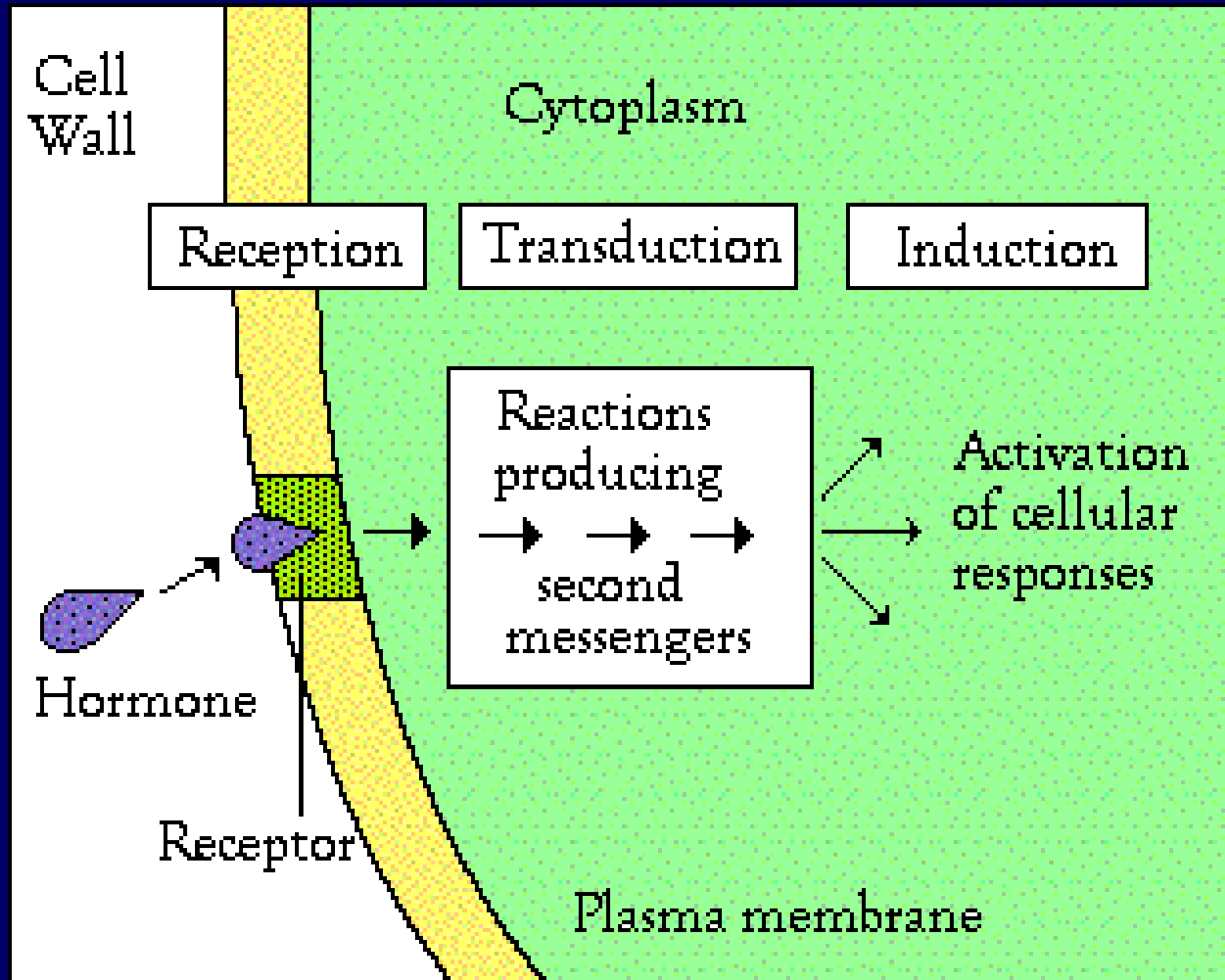
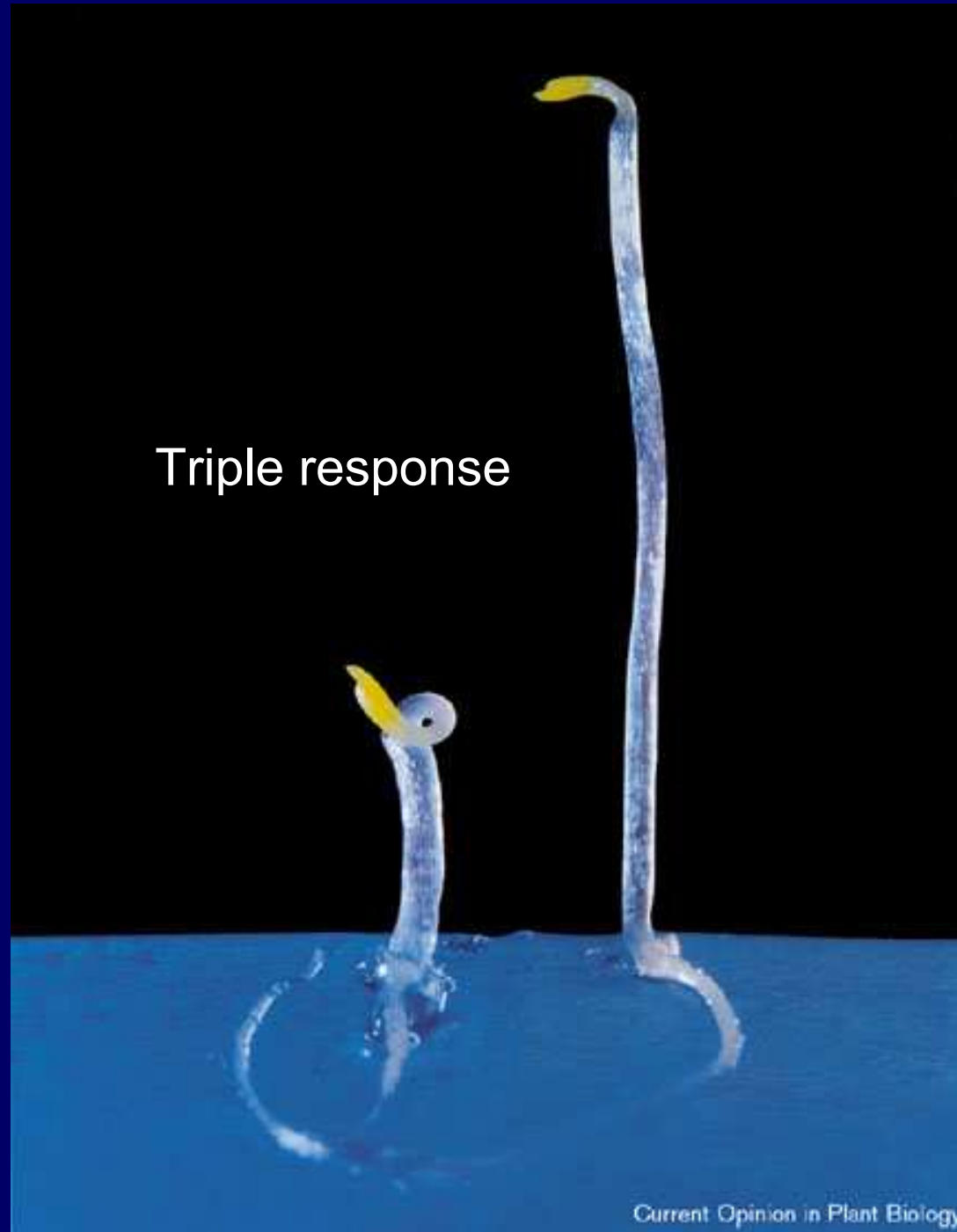


