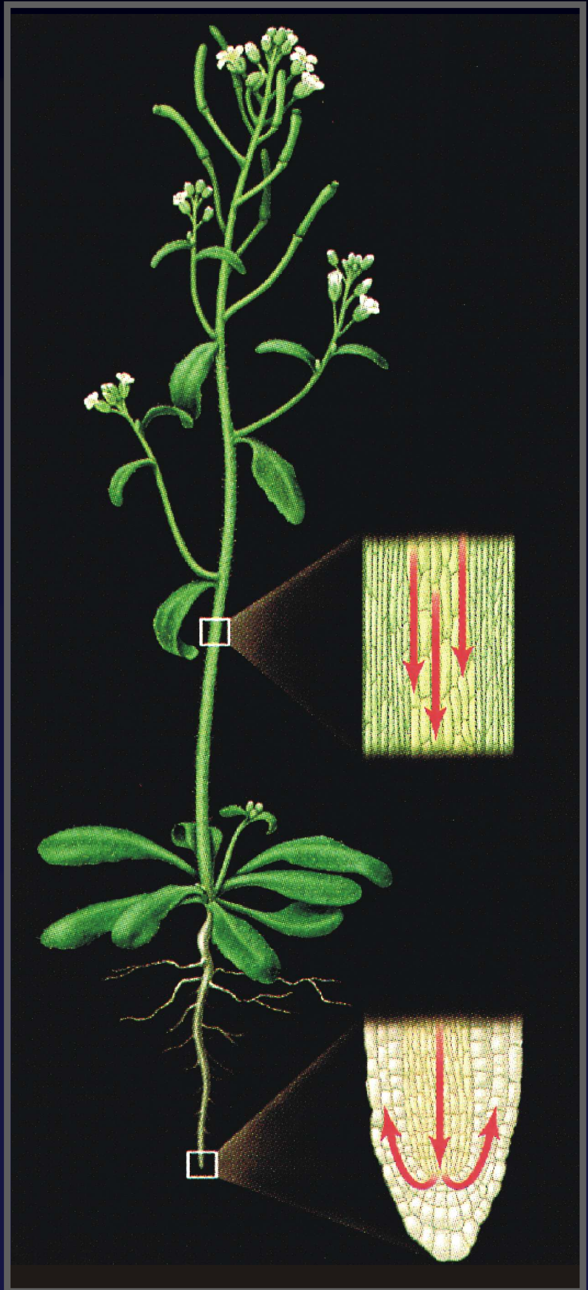


embryos

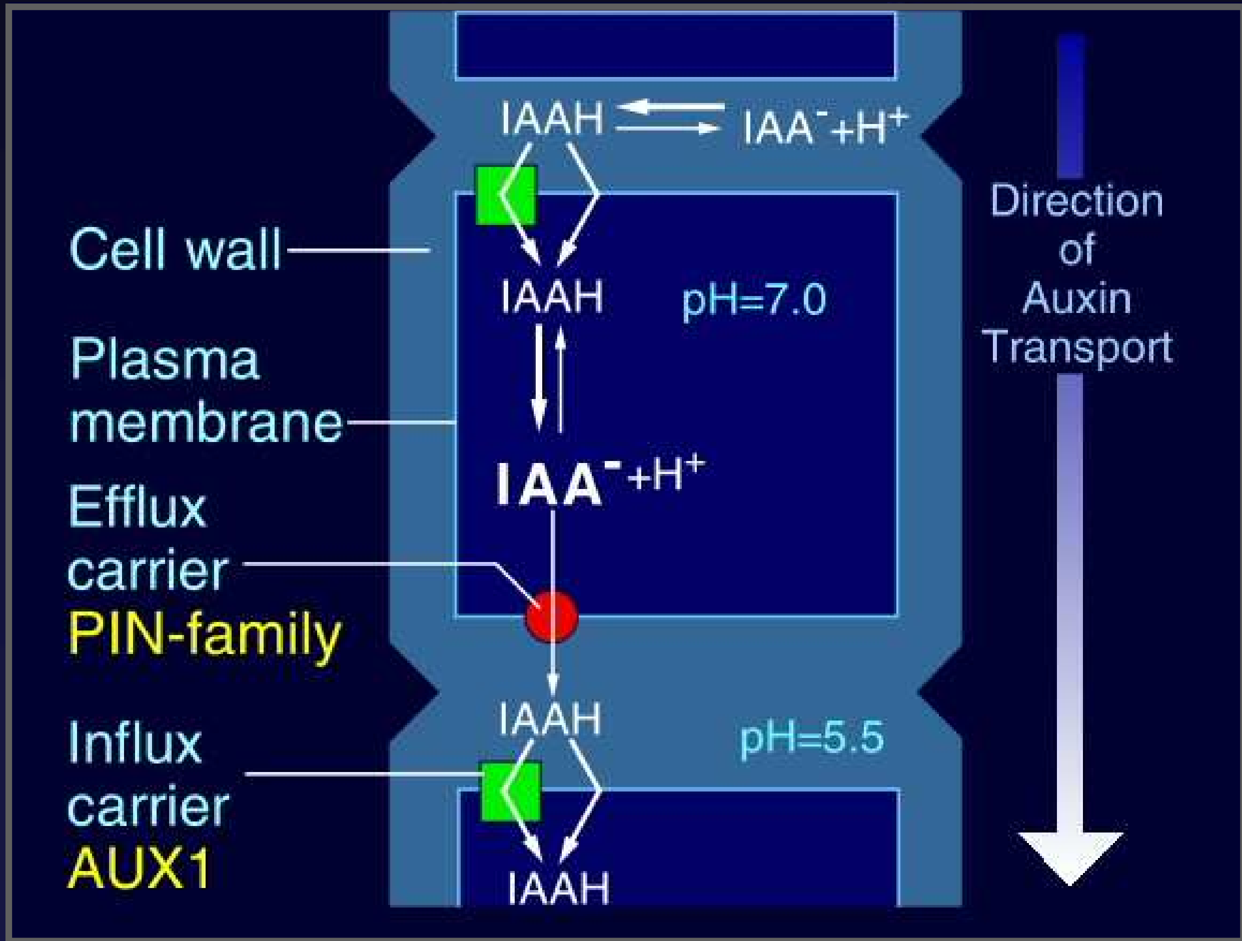


- Embryo development
- Organ initiation and positioning
- Vascular tissue differentiation
- Shoot and root elongation
- Growth responses to light and gravity
- Apical hook formation

Physiology of Auxin Transport



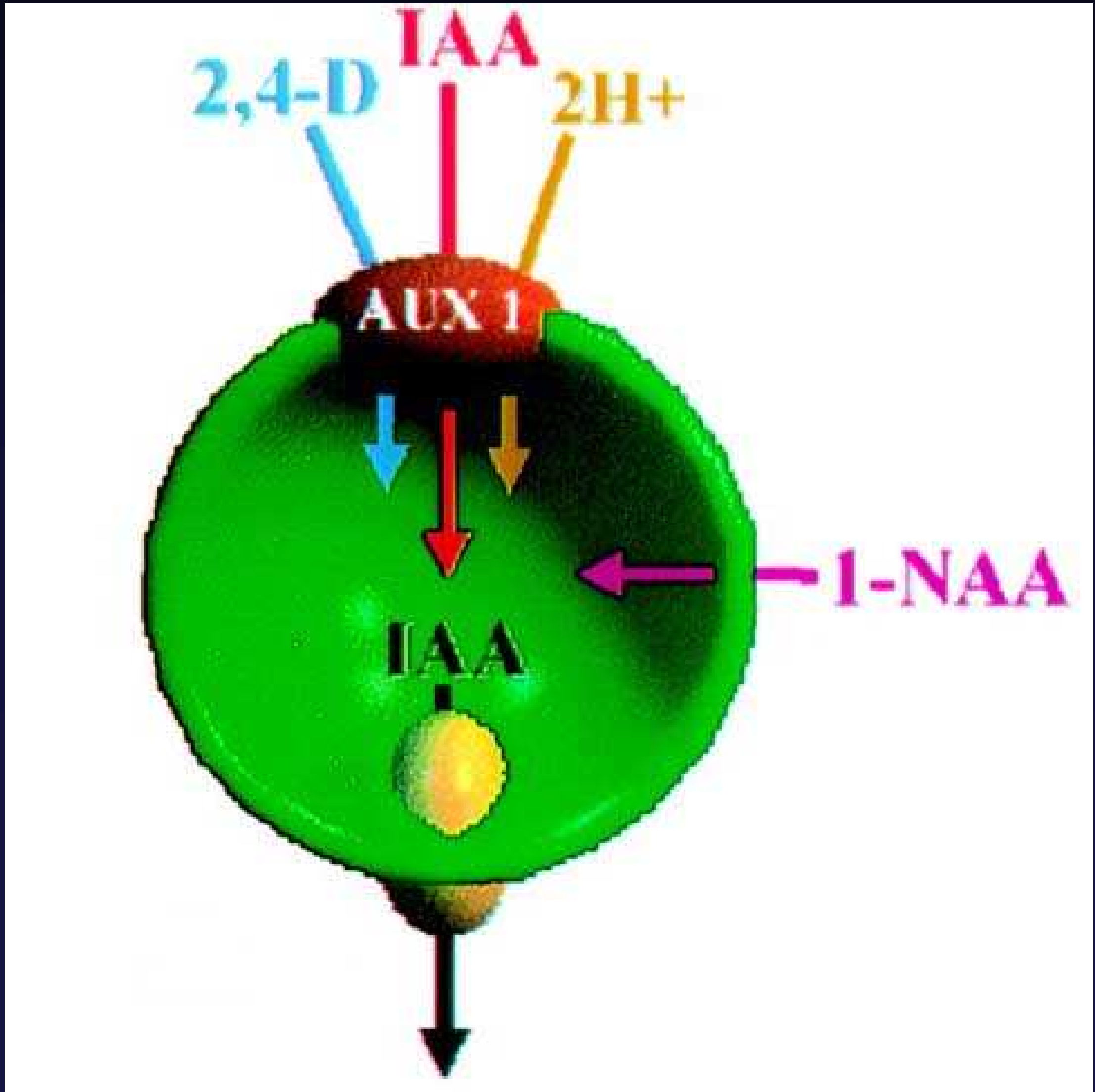
Chemiosmotic hypothesis



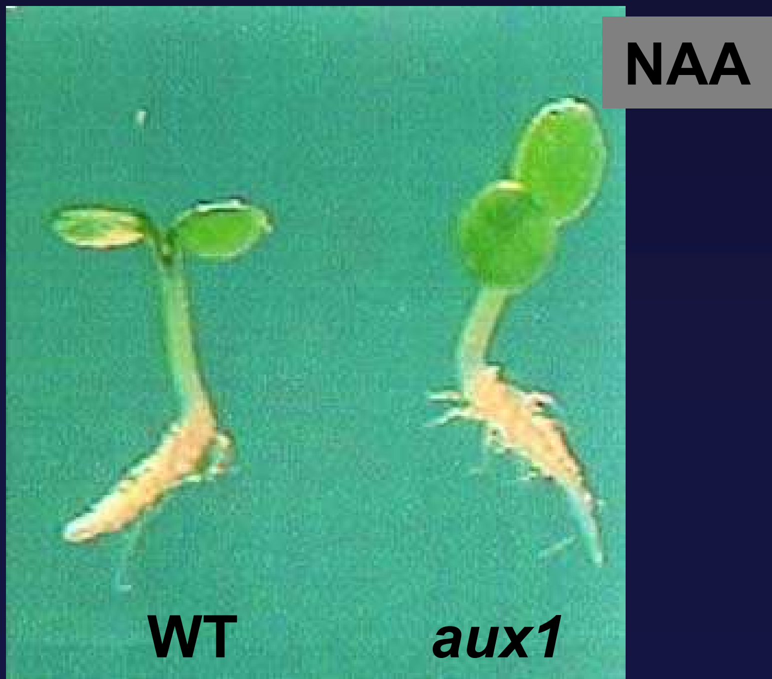
Auxin Influx

aux1 is Resistant to Auxin

Transport properties of different auxins



***aux1* phenotype**



NAA Rescues *aux1* Phenotype

- NAA

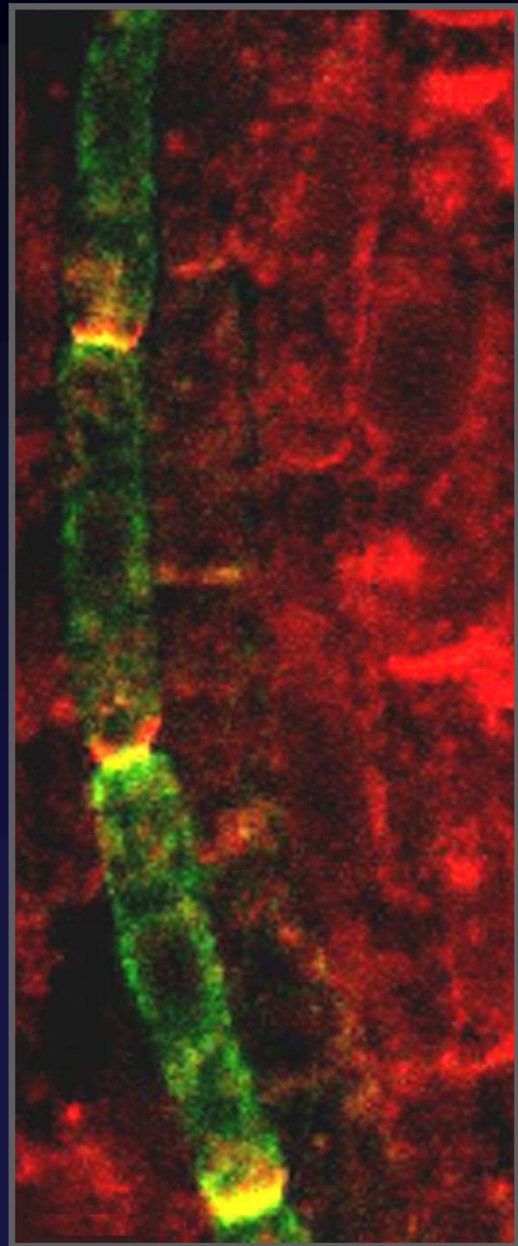
+ NAA

AUX1 – Expression and Localization

AUX1::GUS

AUX1 protein

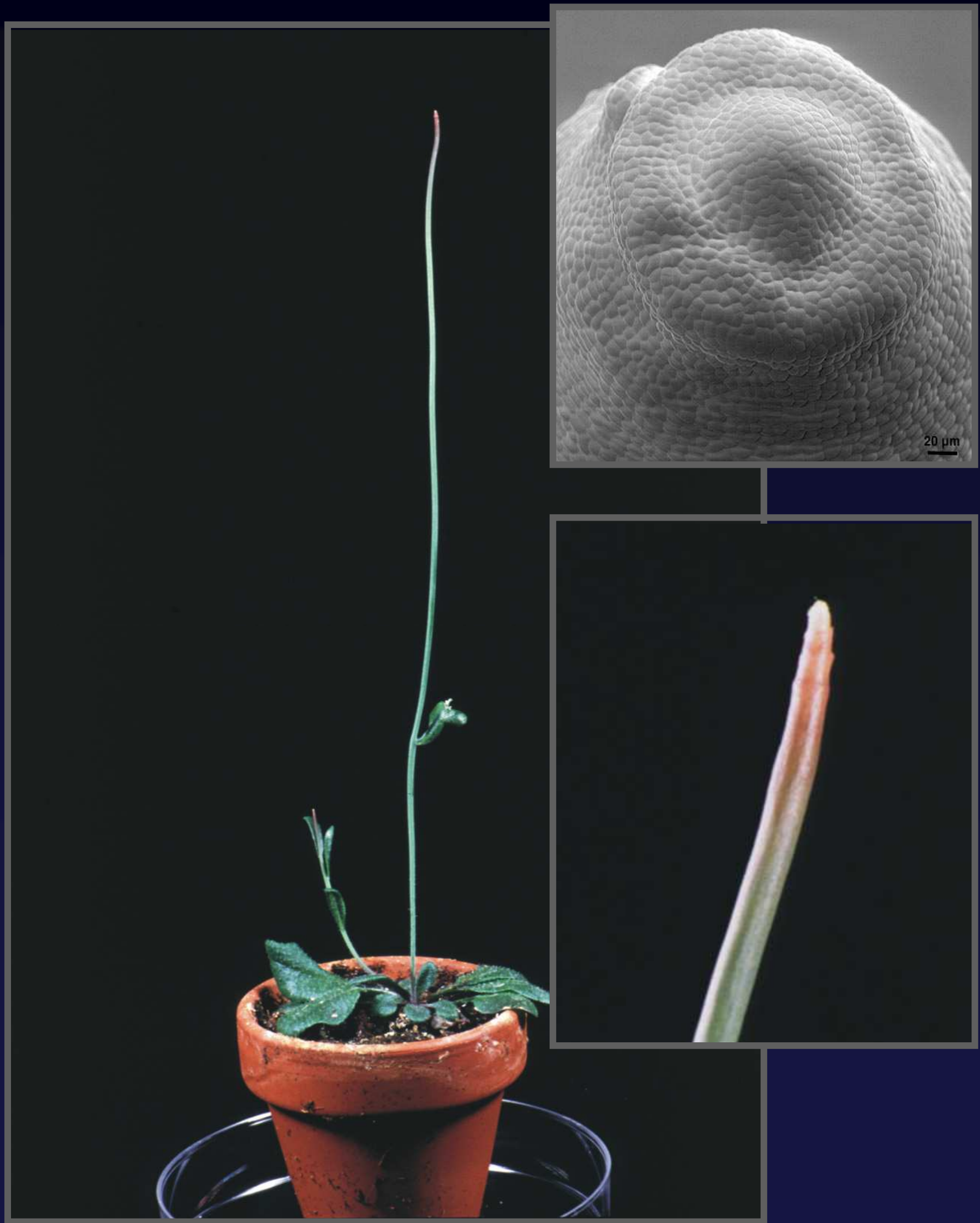
PIN1/AUX1



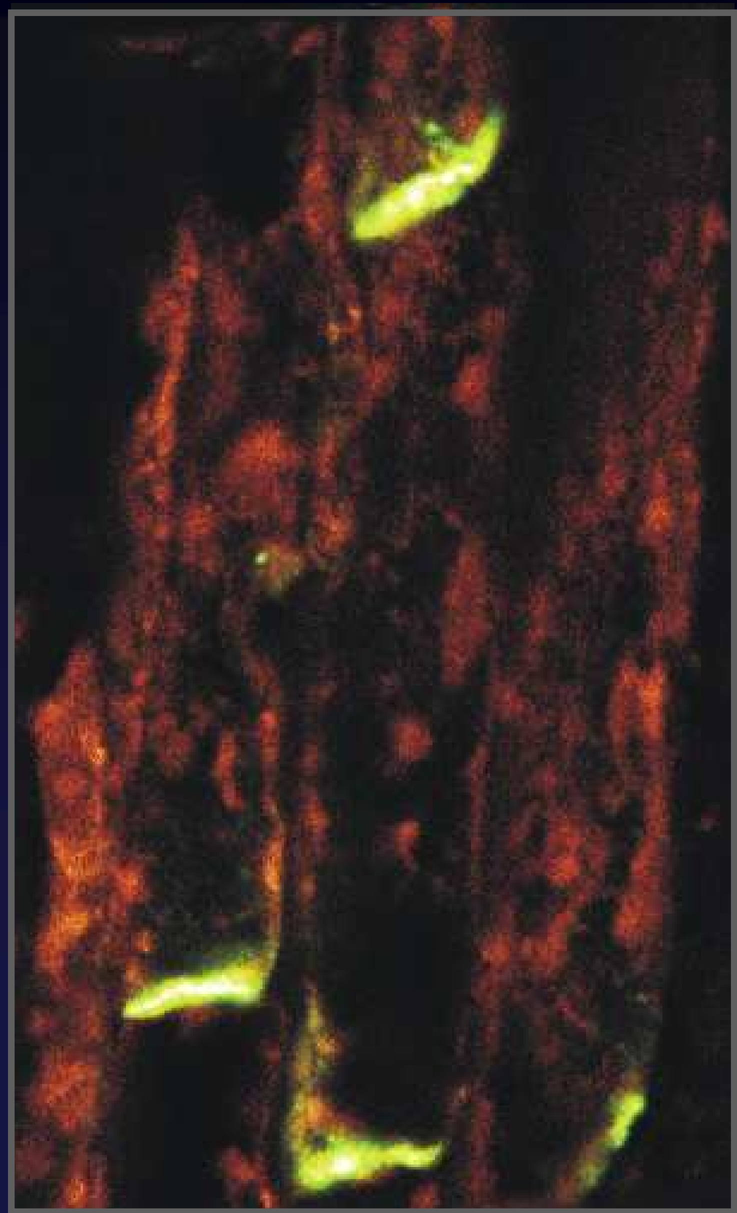
Auxin Efflux

PIN1 – the Auxin Efflux Carrier?

pin1 mutant



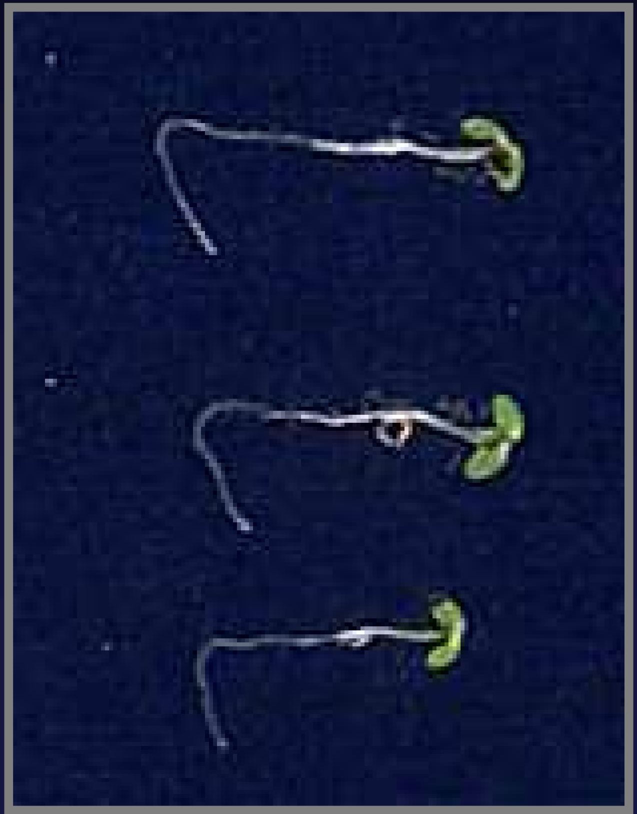
PIN1 protein

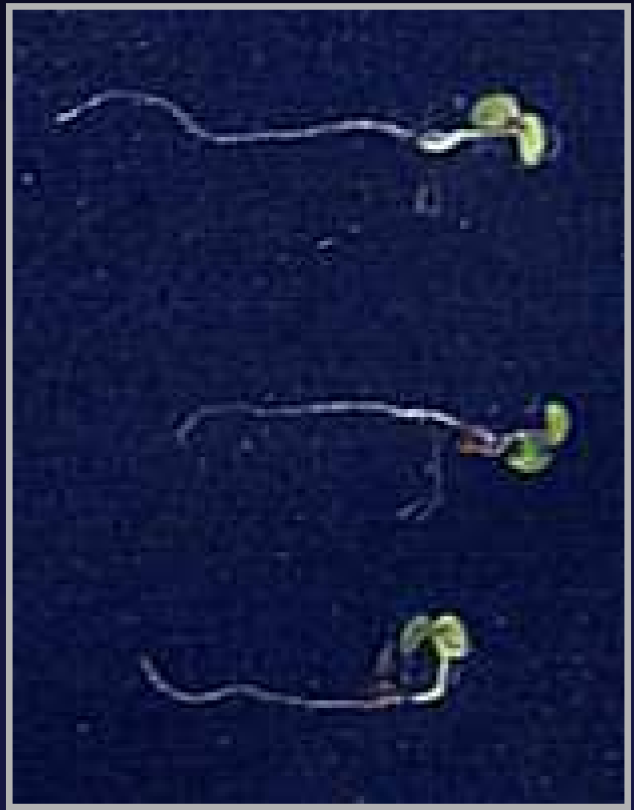


PIN2 – Root Gravitropism

Col-0

pin2

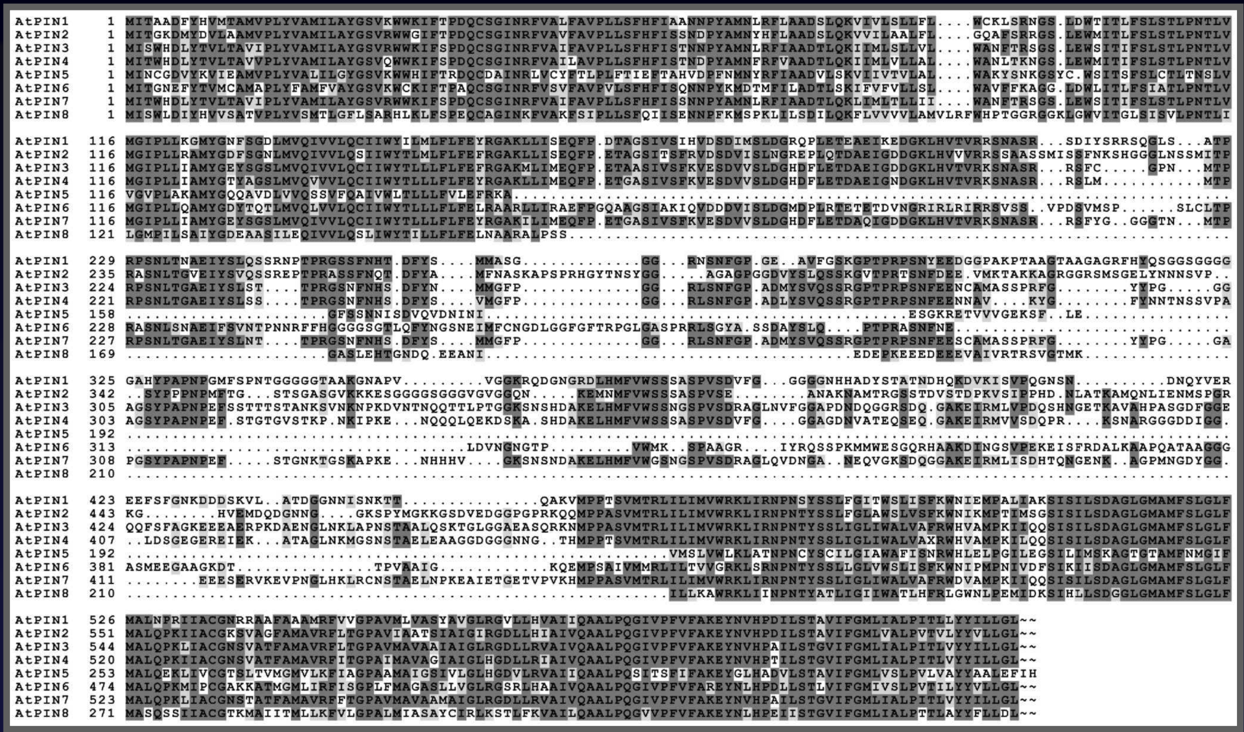




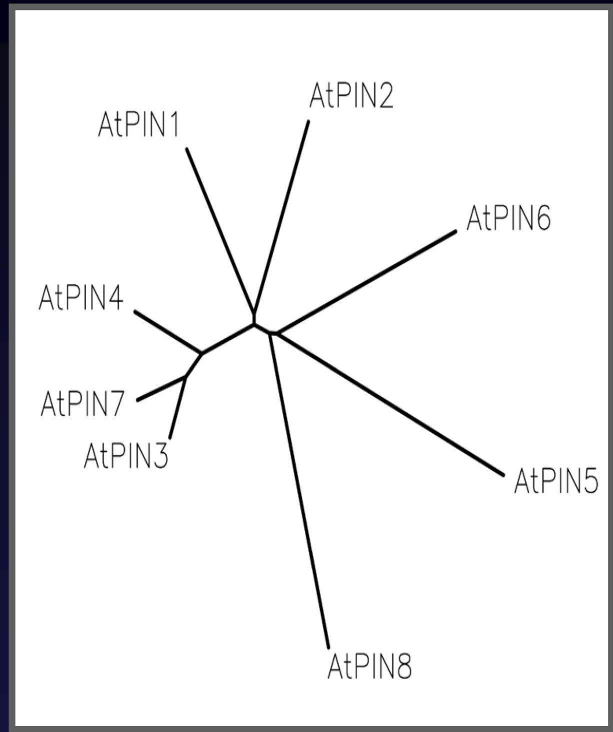
PIN2 protein

The Arabidopsis *PIN* Gene Family

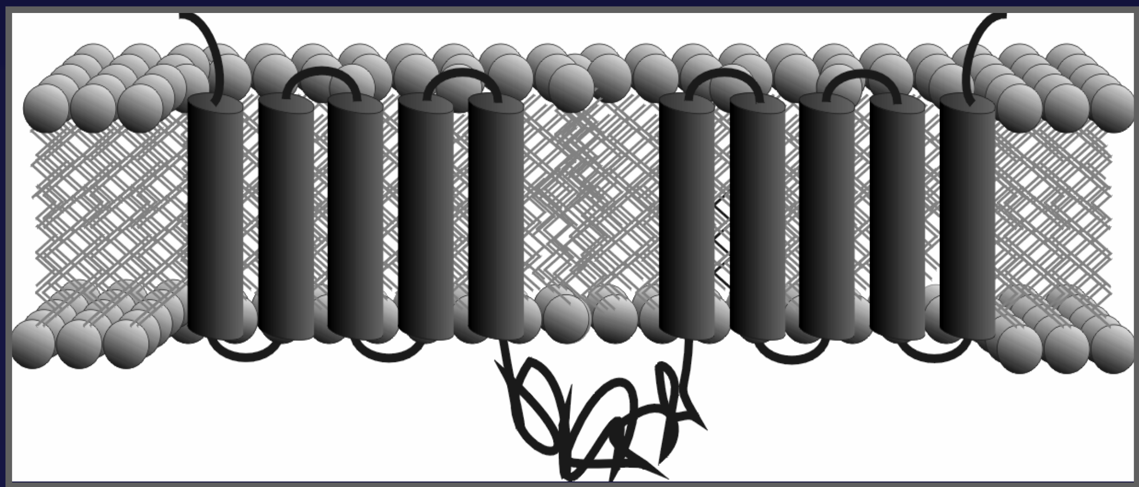
Comparison of *Arabidopsis* PIN proteins