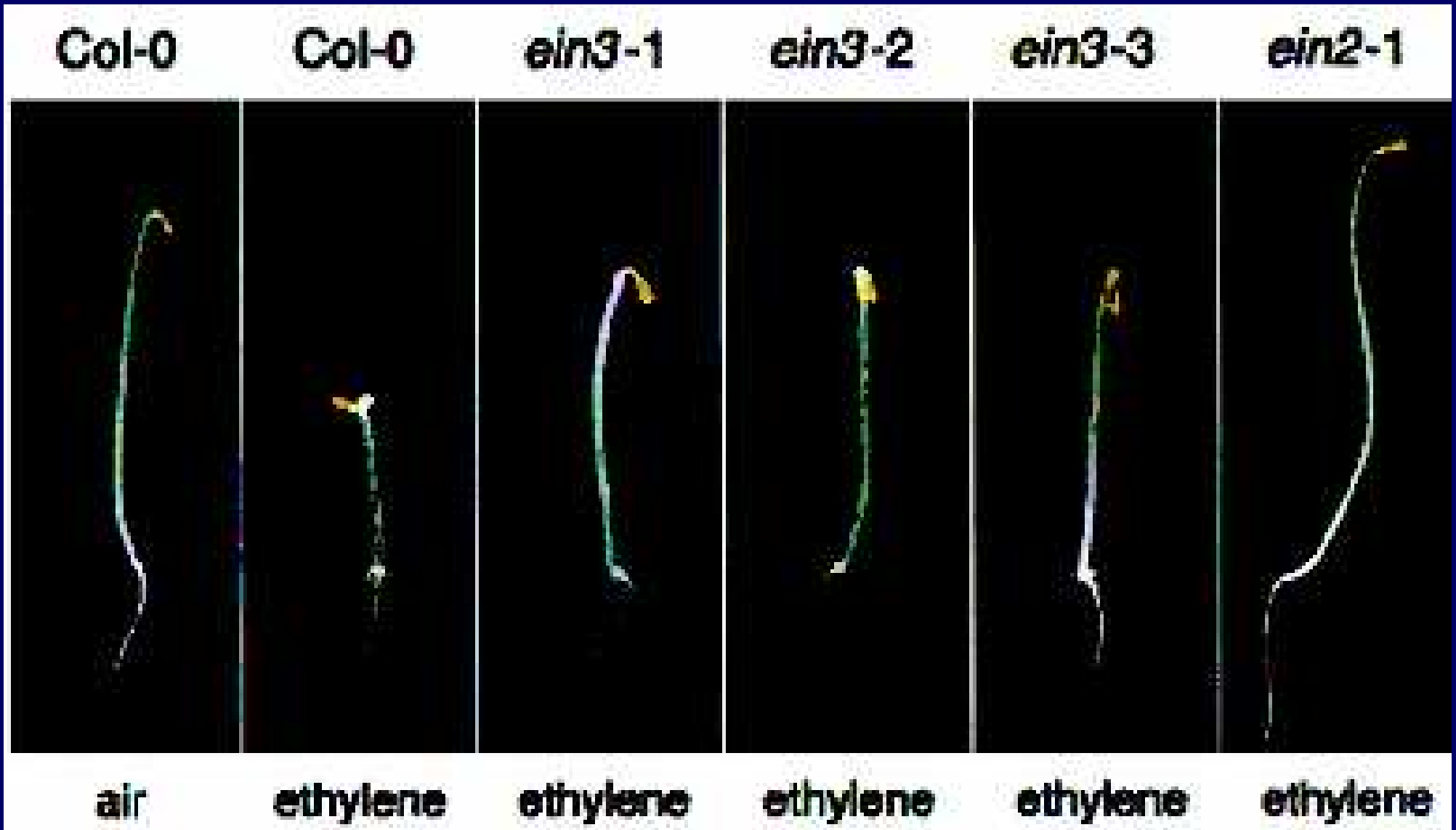
Signal Transduction



Mutant screens for ethylene pathway genes



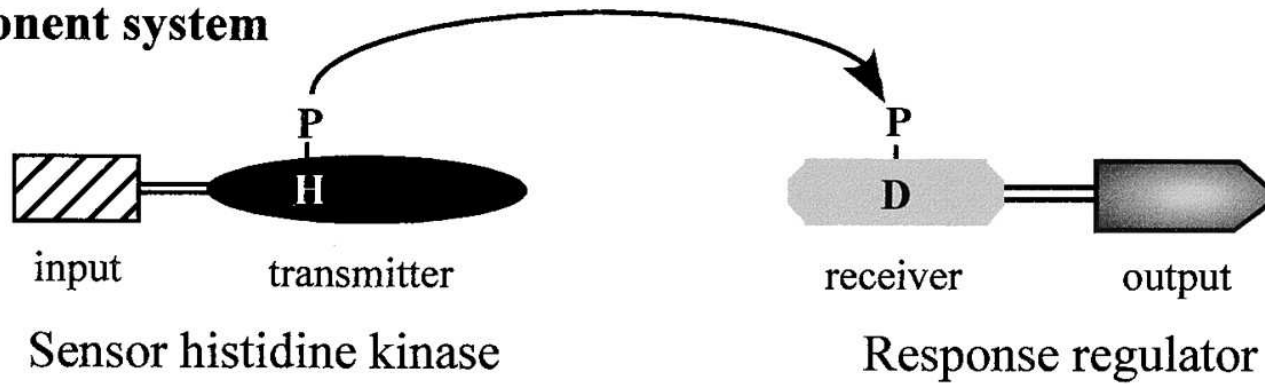
Ethylene signal transduction pathway



Ethylene signal transduction pathway

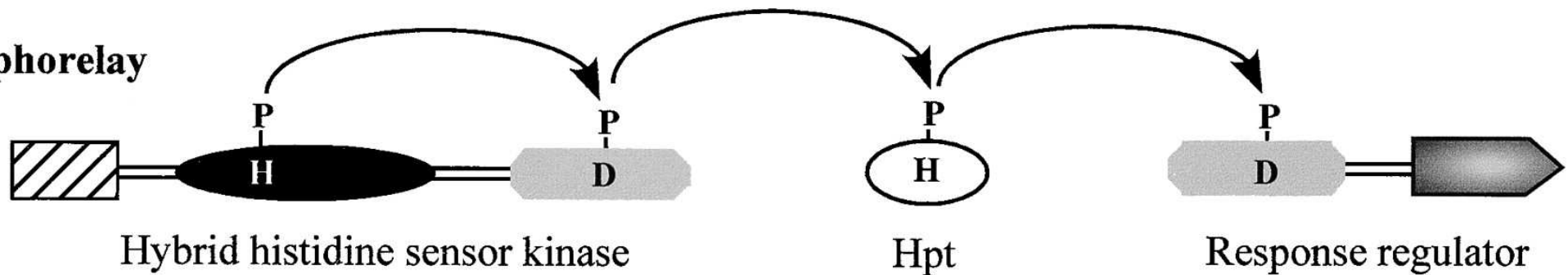
A

Simple two-component system

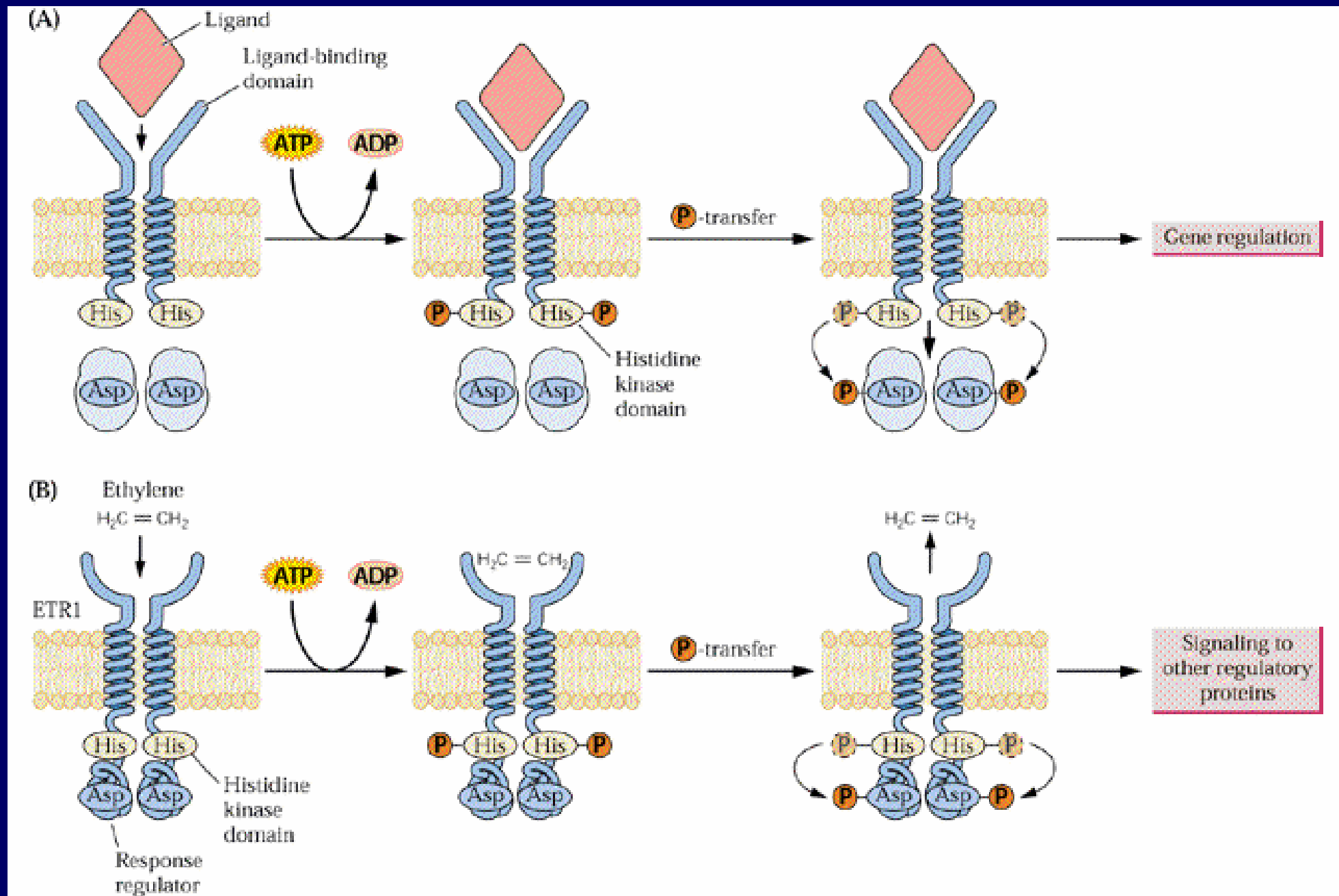


B

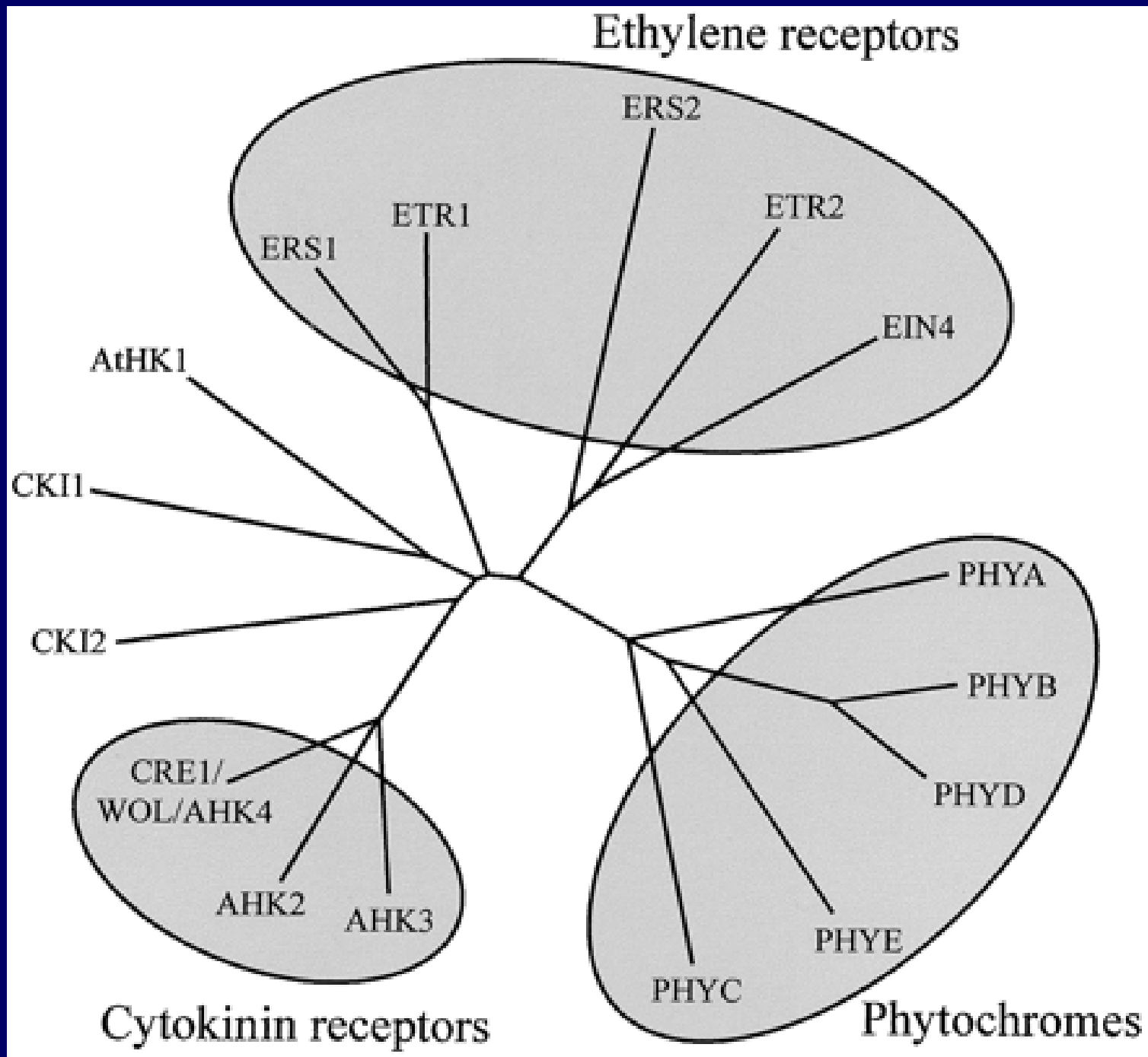
Phosphorelay



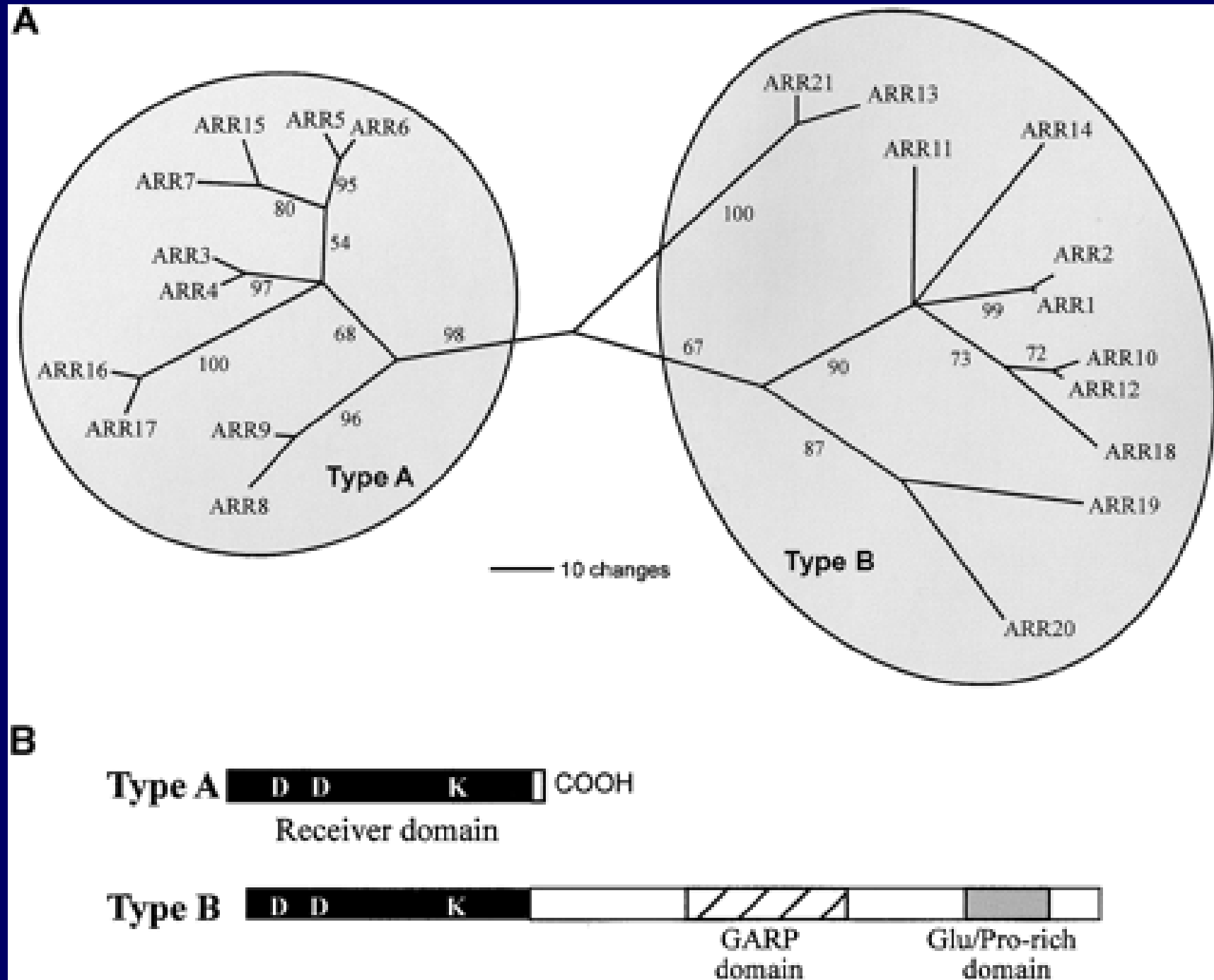
Ethylene signal transduction pathway



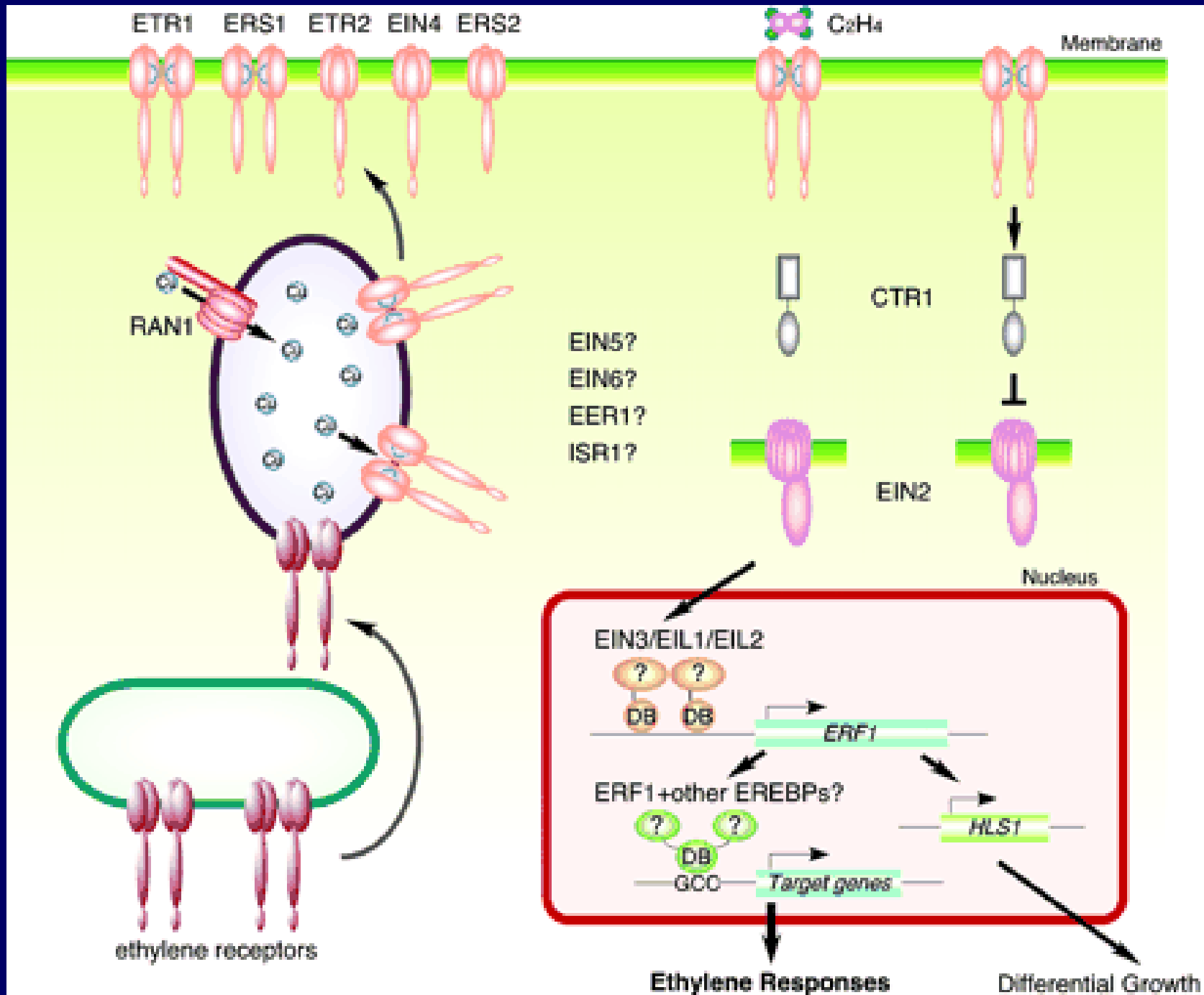
His-kinases in Arabidopsis



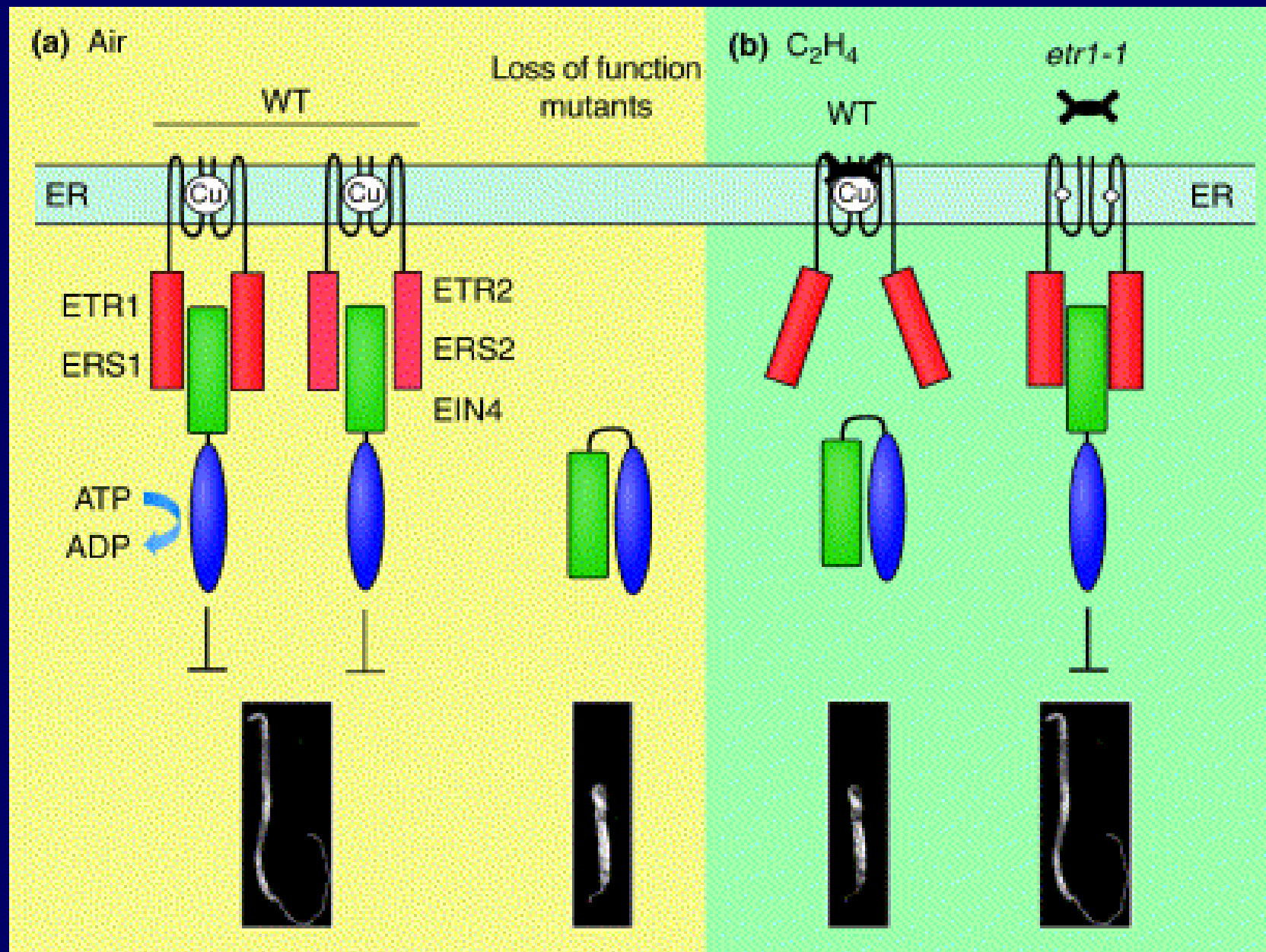
Response Regulators in Arabidopsis



Ethylene signal transduction pathway



Genetic interactions



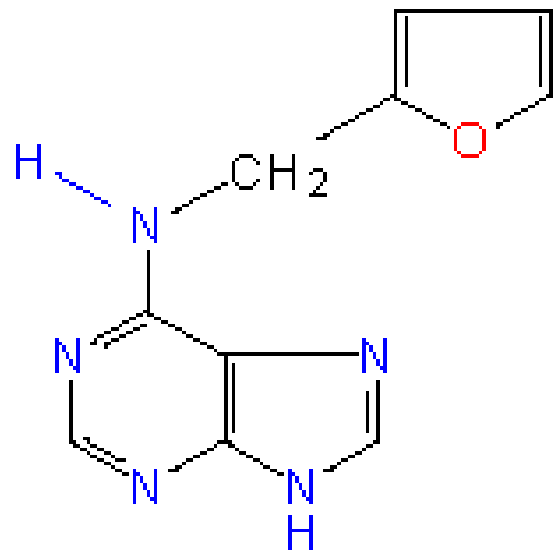
CYTOKININ – what is important?

Synthesis – *IPT* genes

Degradation – CK-oxidase

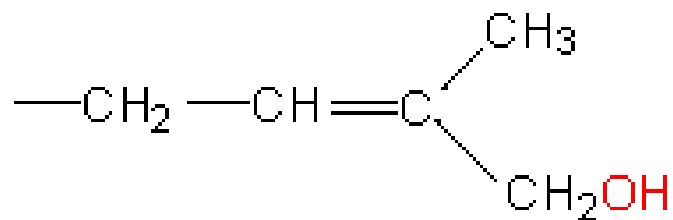
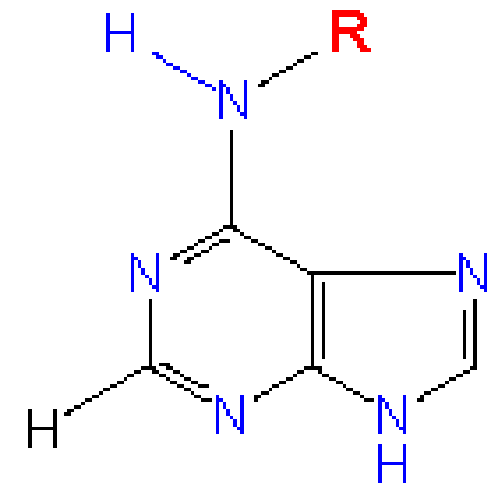
Signal transduction – forward genetics
activation tagging
CK regulated genes

Cytokinins

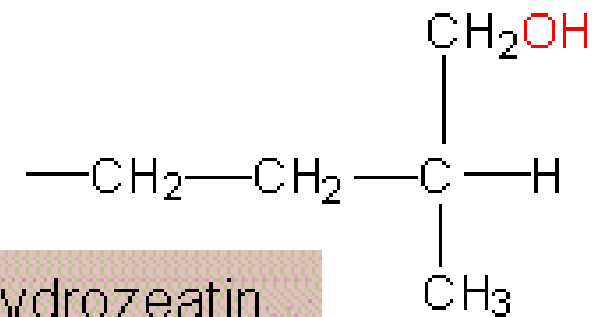


Kinetin:
6 - (2 - Furfuryl -
7 - Aminopurin)

Cytokinin
(Grundstruktur)