Phylogenetic tree



Membrane topology model



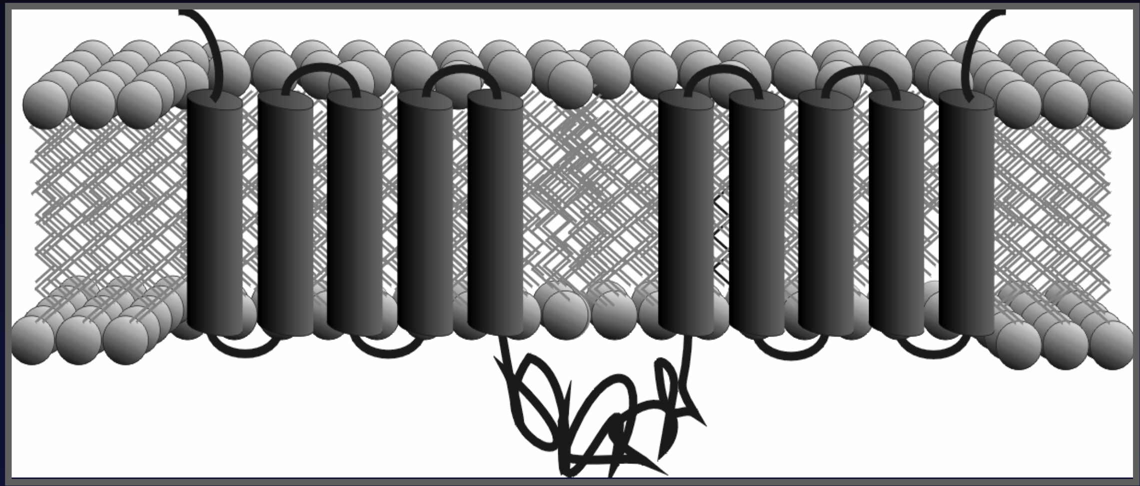
What is Molecular Role

of PIN Proteins

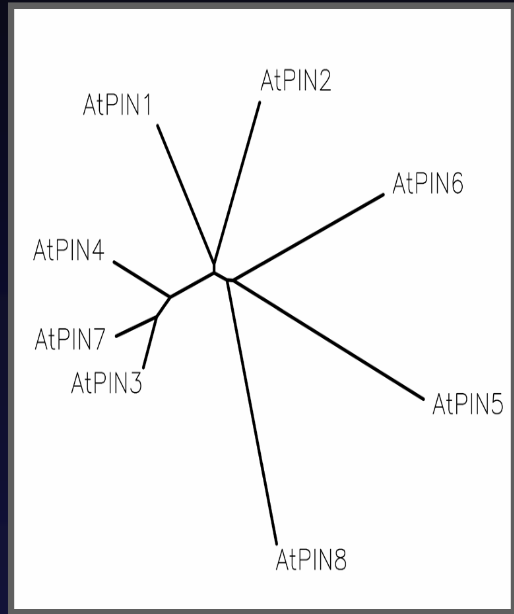
in Auxin Transport?

PINs Are Essential Components of Auxin Transport

Putative topology of PIN proteins



Phylogenetic tree



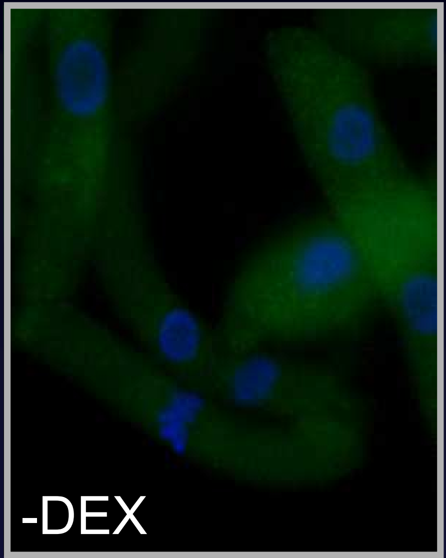
- All defects in *pin* loss-off-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors

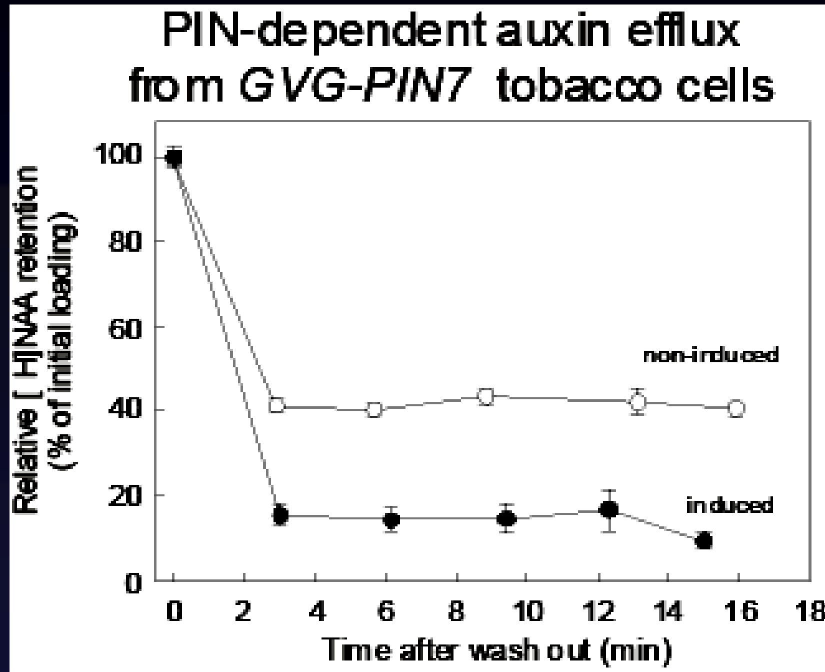
- Local auxin distribution (gradients) are affected in *pins*

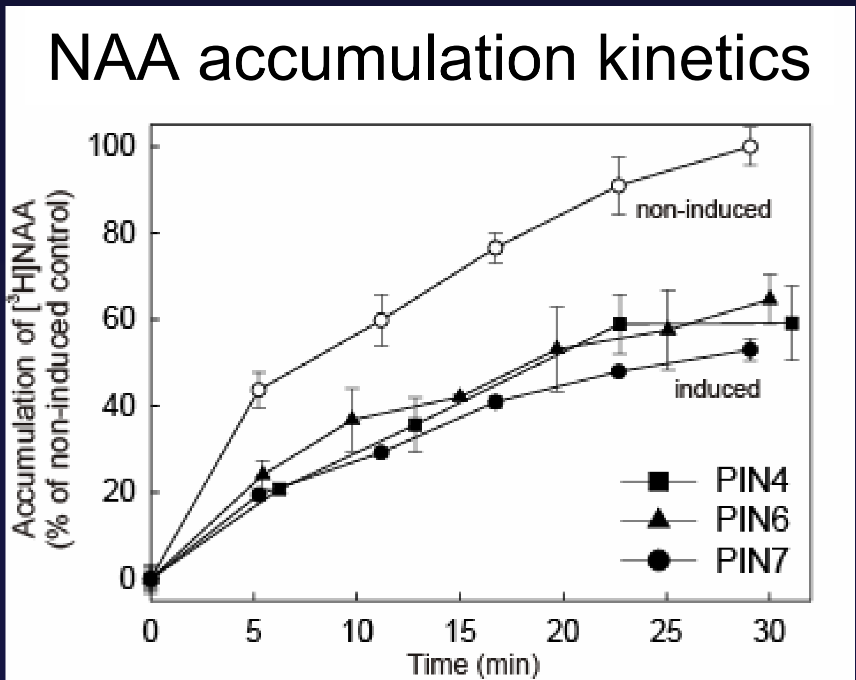
- Polar PIN localization determines direction of auxin flow

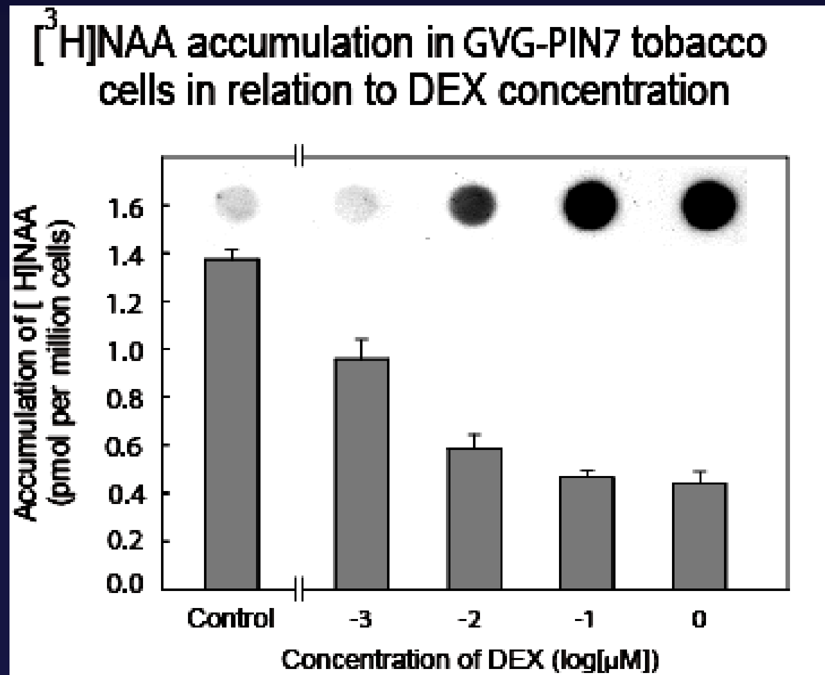
PINs Are Rate-limiting Factors in Auxin Efflux

Inducible PIN1 expression



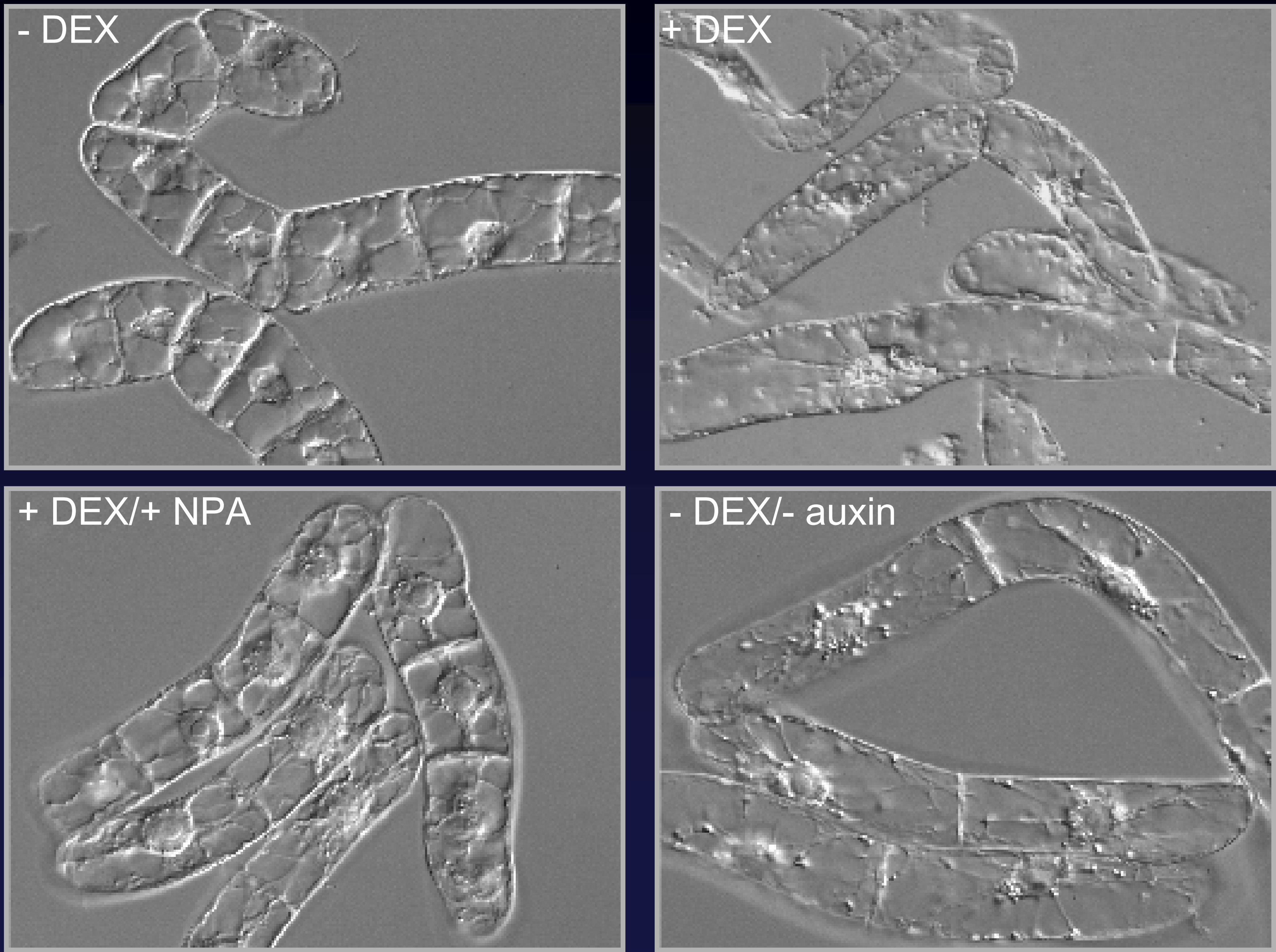






Petrasek et al., 200(

PIN-induced Phenotypes in BY-2 Cells

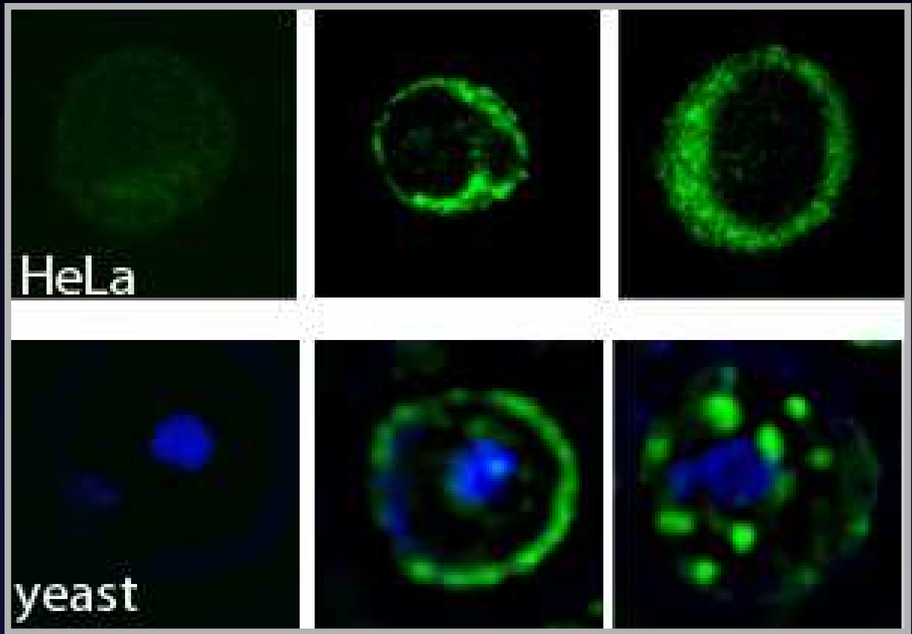


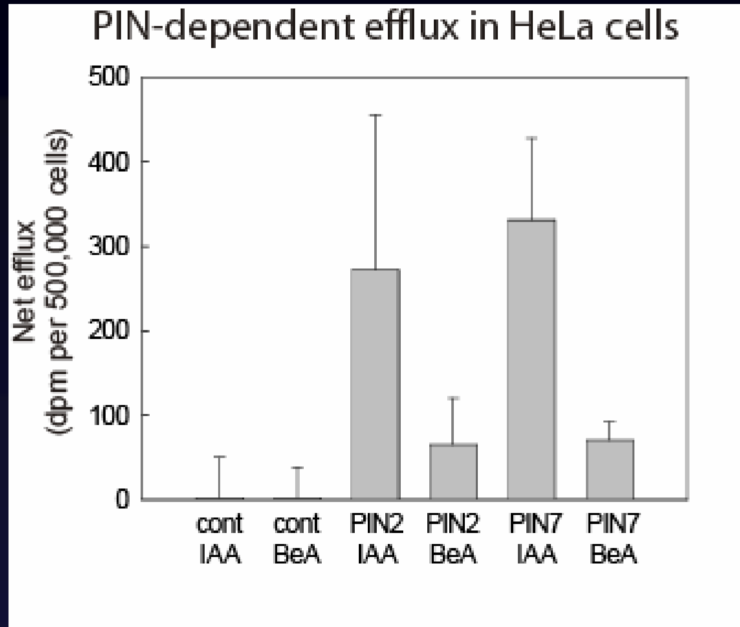
unpublished

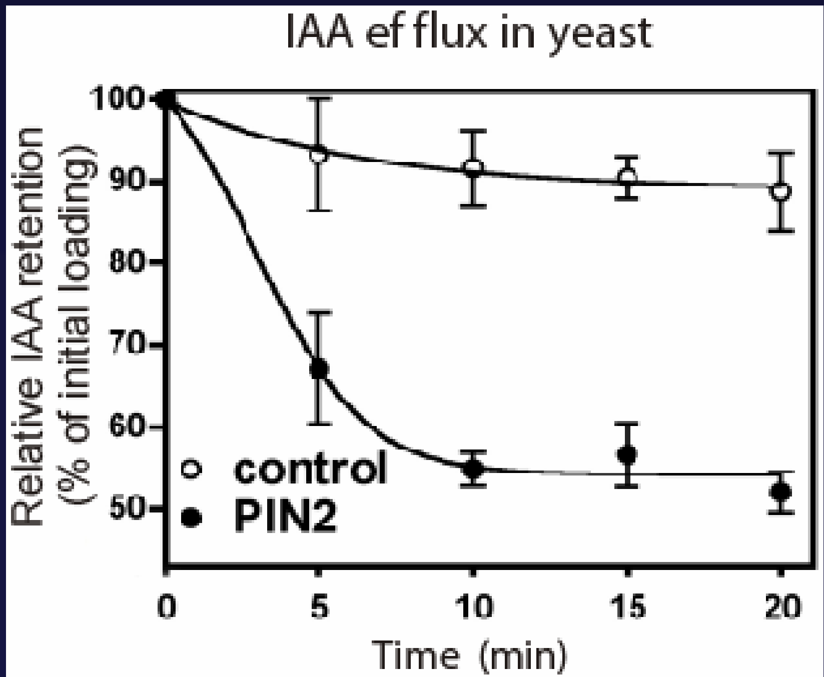
Expression of PINs in HeLa and Yeast

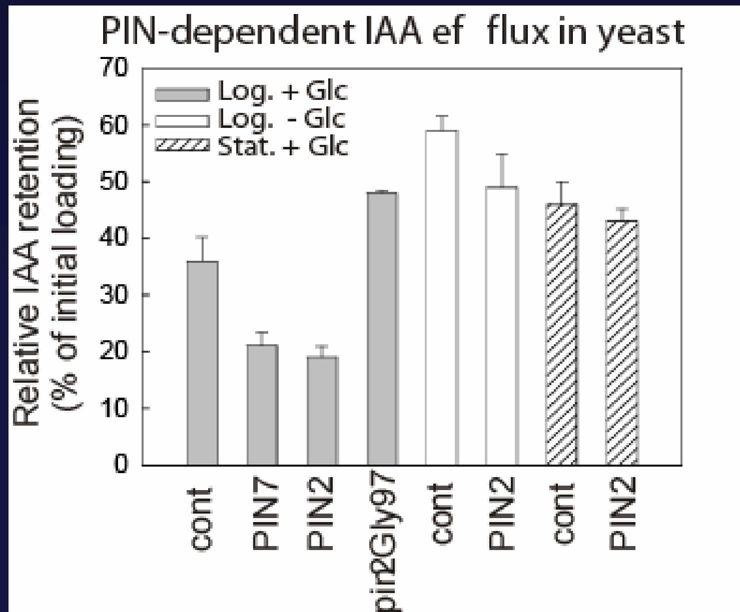
Heterologous PIN2 expression

auxin efflux activity



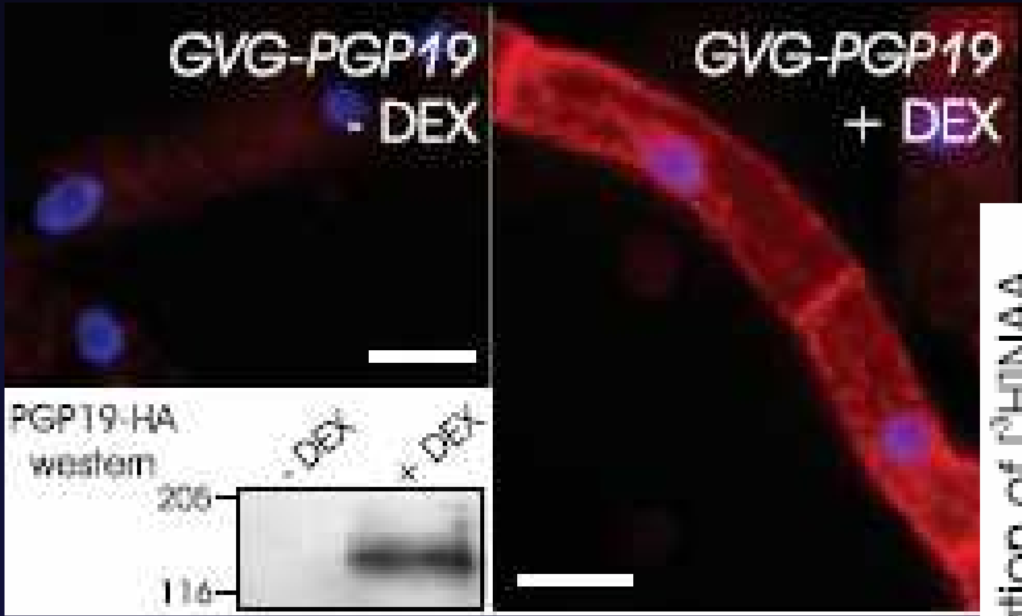




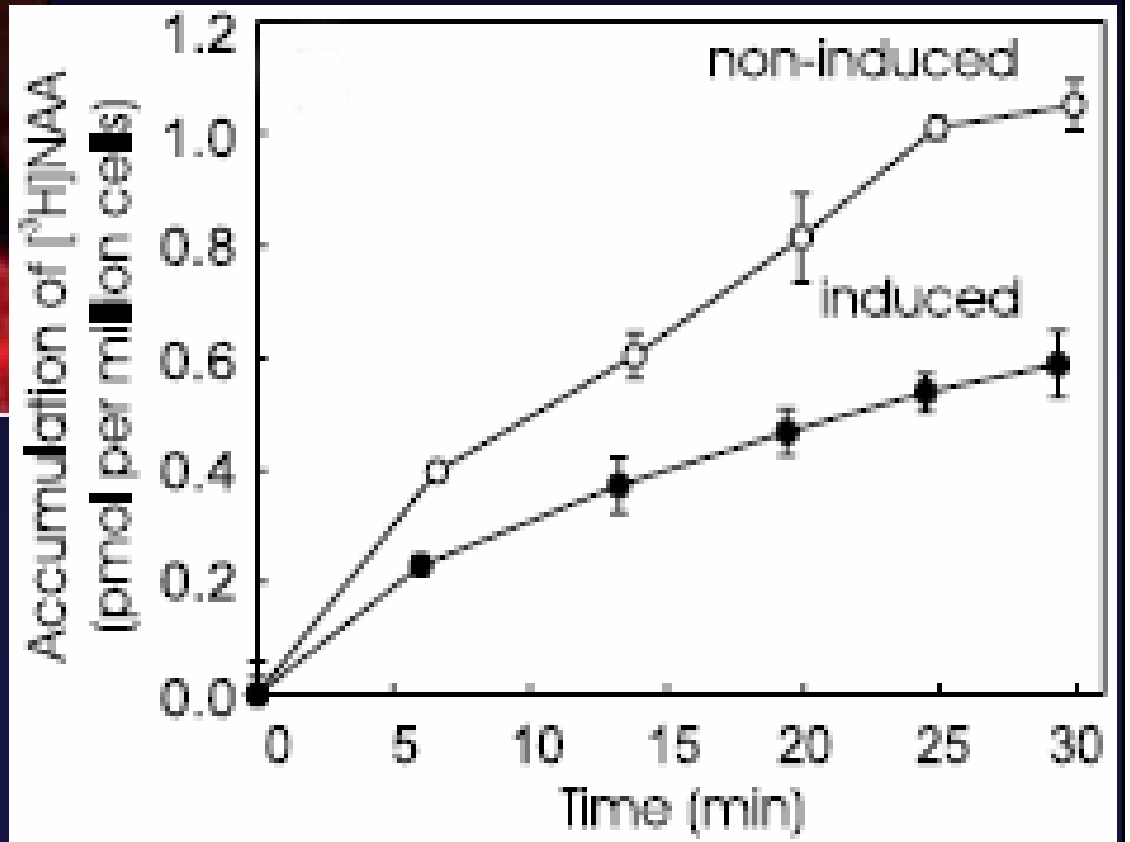


Petrasek et al., 2006

PGP19 Mediates Auxin Efflux in BY-2 Cells



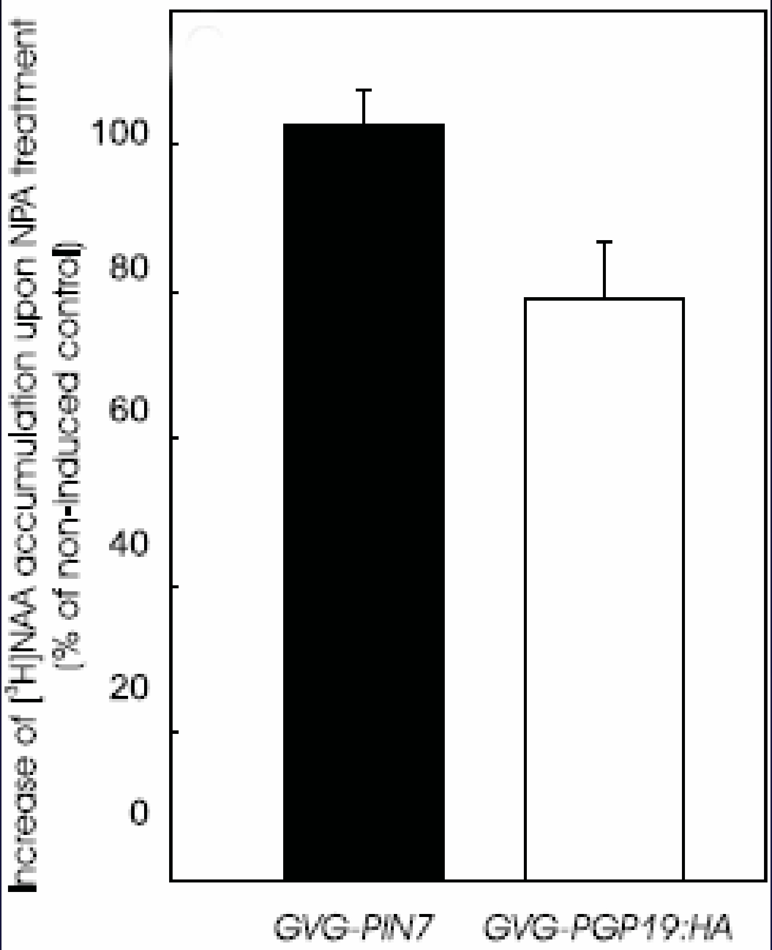
NAA Accumulation in BY-2 Cells