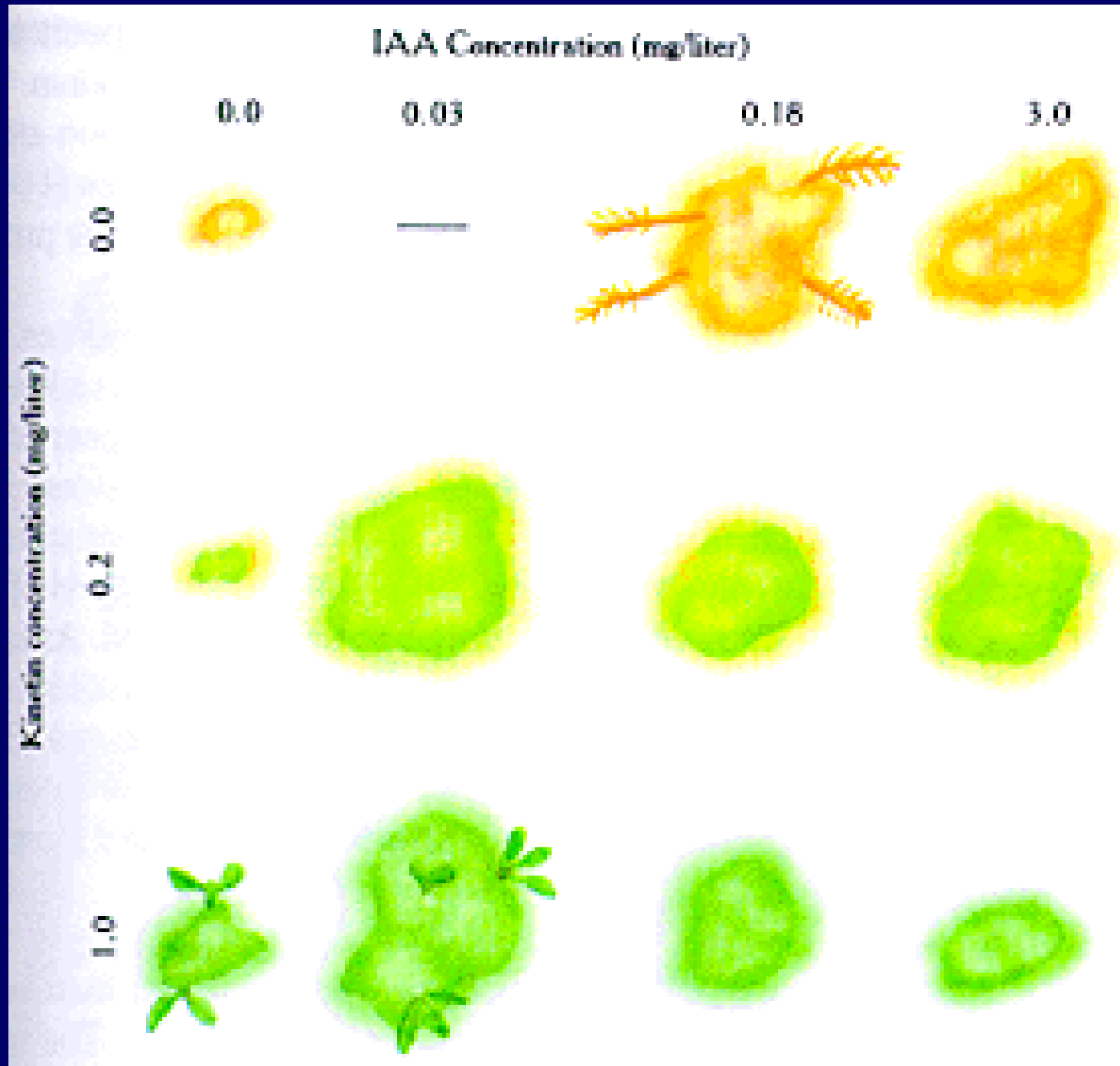


Zeatin

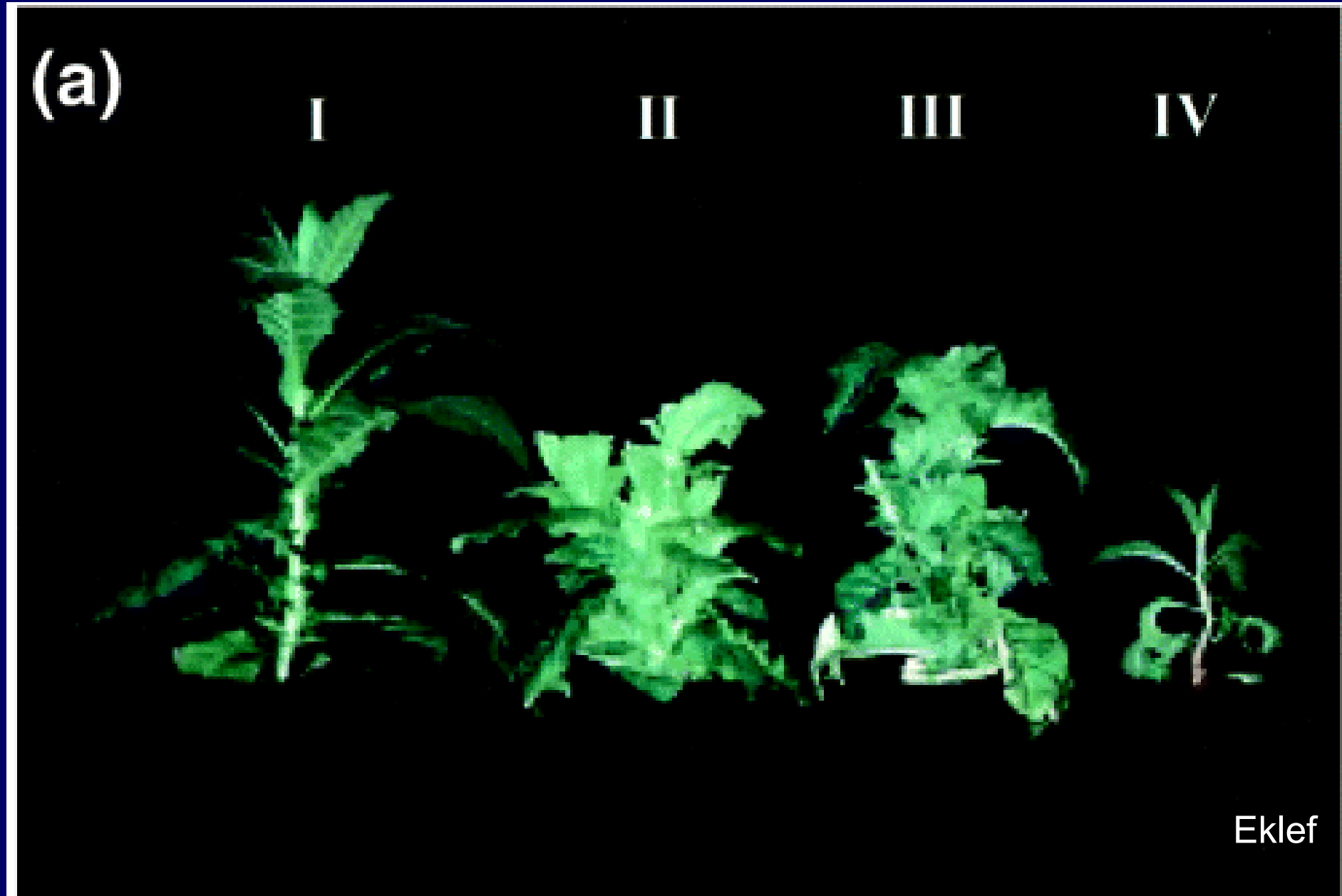


Dihydrozeatin

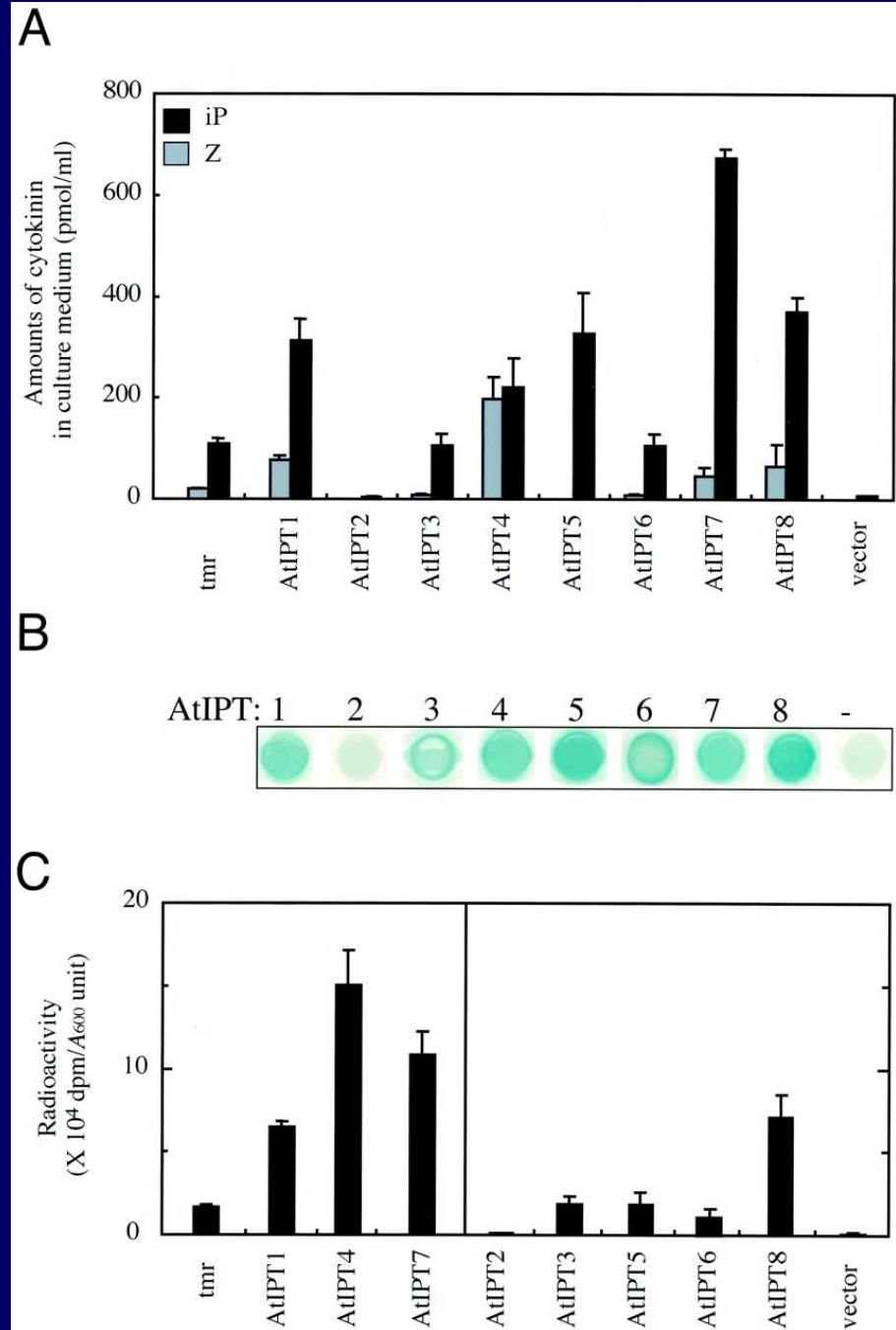
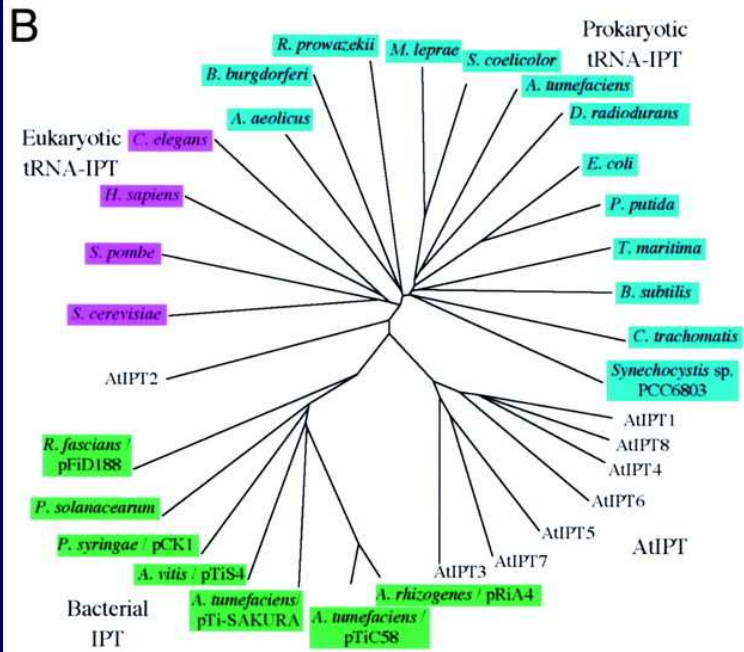
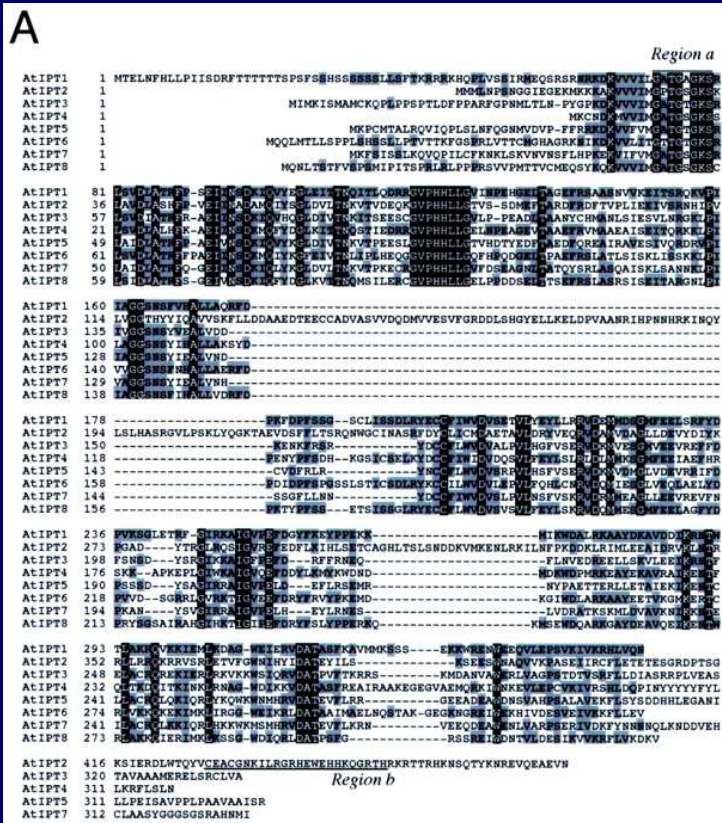
Effect of CK on regeneration



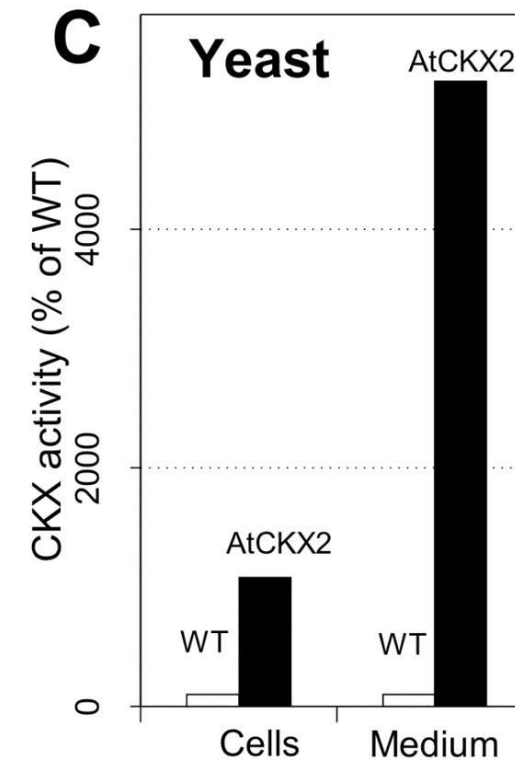
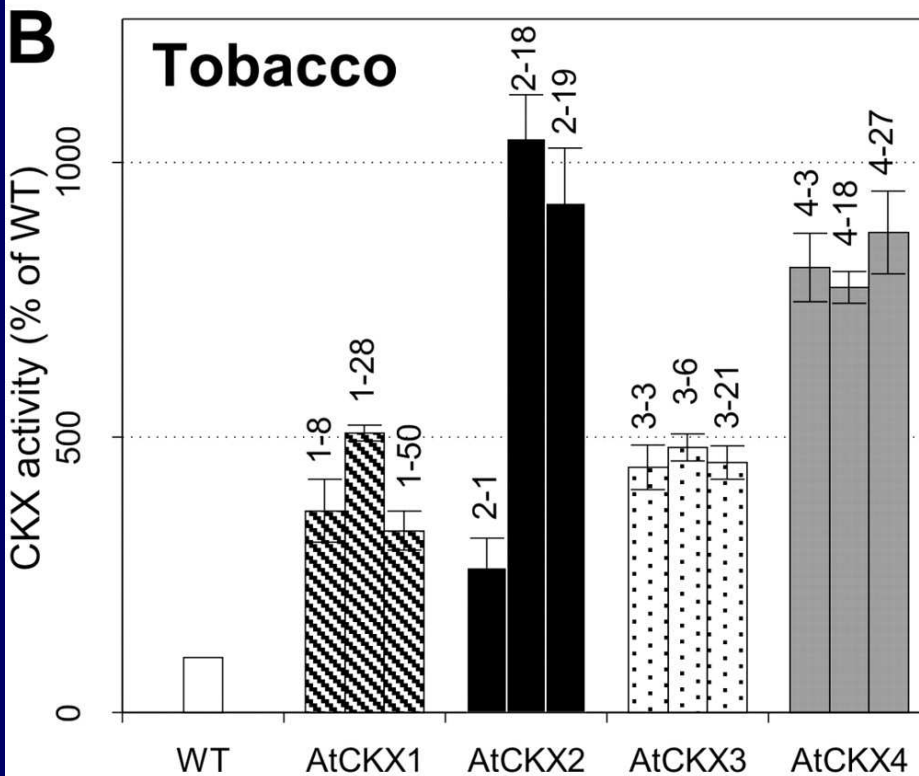
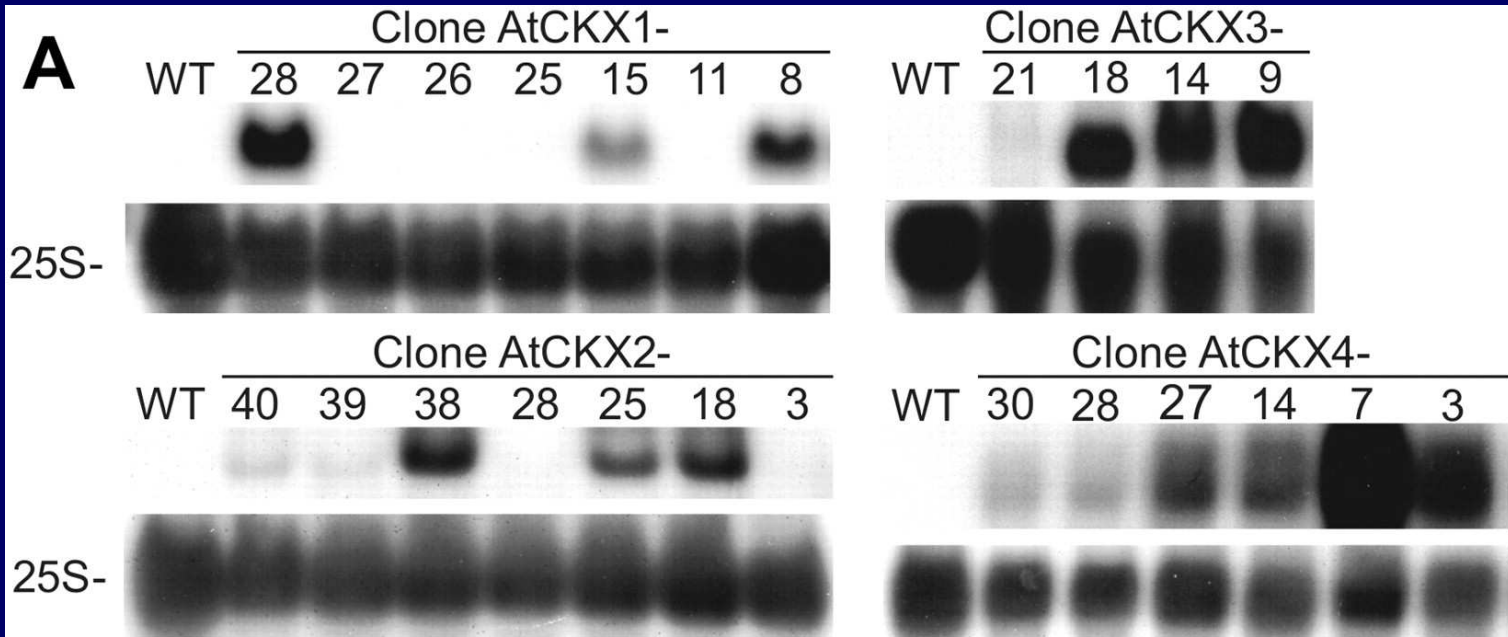
Manipulating of CK levels by overexpression of bacterial *IPT*