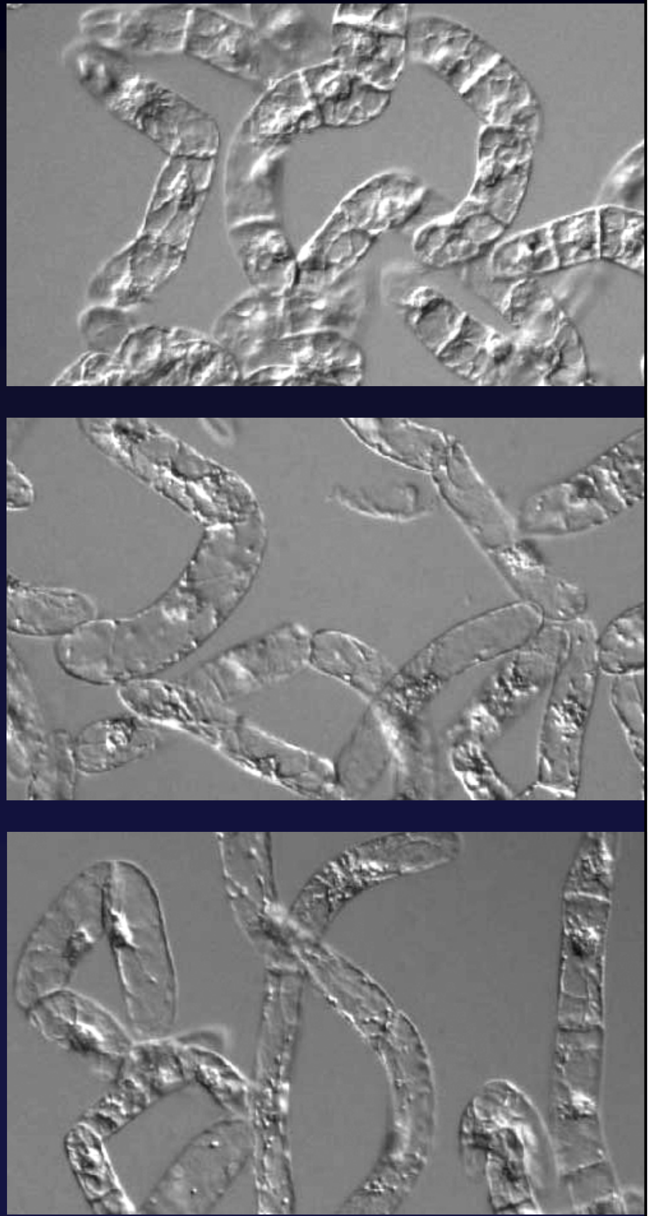
Petrasek et al. 2006

PGP19-induced Phenotypes in BY-2 Cells

NPA Sensitivity of PIN- and PGP-mediated Auxin Efflux

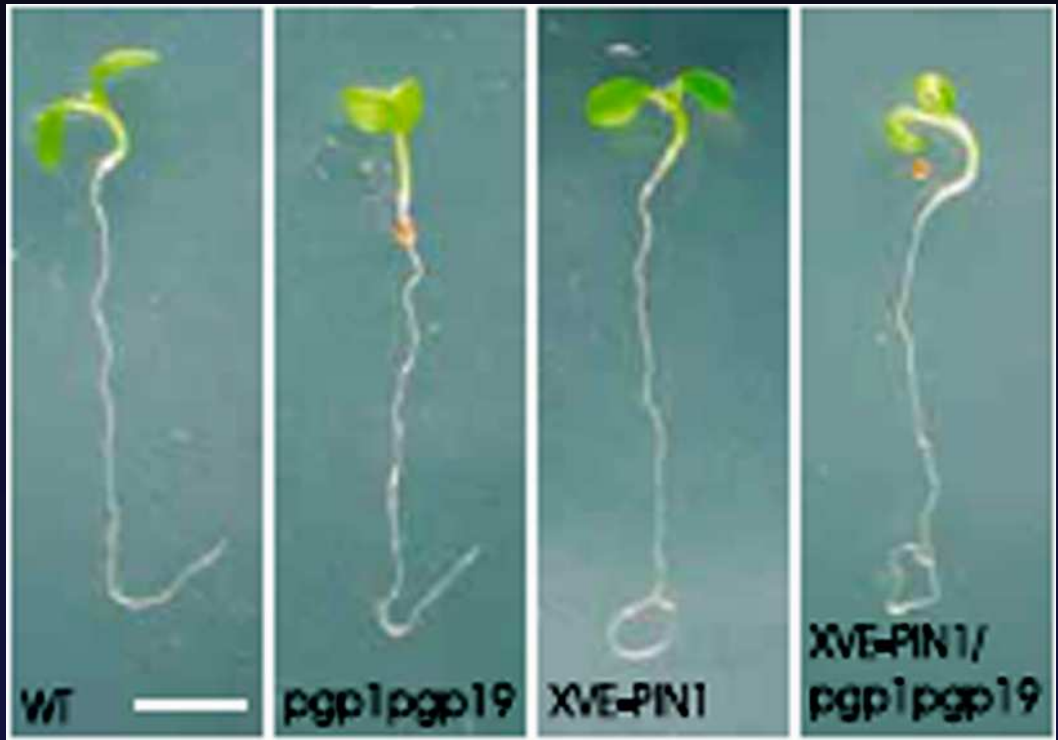


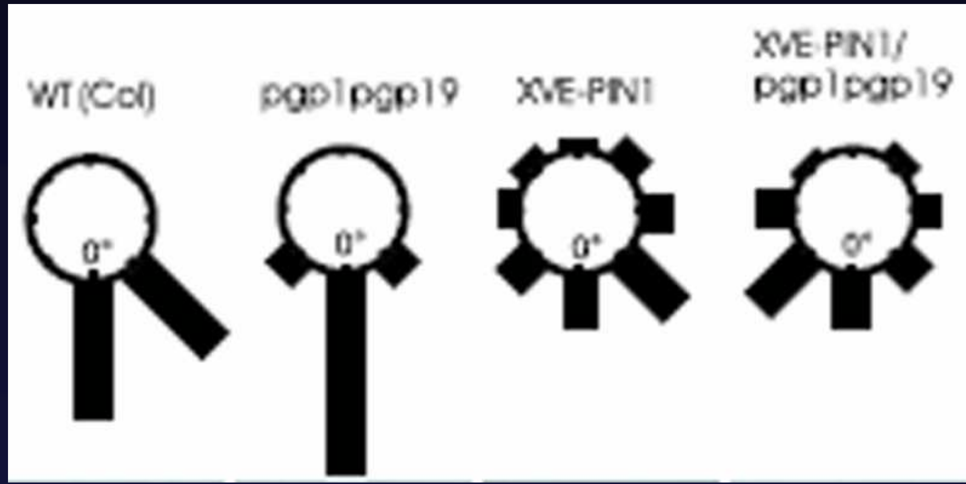
PGP19



unpublished

PIN1 is Functional in Absence of PGP1 and PGP19

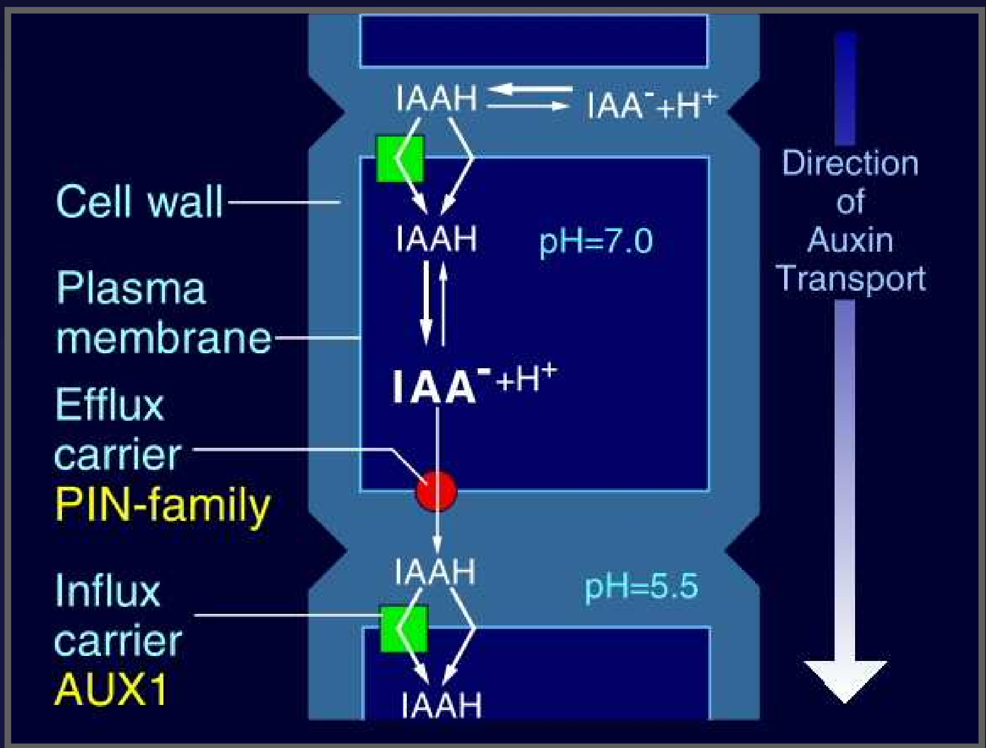


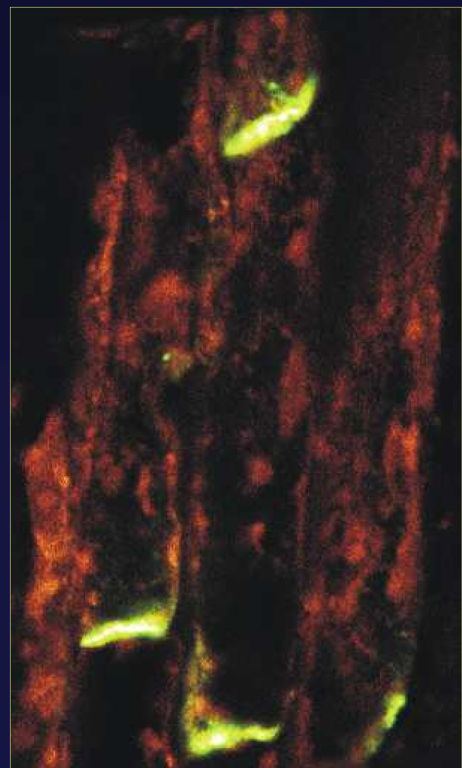


PINs and PGPs define distinct,
functionally independent transport systems

Petrasek et al. 2006

Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow





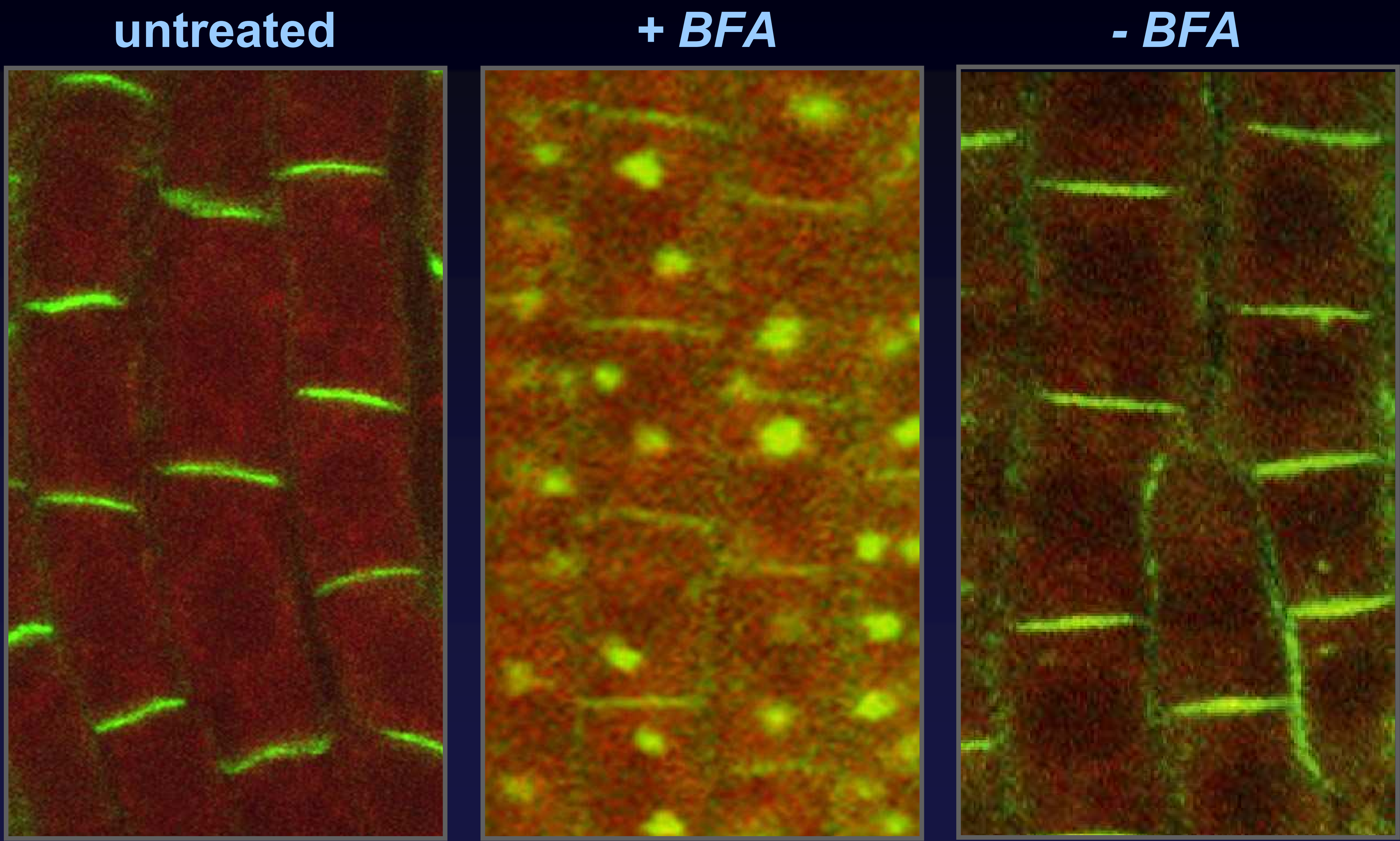
PIN proteins are rate-limiting factors in auxin efflux from cells

and

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

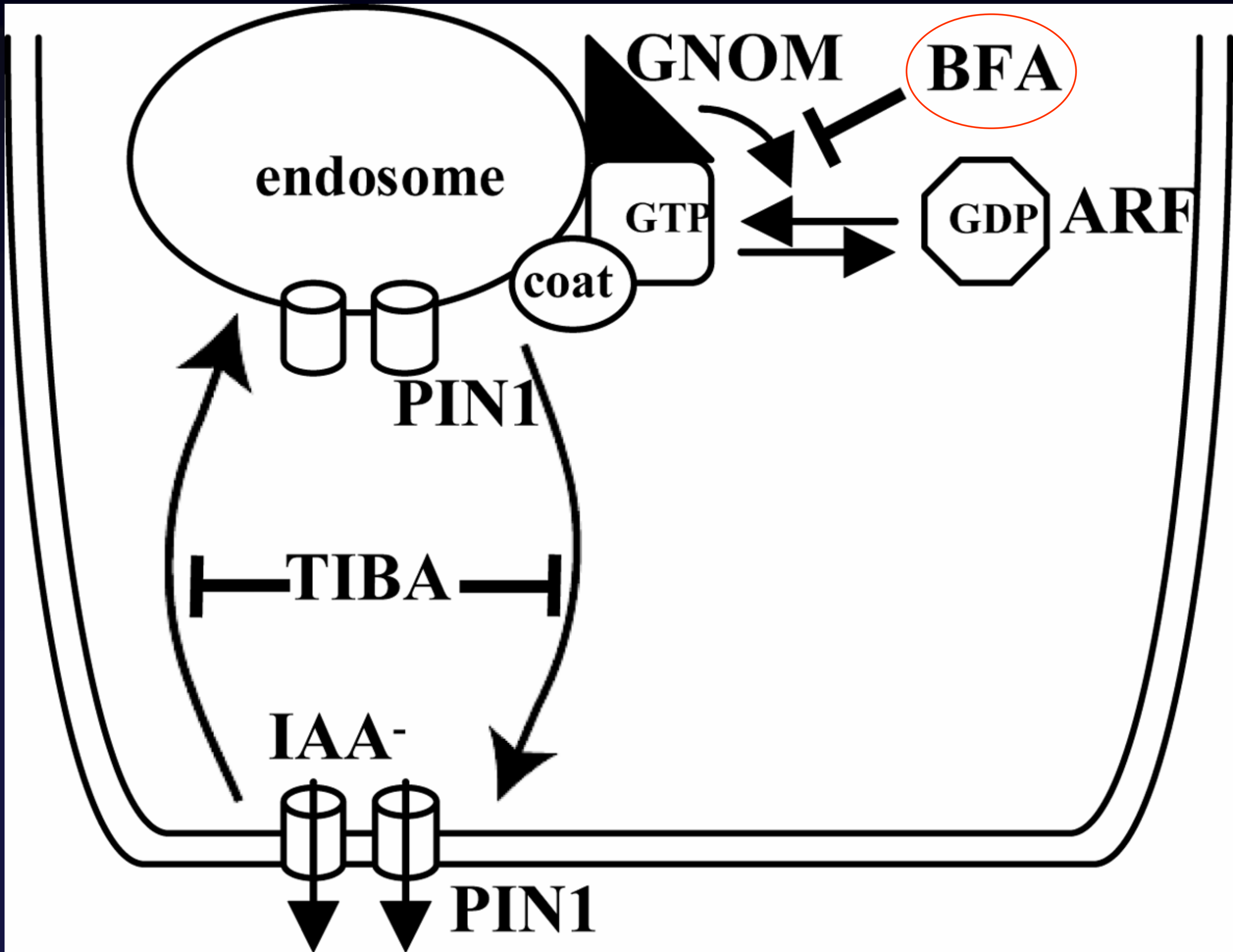
Constitutive Cycling of PINs

PIN1 Subcellular Movement

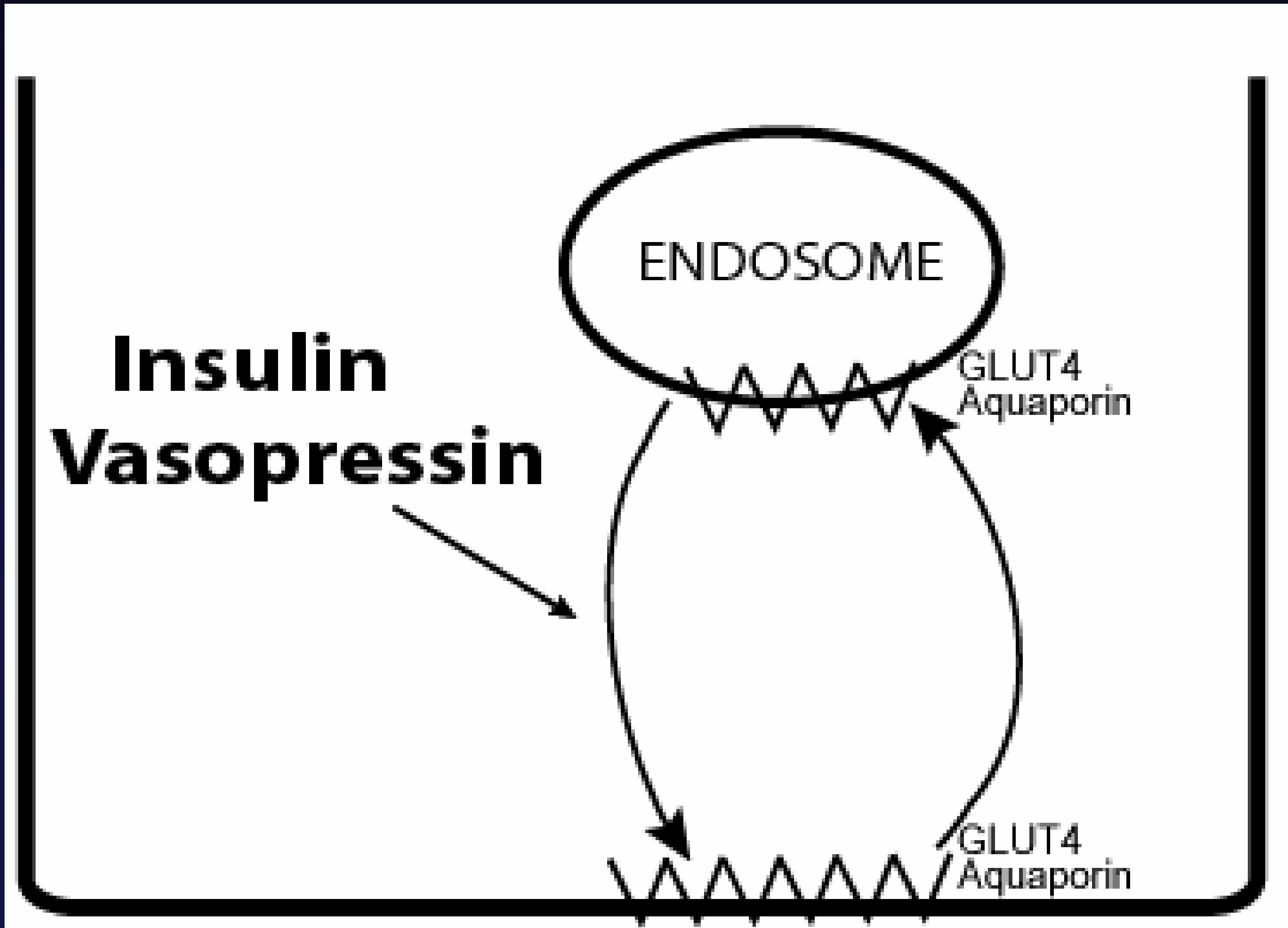


Geldner et al., 2001

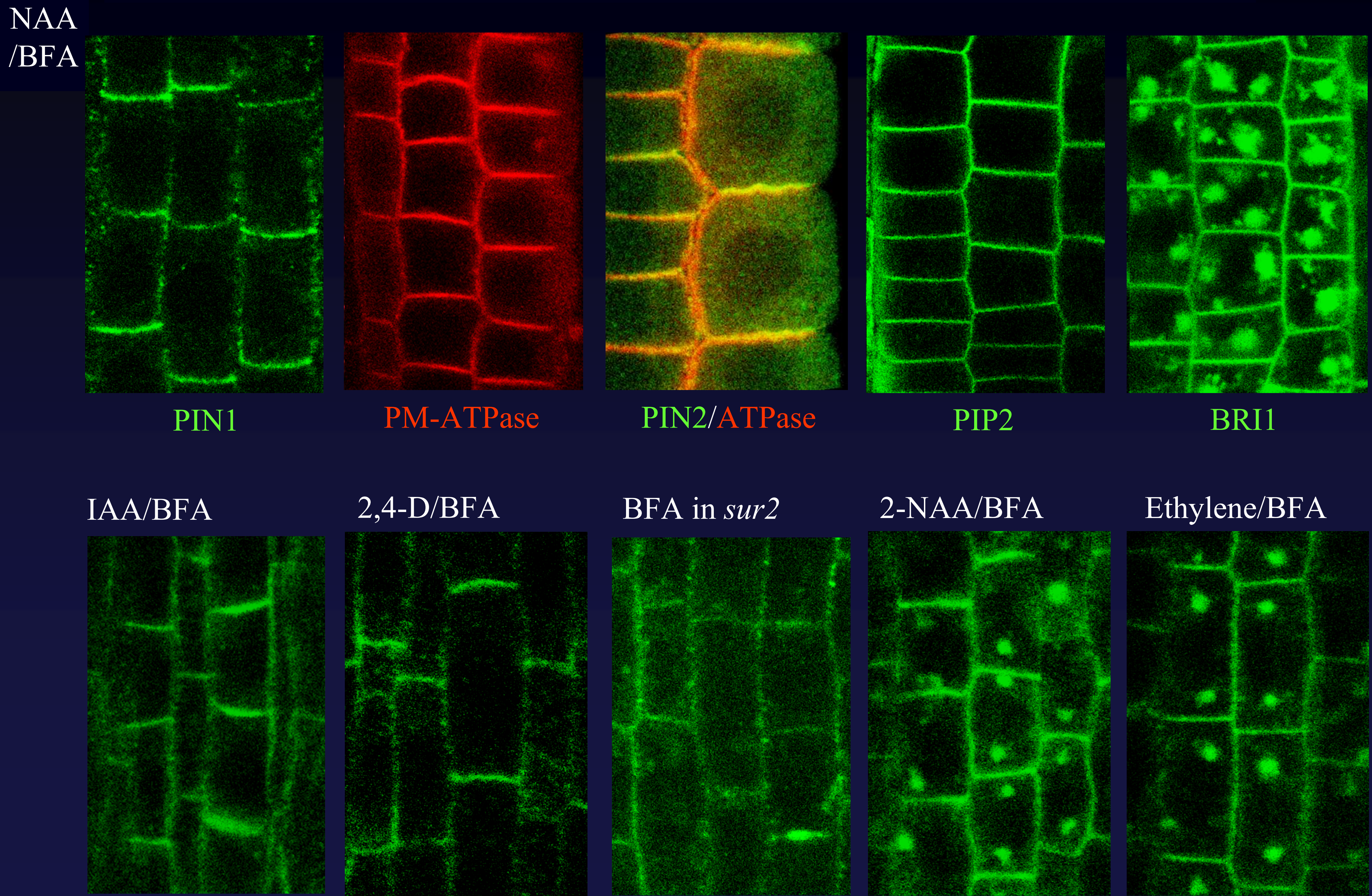
Dynamic Movement of PIN Proteins



Subcellular Cycling – Means to Modulate Protein Activity?