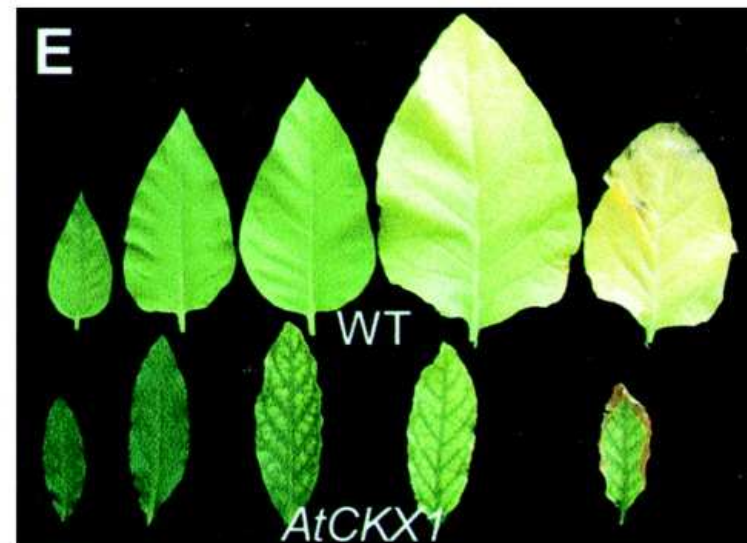
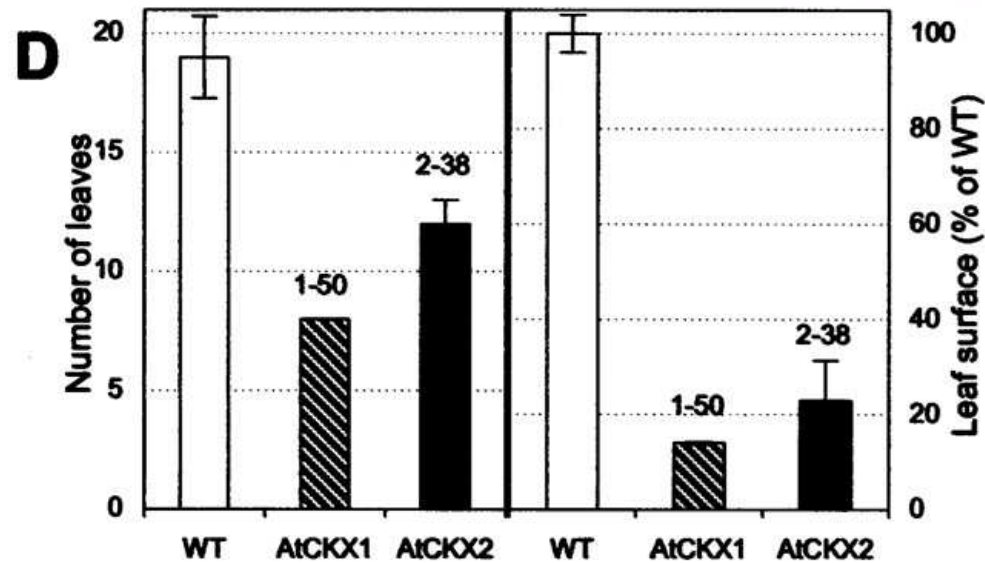
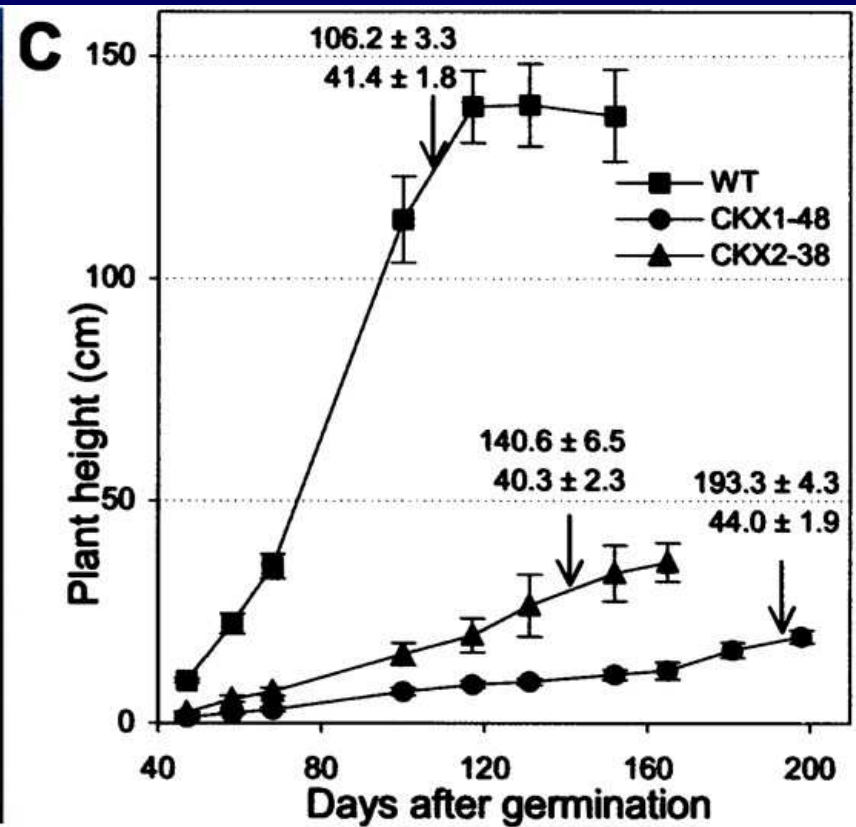
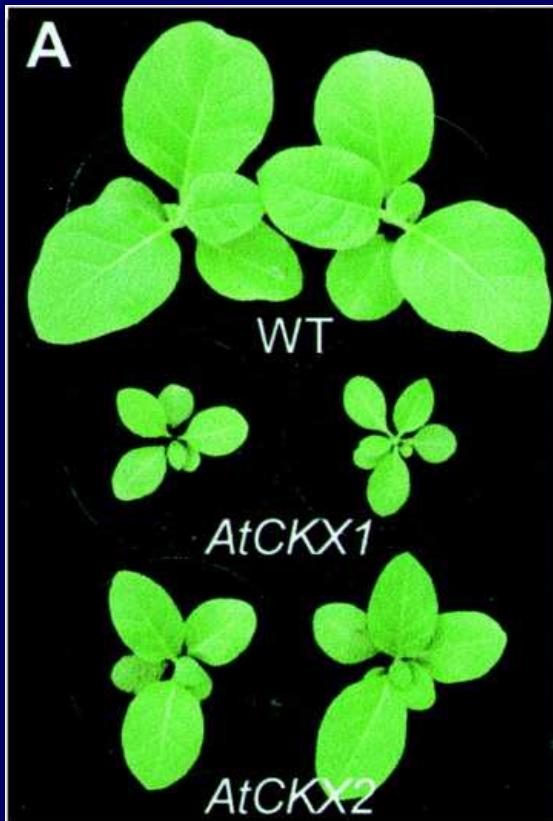
Isolation of Arabidopsis IPTs



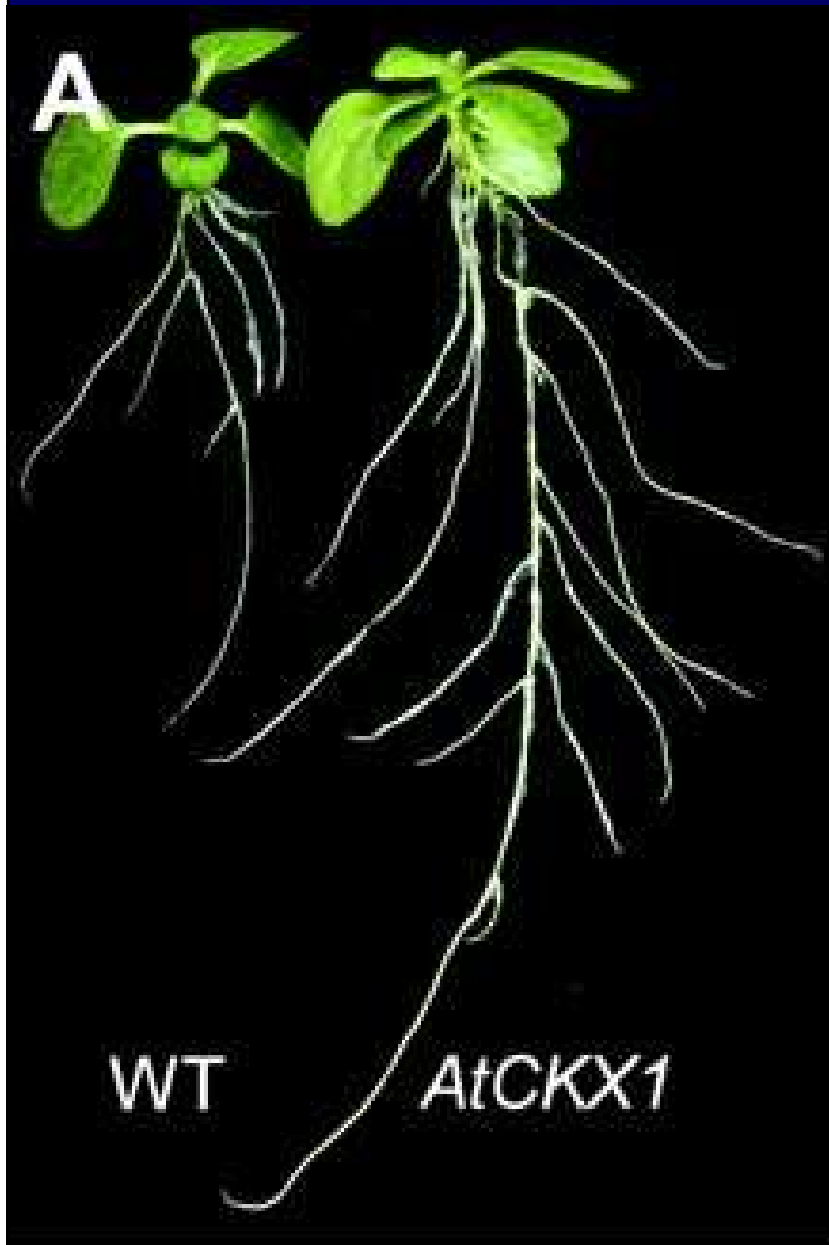
Isolation of CK-oxidase (AtCKX)



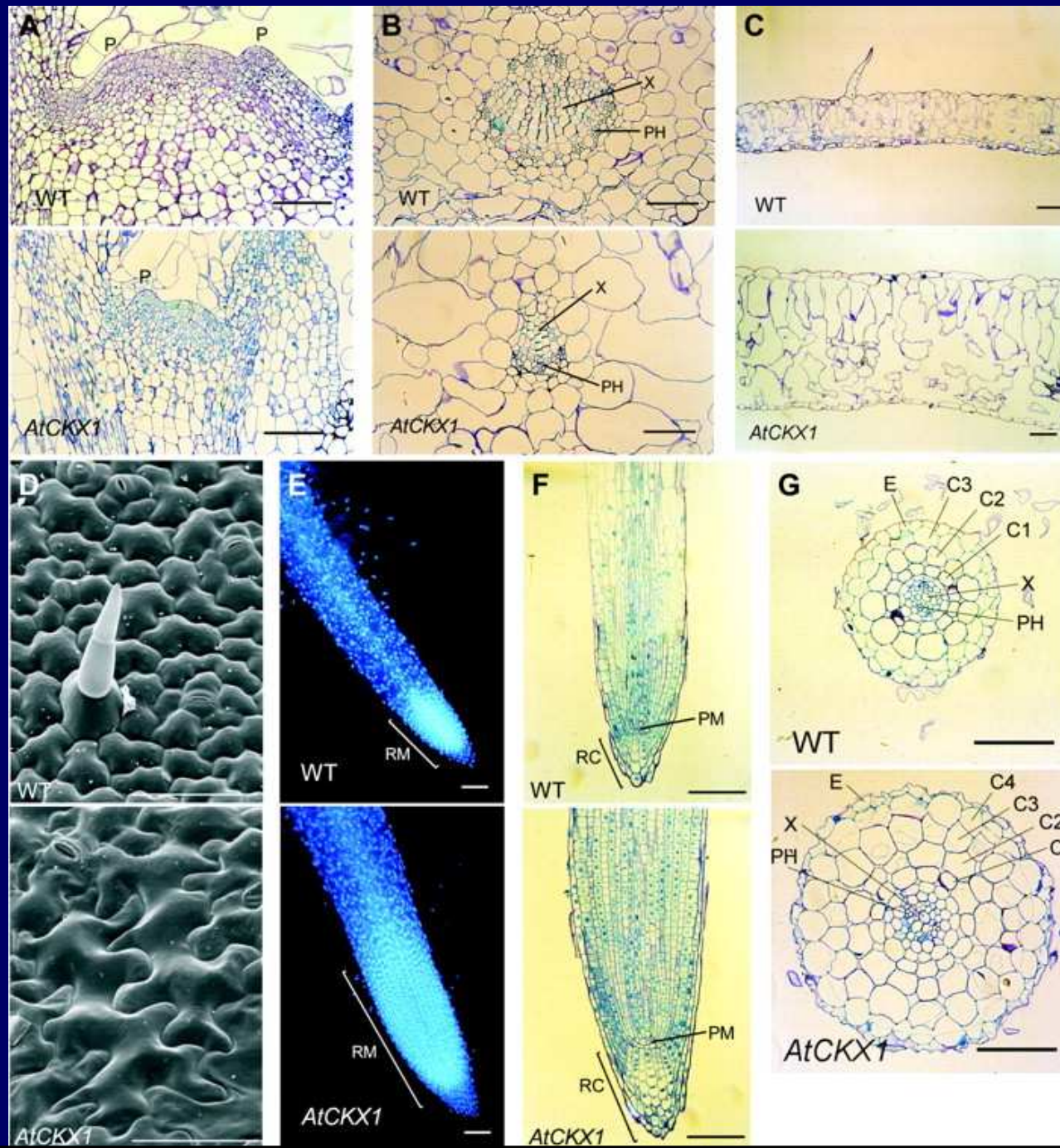
AtCKXs overexpression in tobacco



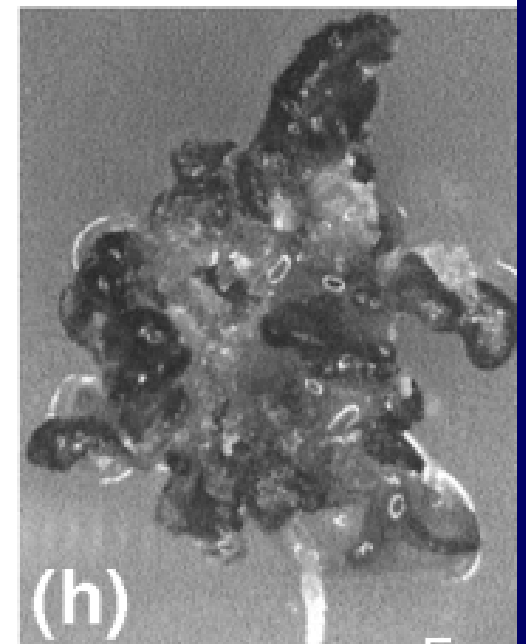
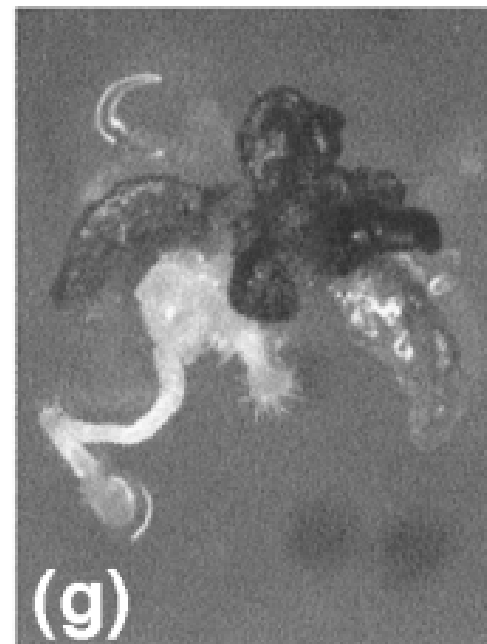
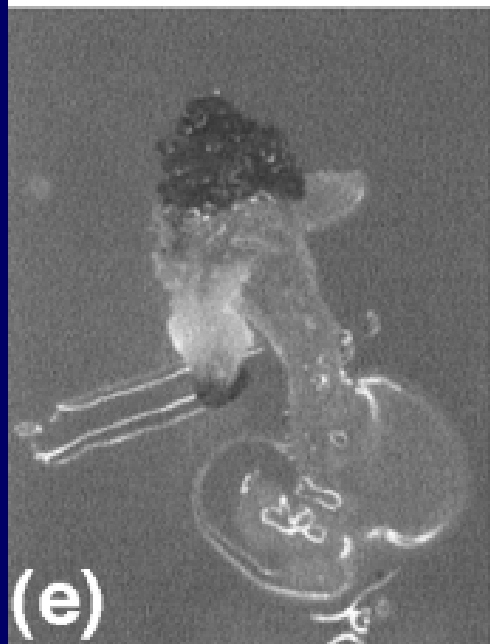
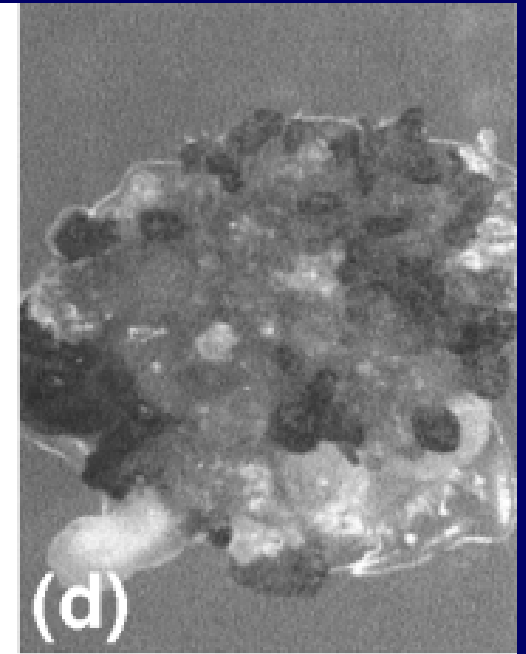
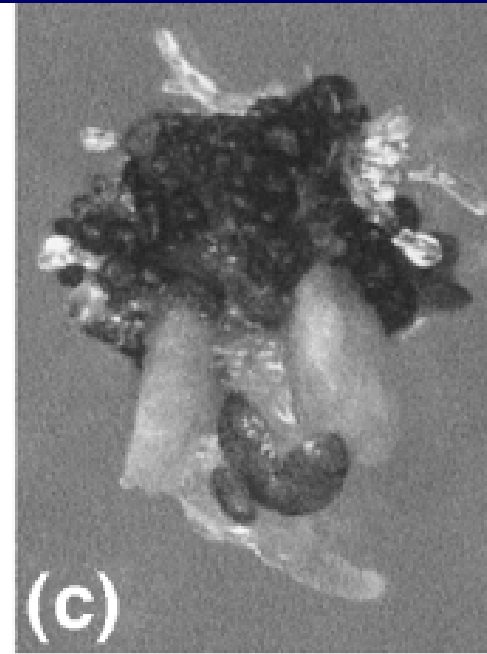
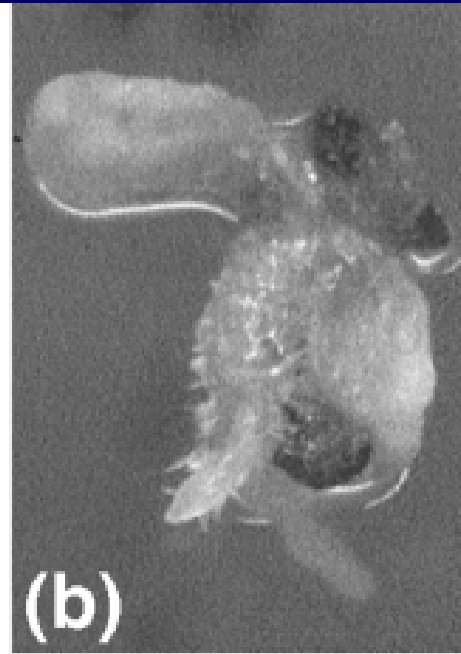
Effect of AtCKX on tobacco root