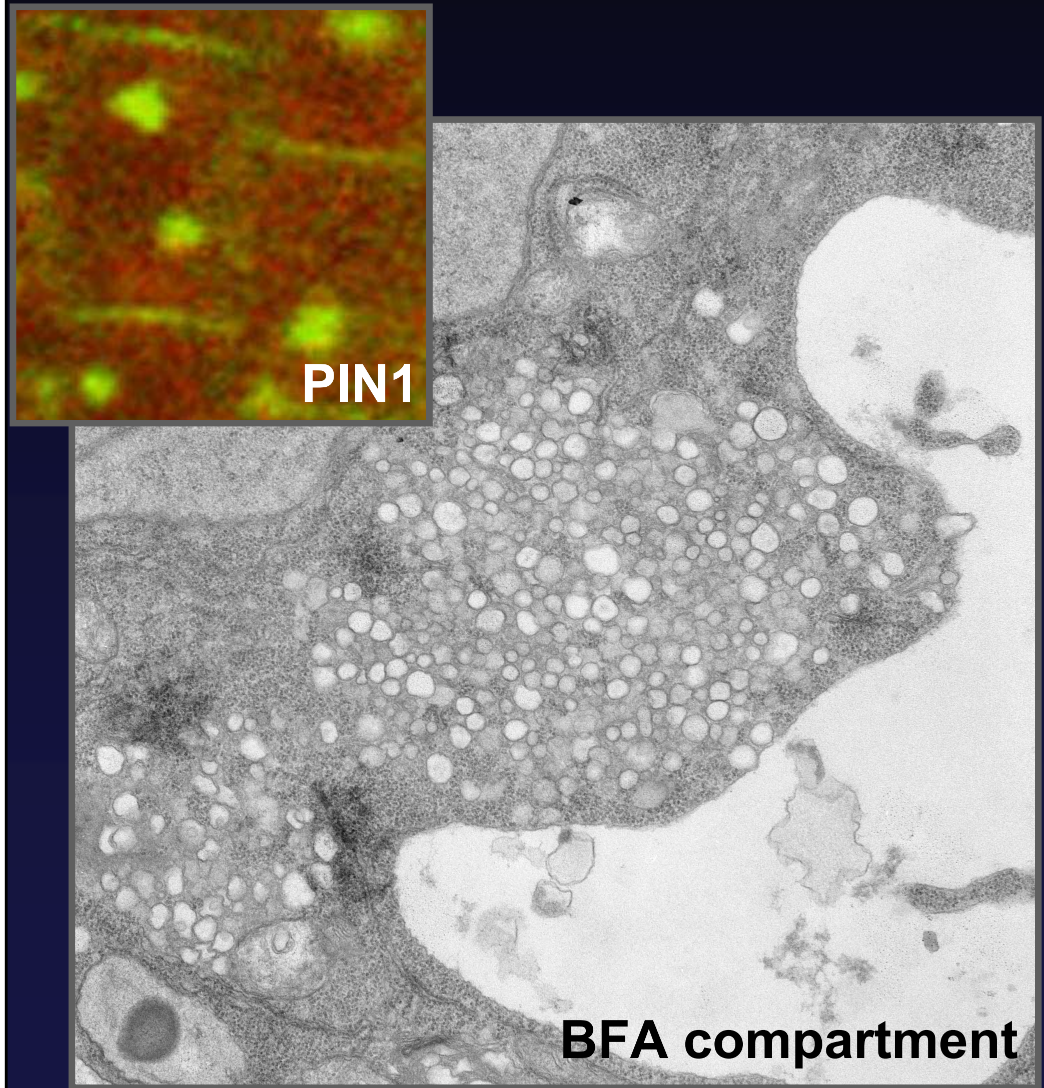


Auxin Inhibits Internalization of Plasma Membrane Proteins

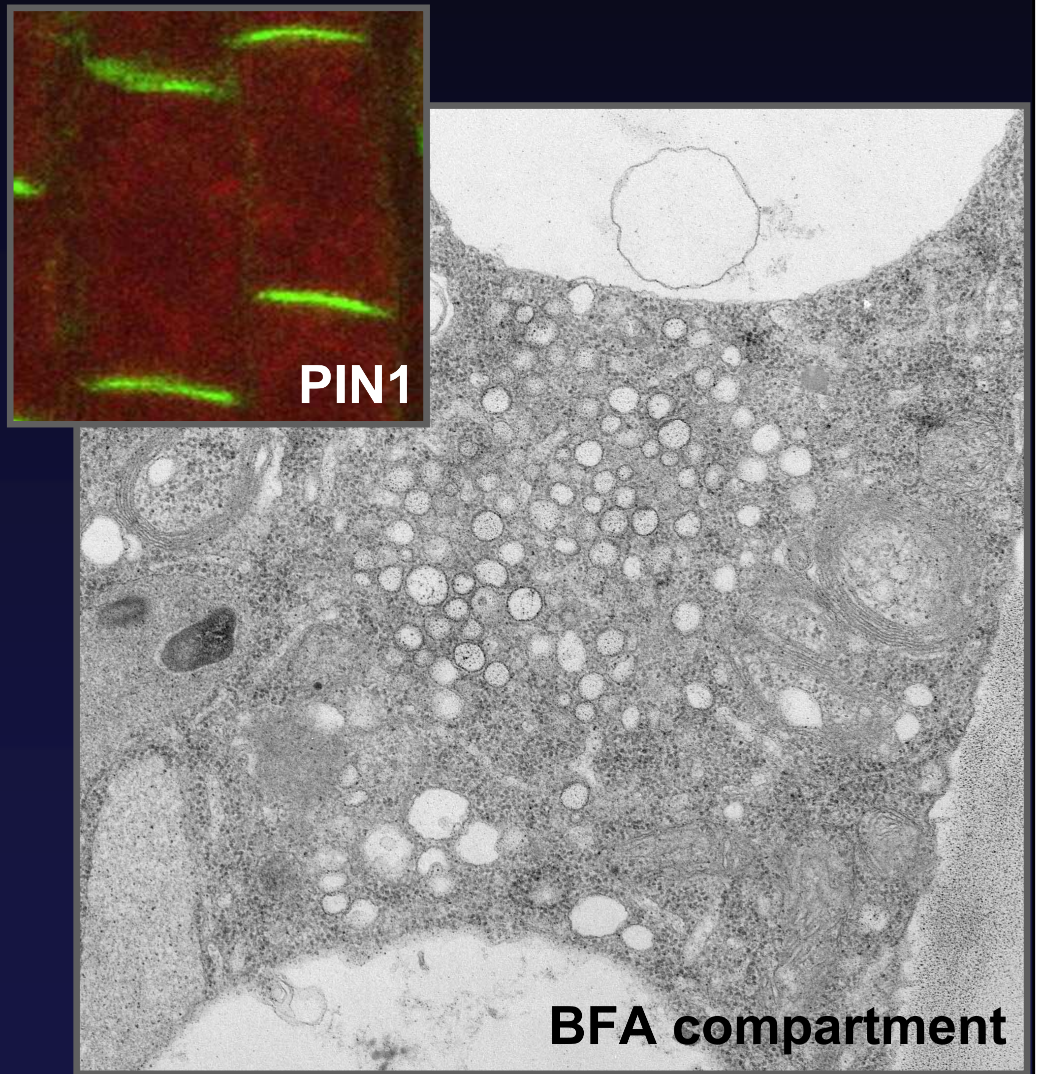


Place of Auxin Action in Protein Cycling

BFA



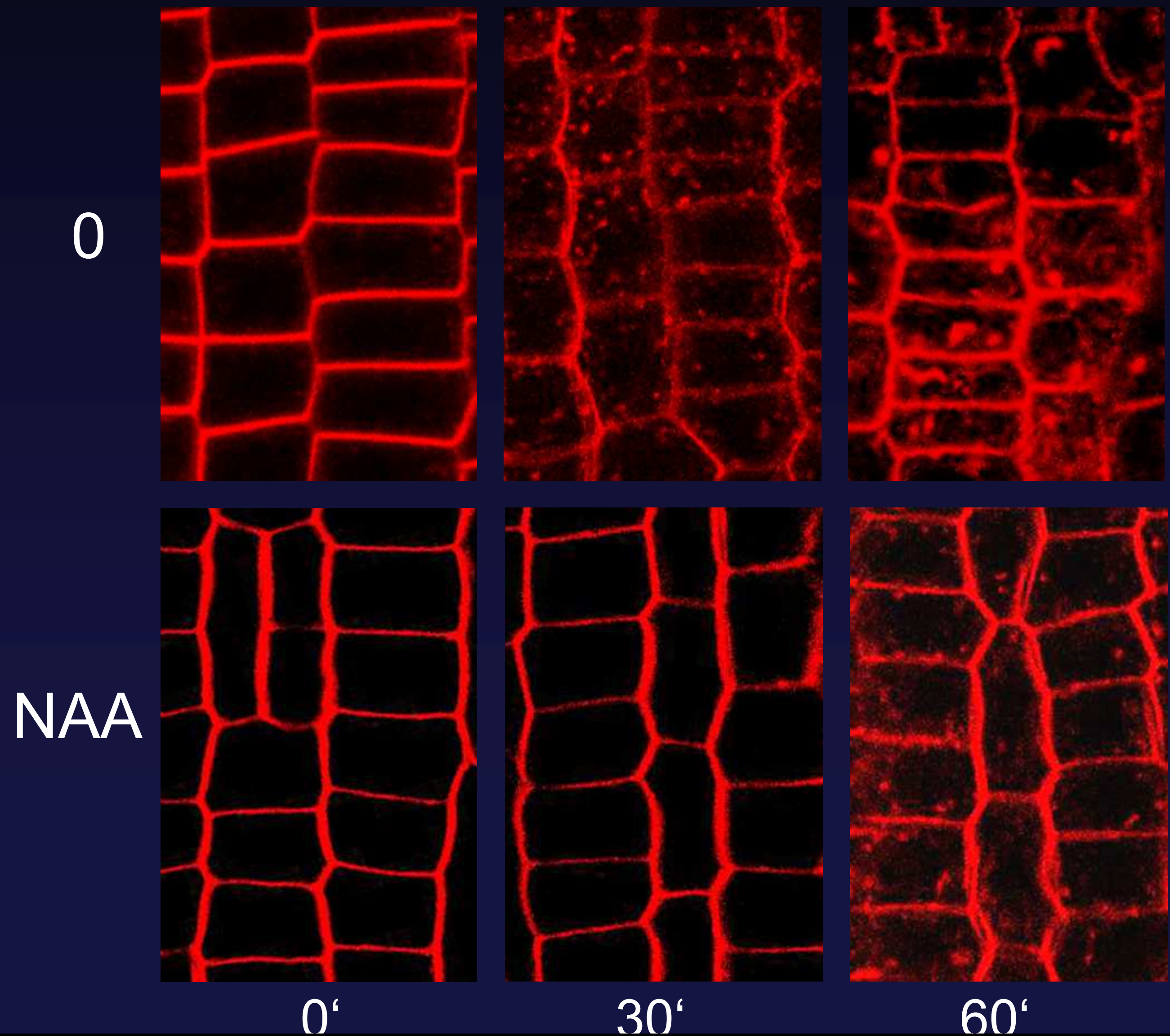
Auxin + BFA



Paciorek et al., 2005

Auxin Inhibits Endocytosis

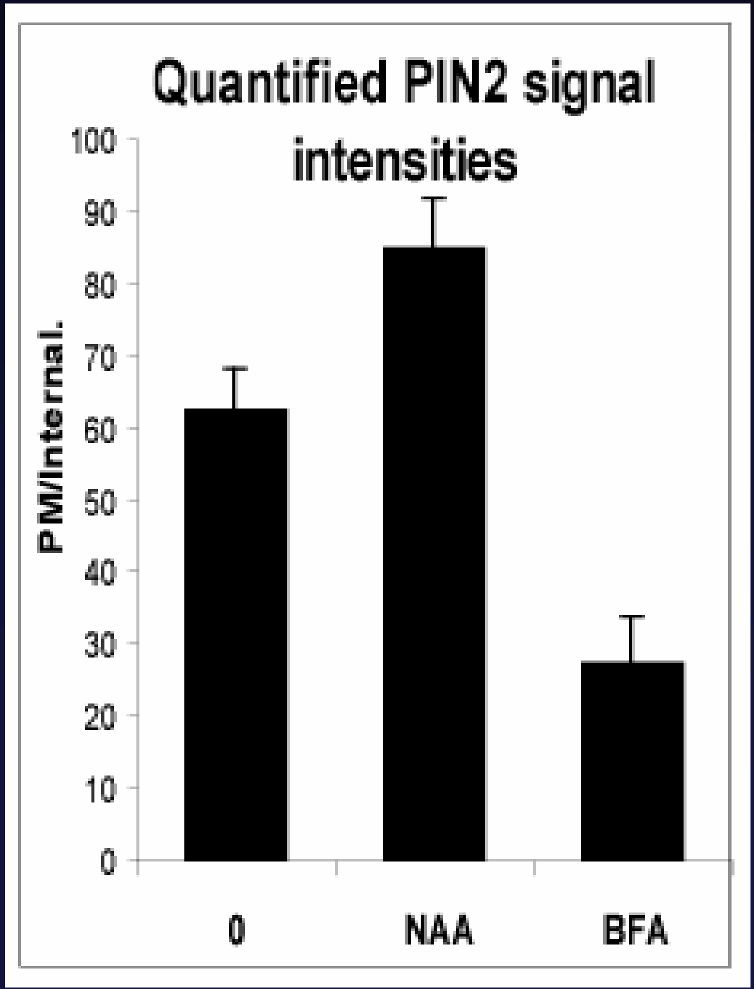
Uptake of endocytic tracer FM4-64



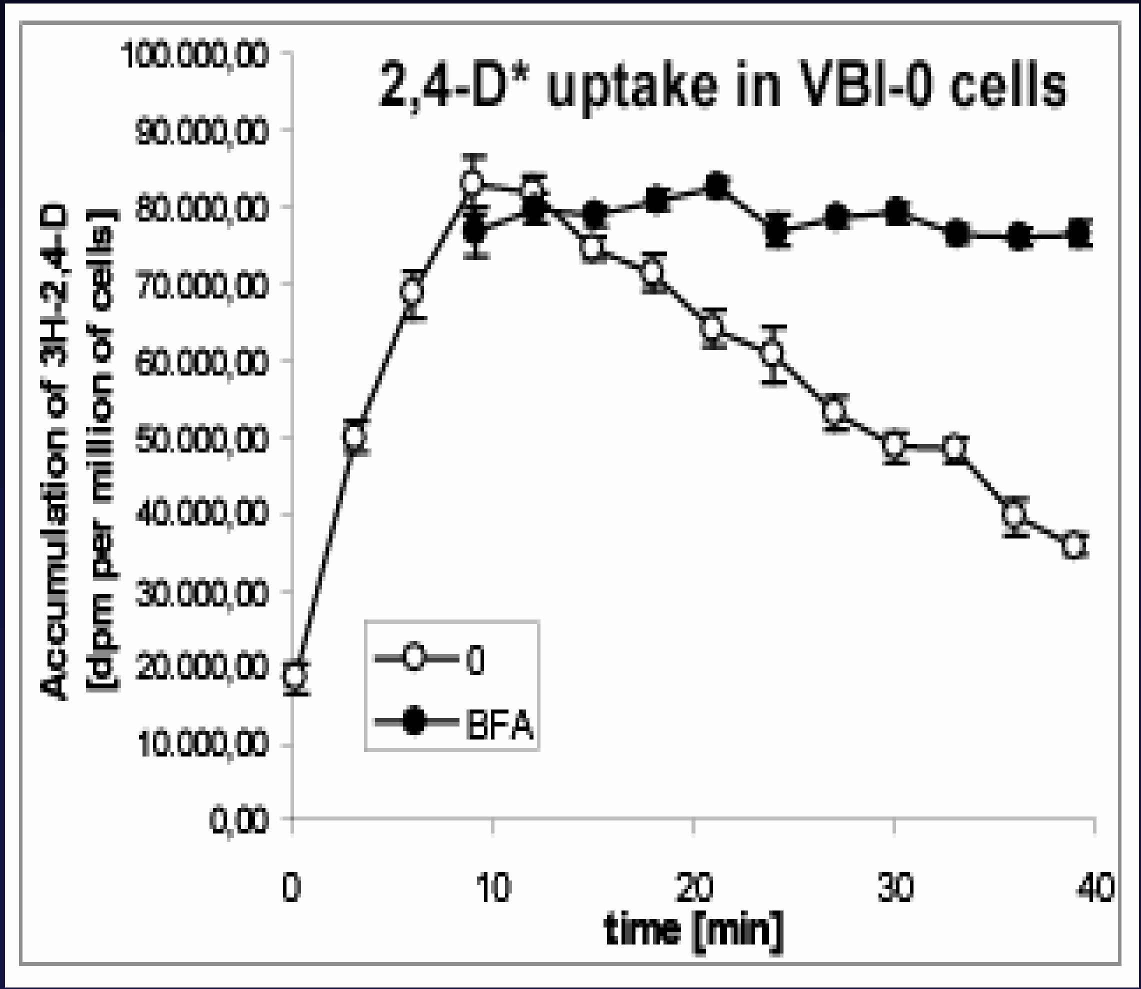
Paciorek et al., 2005

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

PIN2 levels at PM

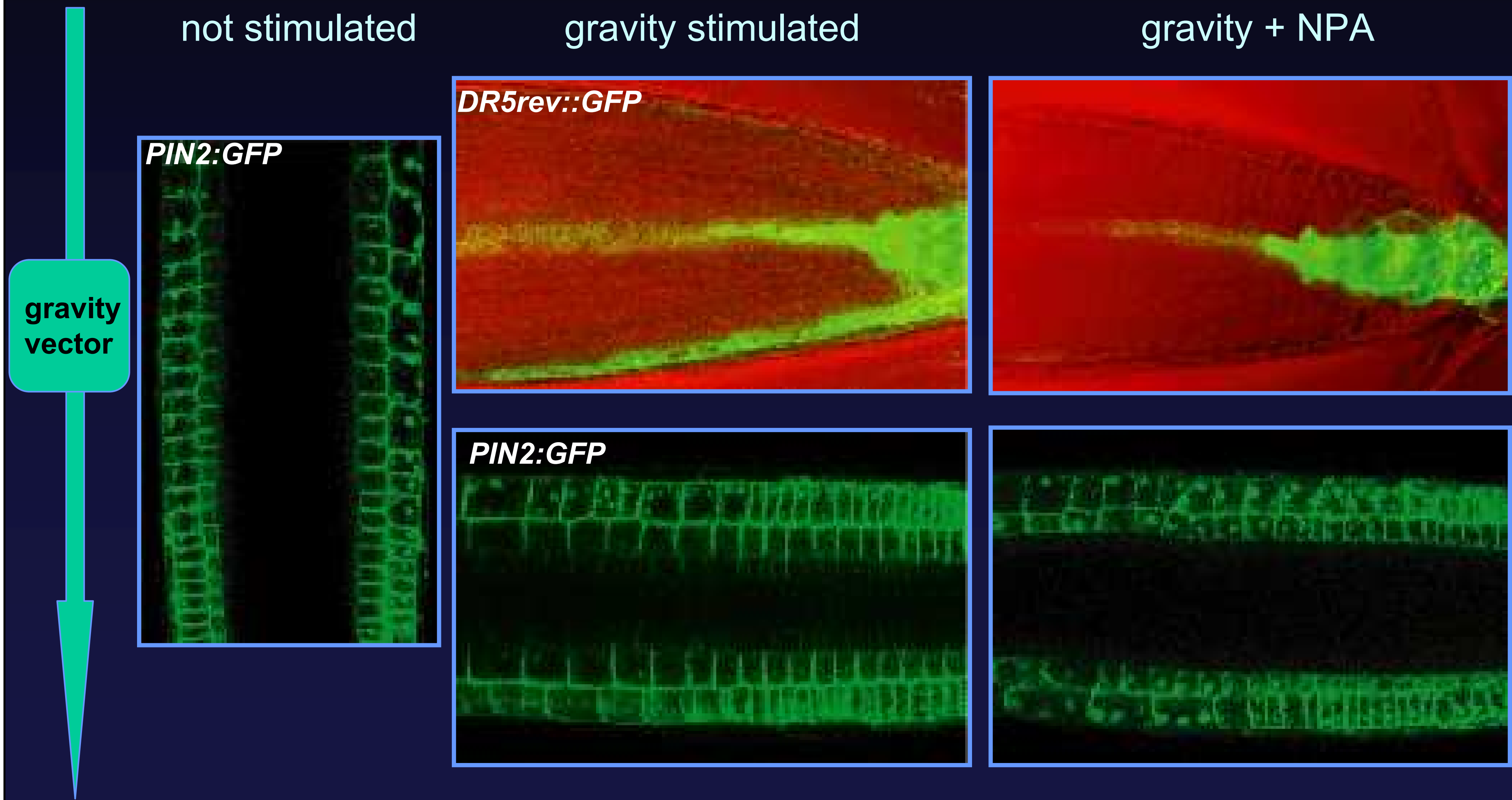


Auxin efflux in tobacco cells



Paciorek et al., 2005

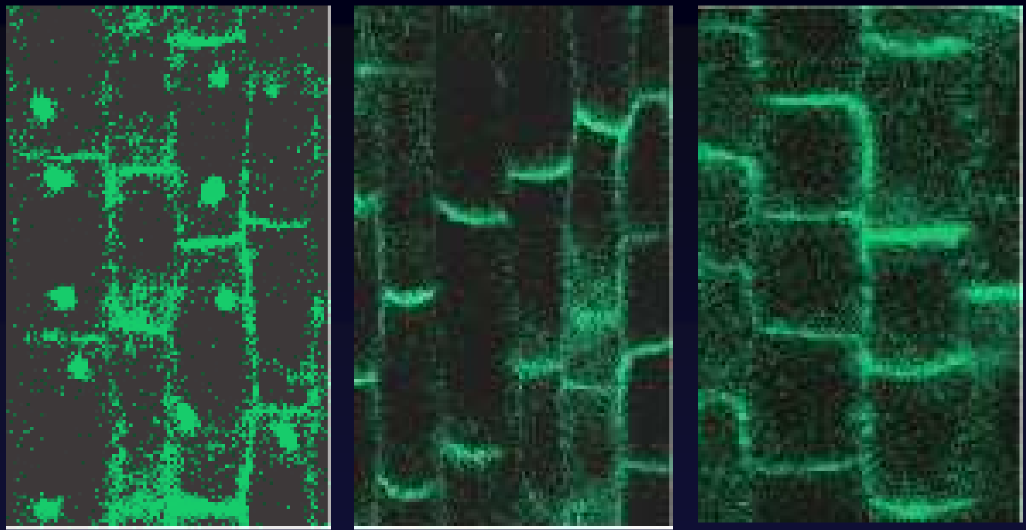
in planta Correlation between Cycling and Auxin Flow



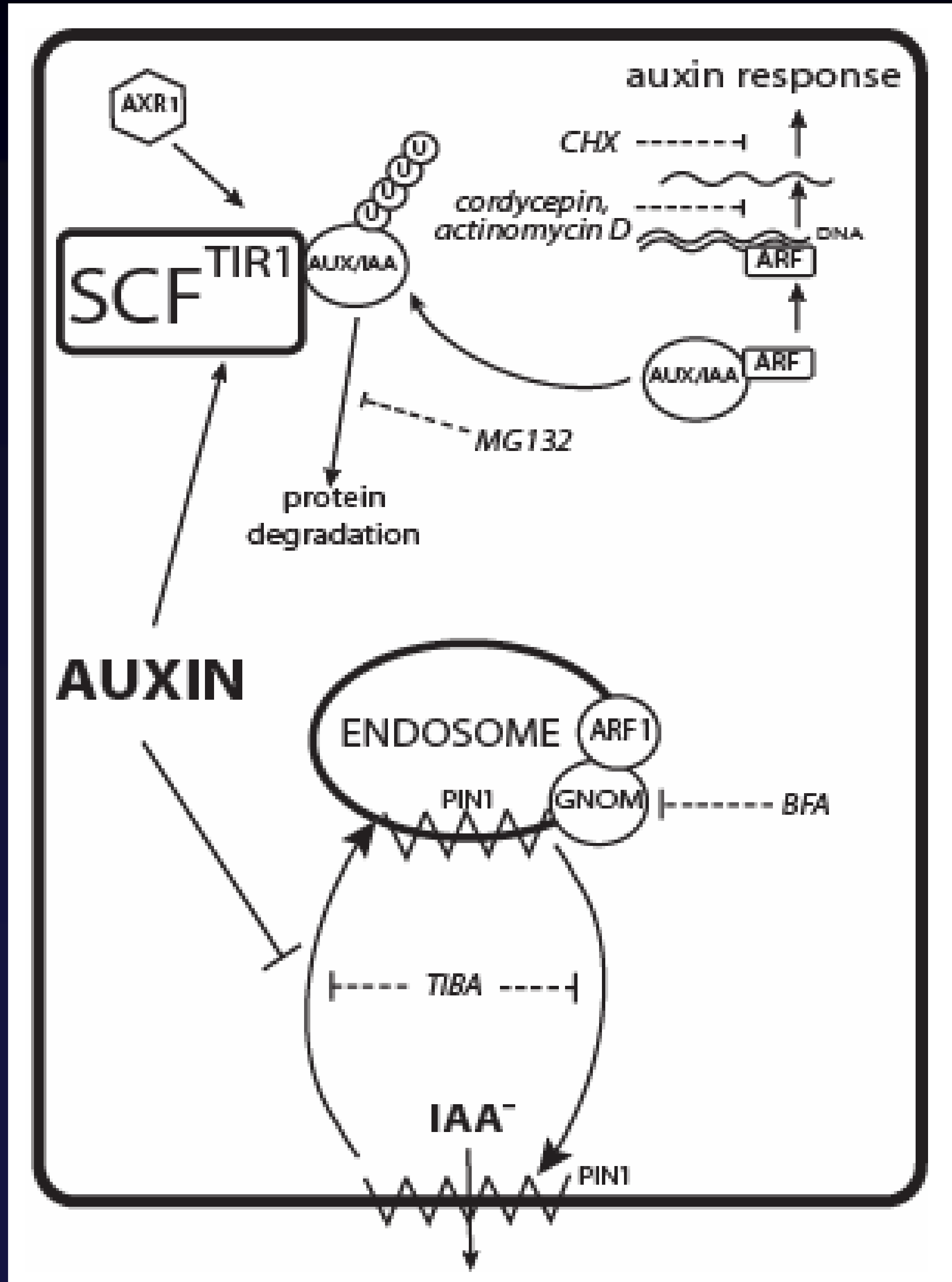
Paciorek et al., 2005

Novel Pathway of Auxin Action