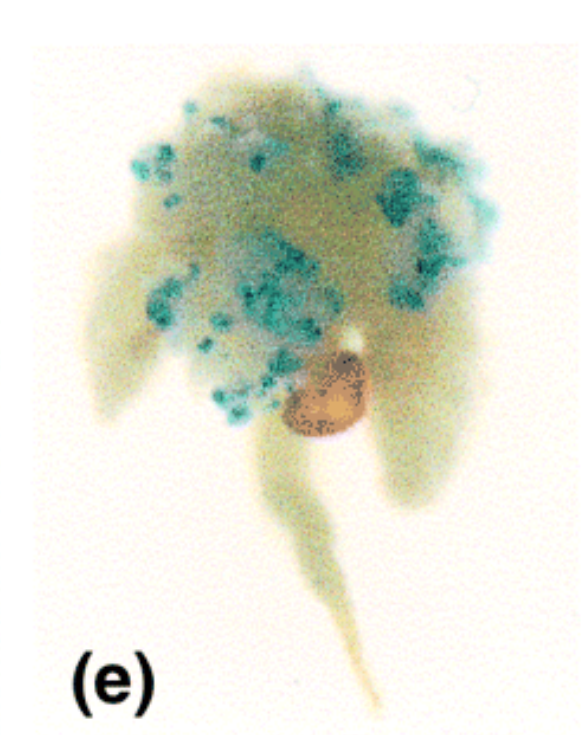
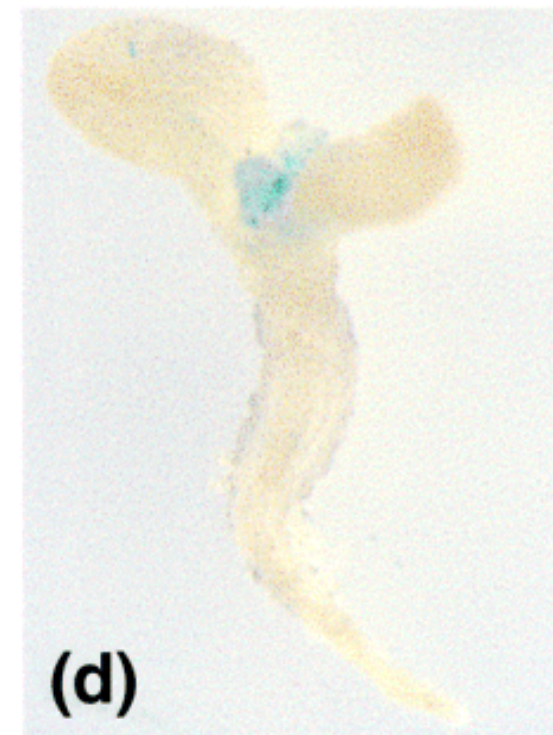
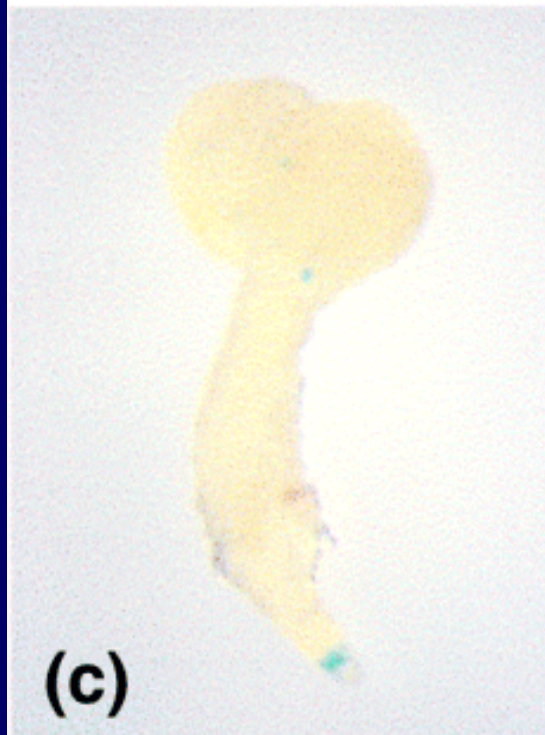
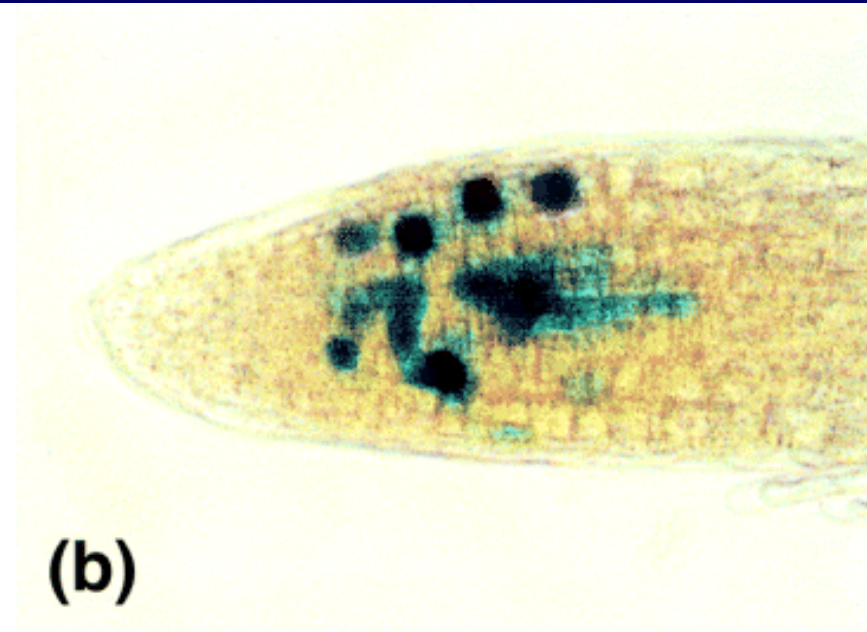
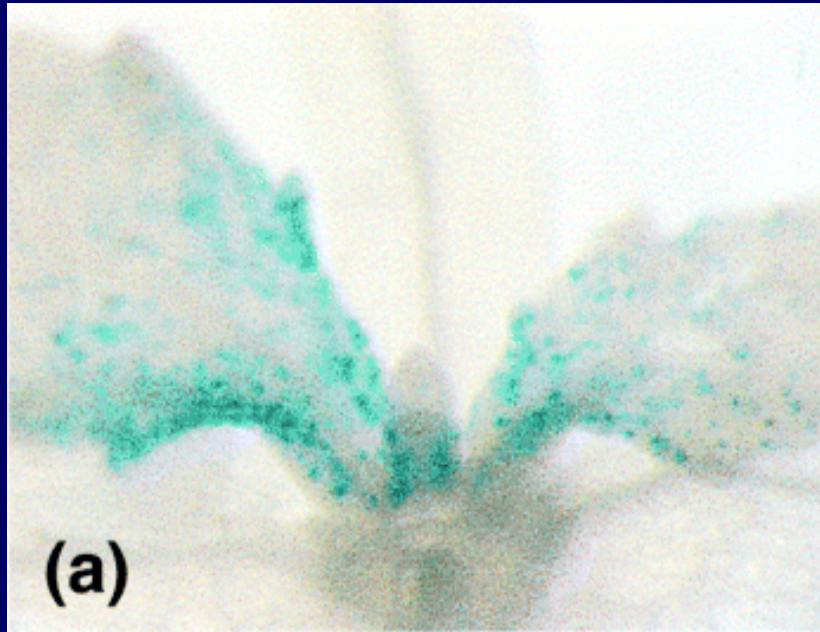
Morphology of AtCKX tobacco plants



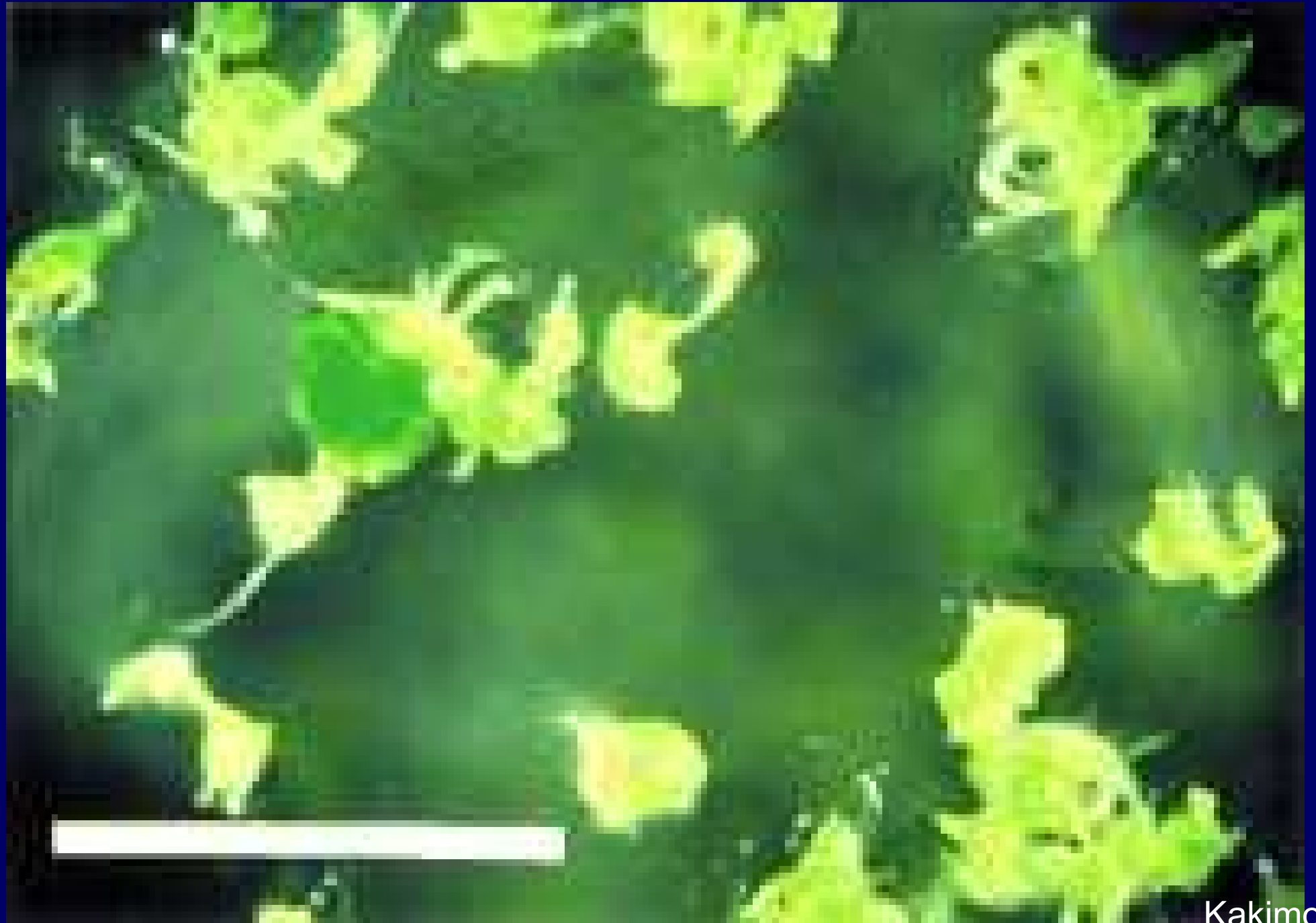
Tumor shoot development (*tsd*) and *pasticcino* (*pas*) mutants