PIN1 BFA 2,4-D/CHX /BFA 2,4-D/MG132 /BFA

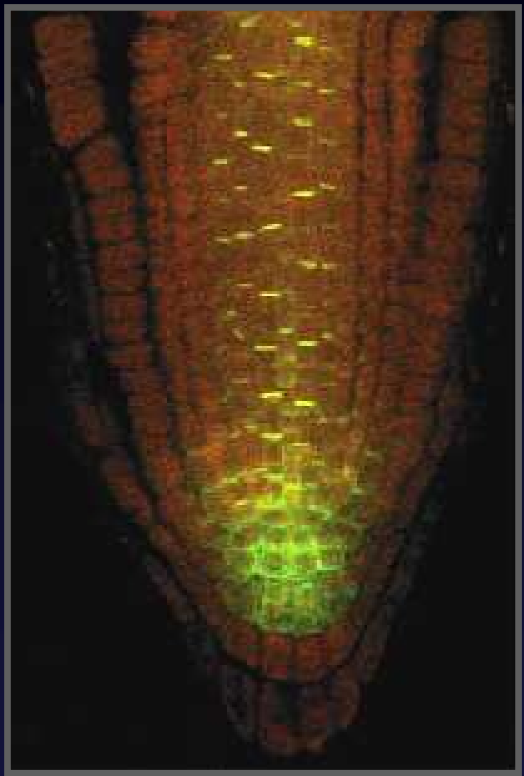


DR5 0 2,4-D 2,4-D/ CHX 2,4-D/ MG132



unpublished

Mutant Screen for Components of PIN Polarity and Cycling



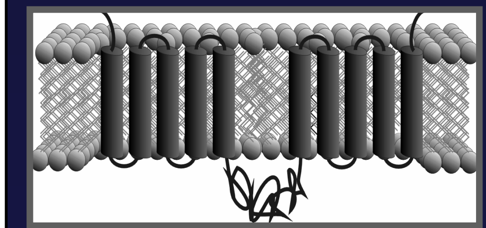
PIN:GFP

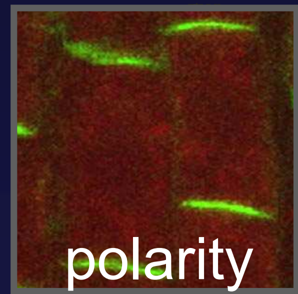
EMS mutagenesis. Screening for polarity and cycling defects.

mutant lines

intragenic → sequencing → **important residues**

extragenic → cloning → **novel genes**



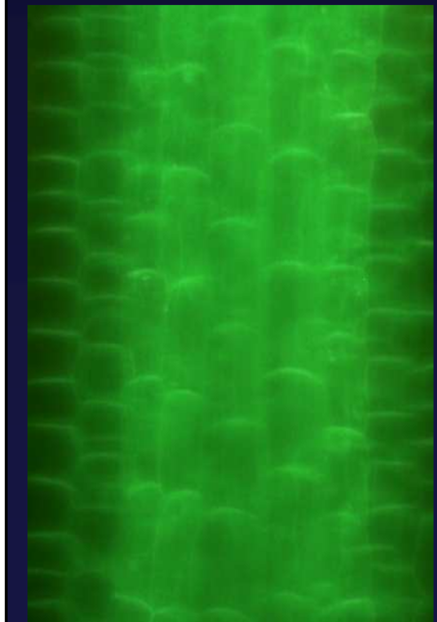


unpublished

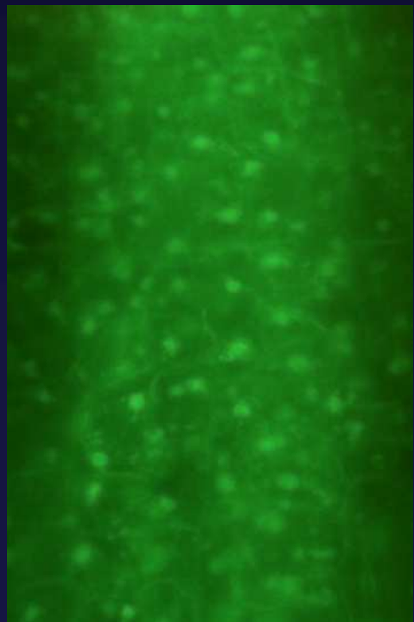
“Cell Biological” Mutant Screens in Progress:

Auxin effect on endocytosis: 3 confirmed mutants

30´ NAA 30 µM/90´BFA 50 µM

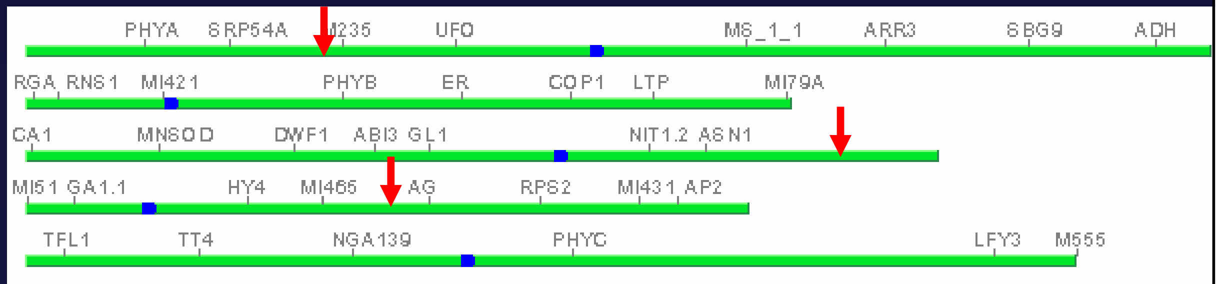


wt



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