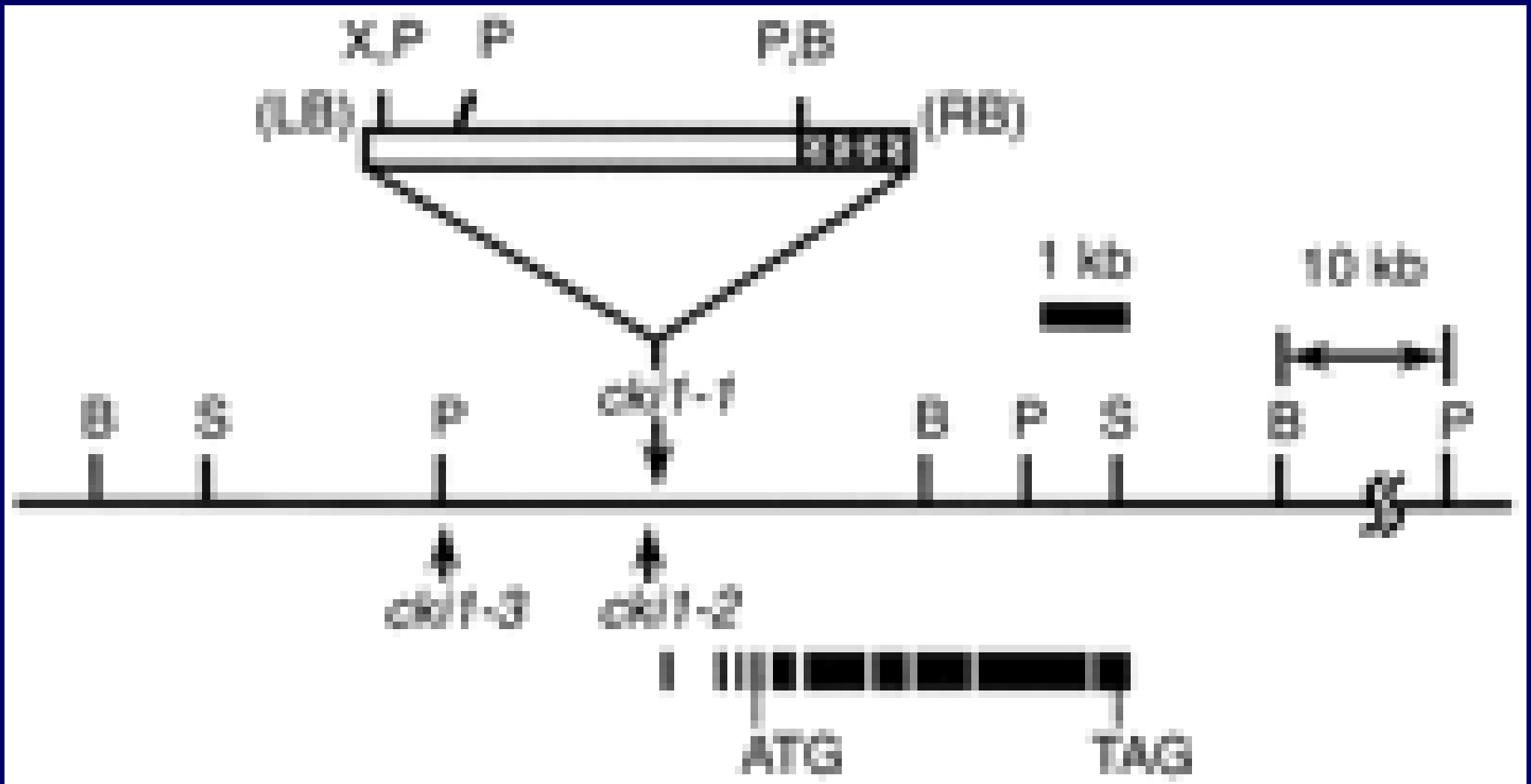
Use of *CyclinB::GUS* for cell division monitoring



Isolation of CK independent (*cki1*) mutant



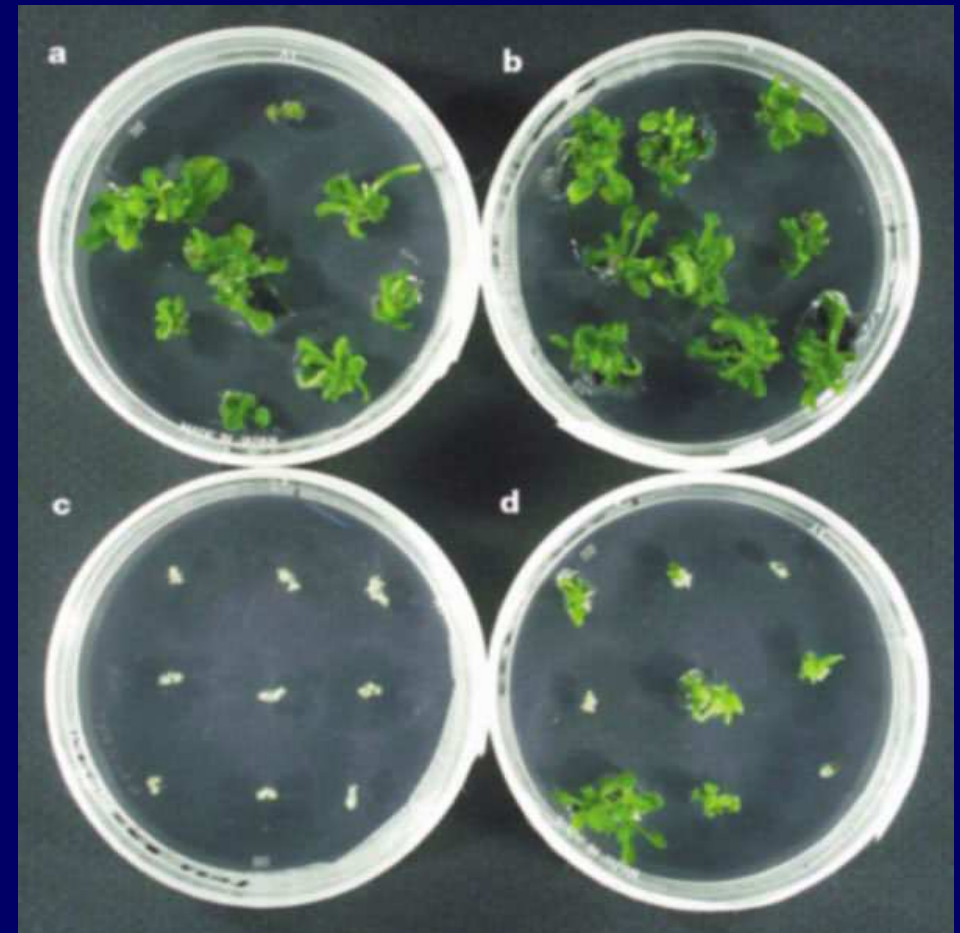
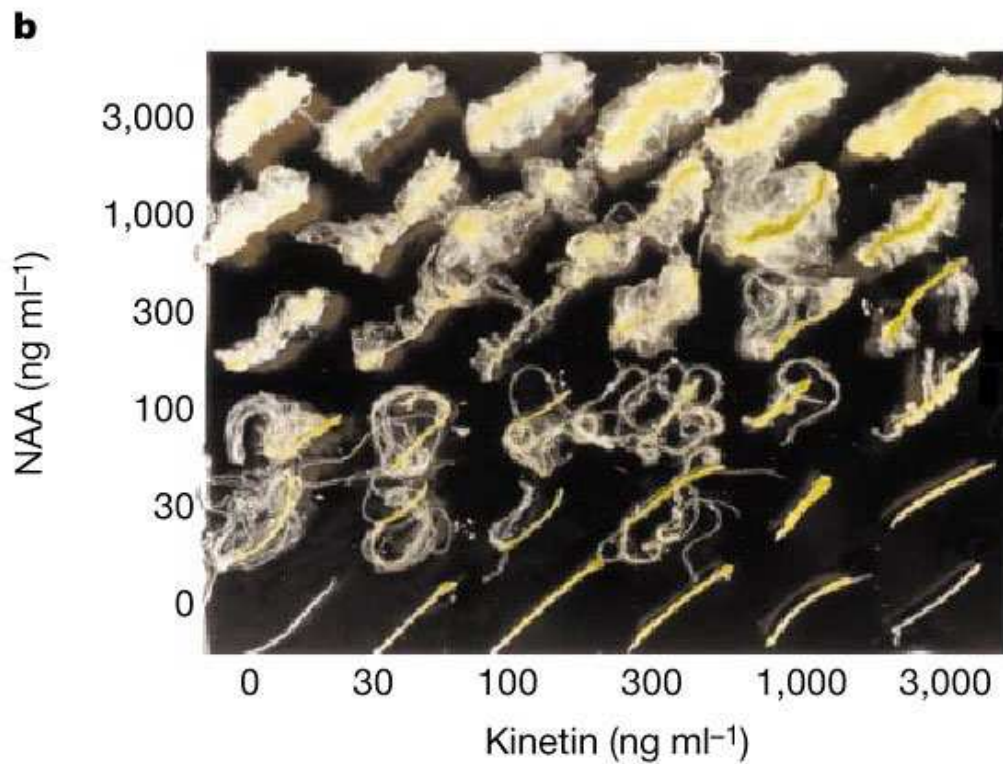
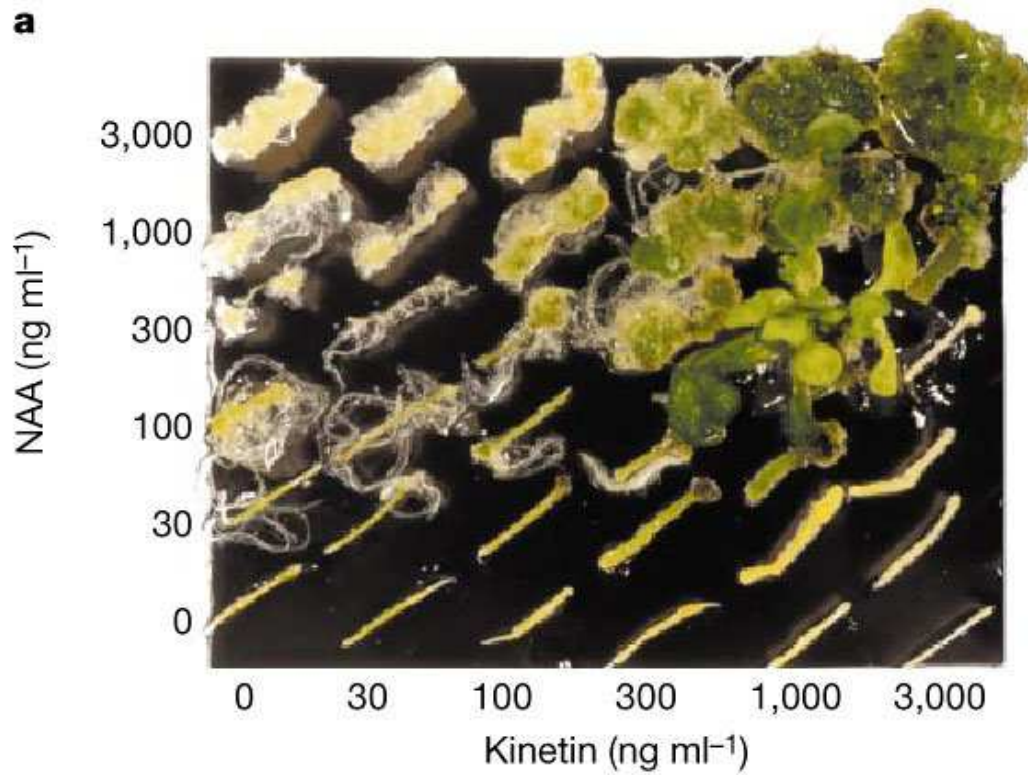
Identification of *CKI1* gene



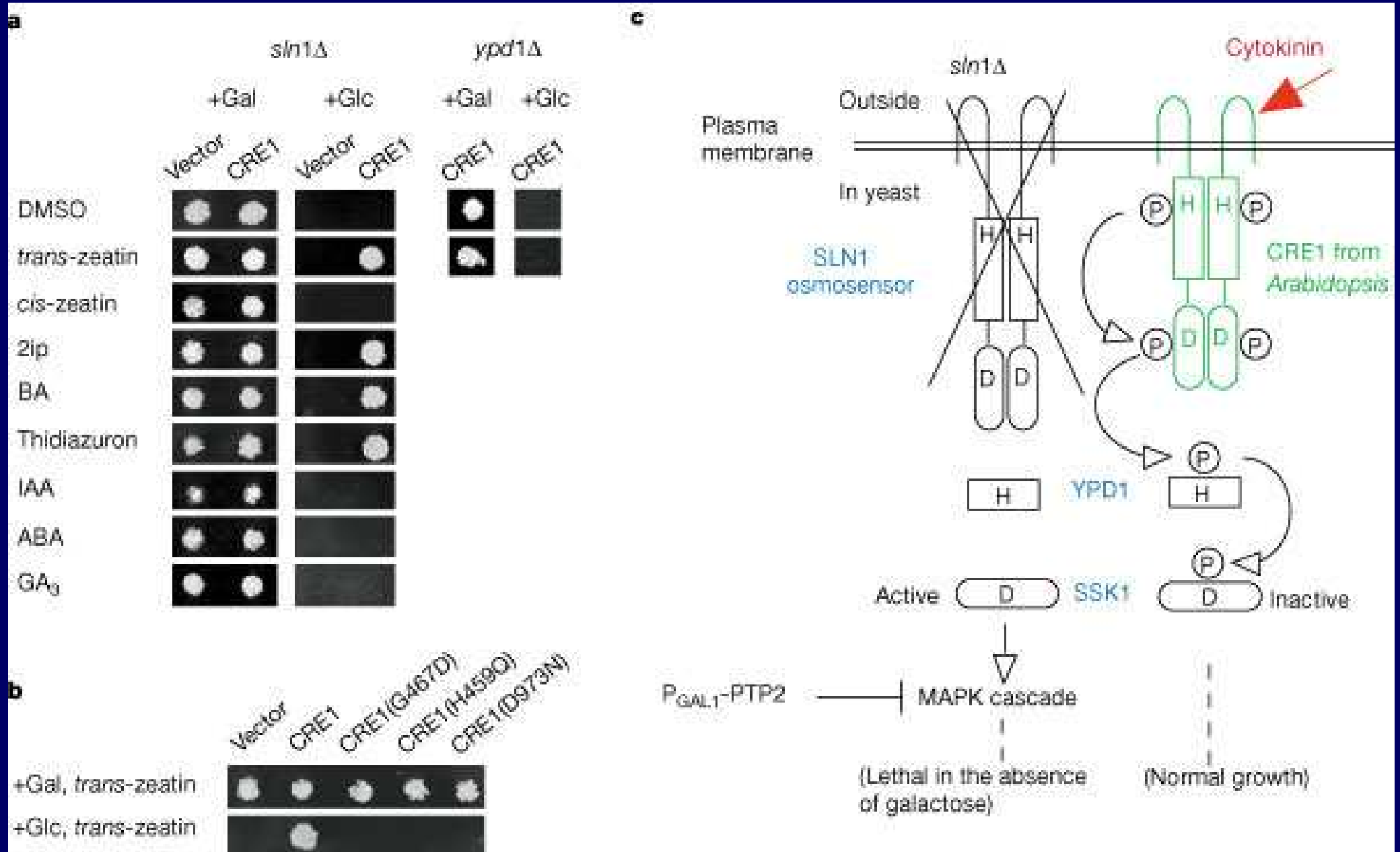
Verification - *35S::CKI1* transgene



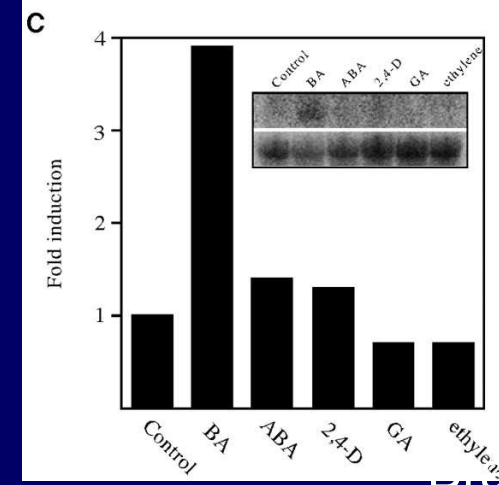
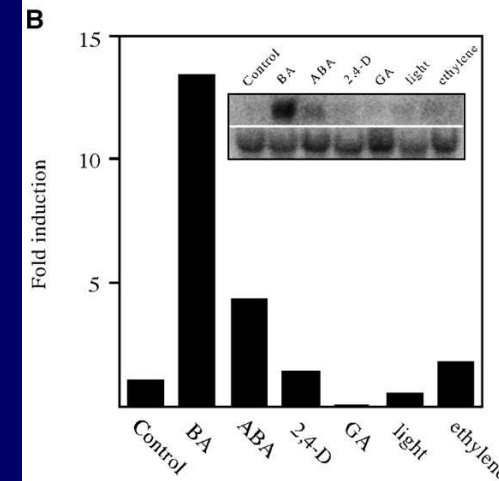
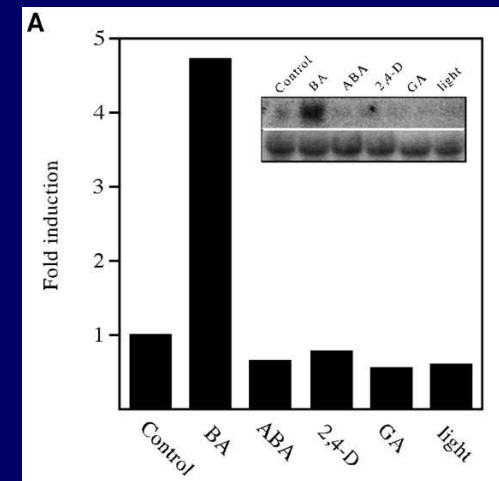
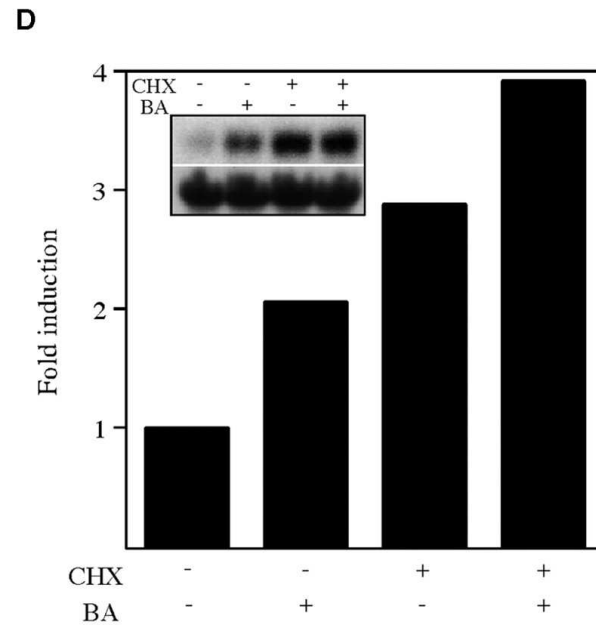
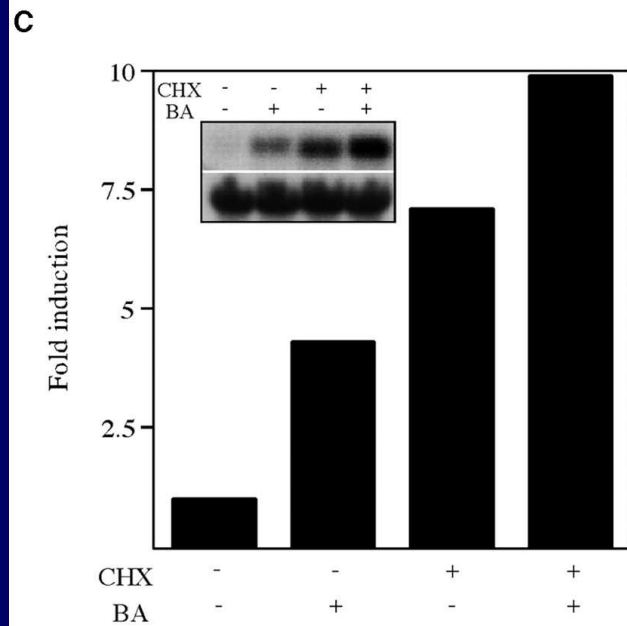
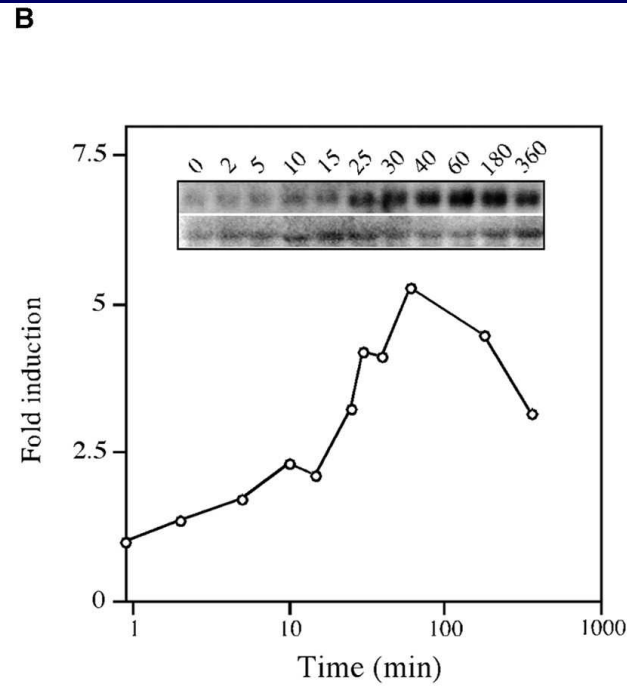
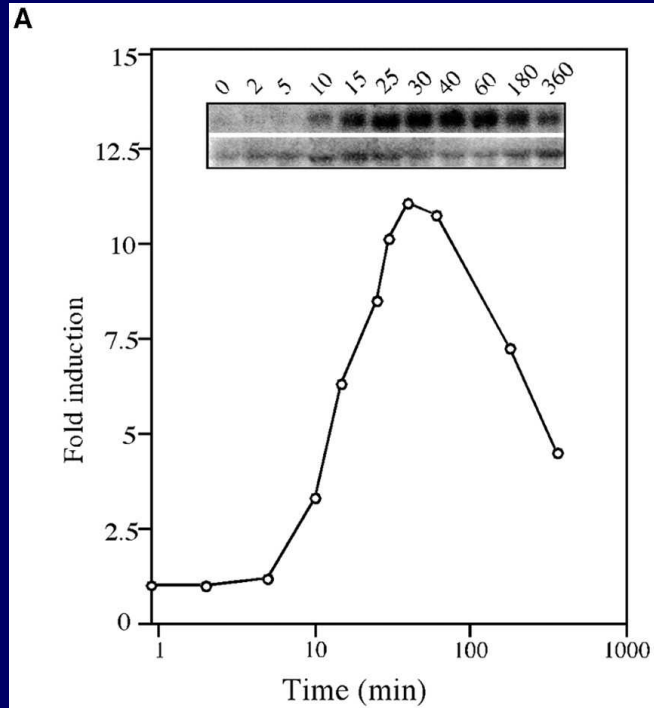
Next strike - CK response mutant (*cre1*)



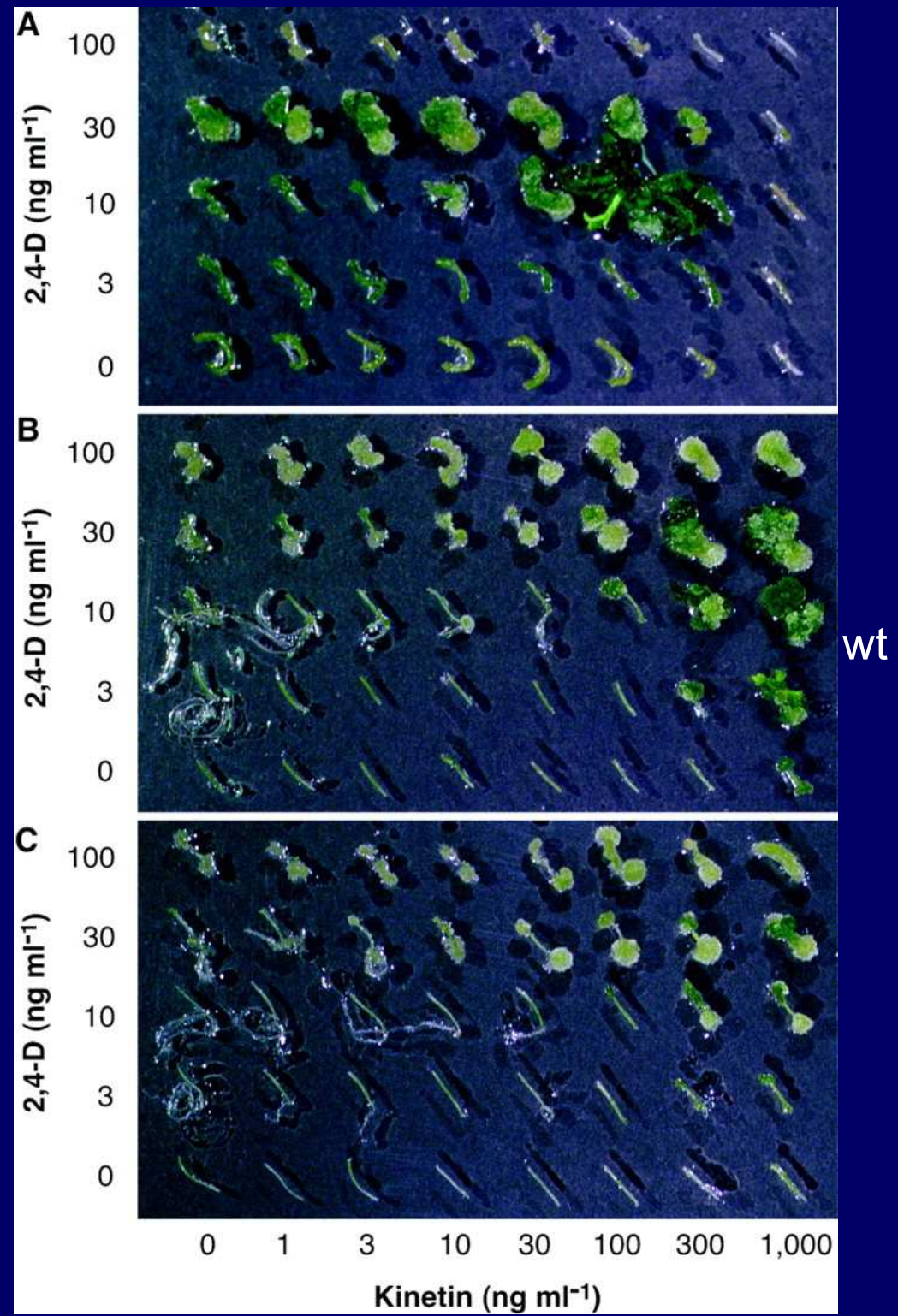
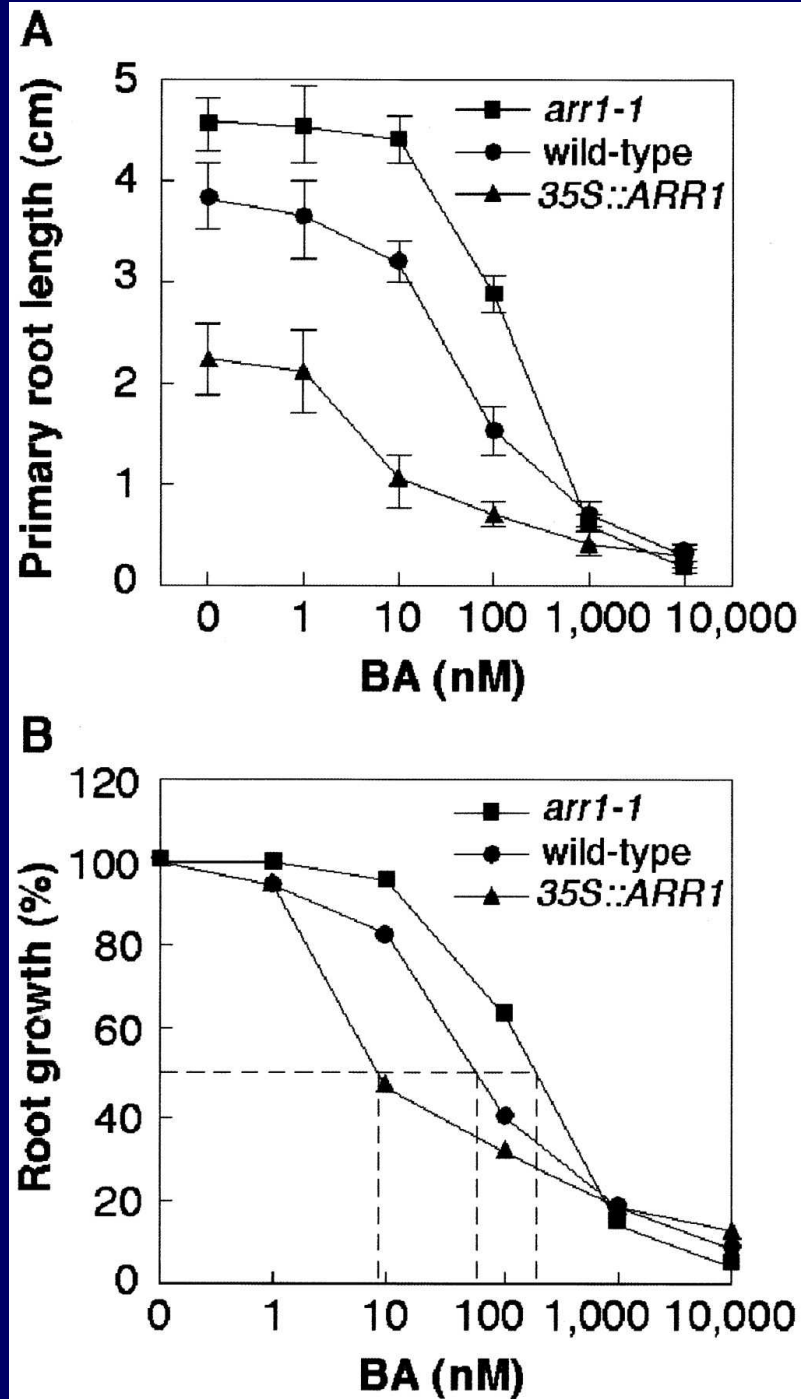
Piece of genius - complementation



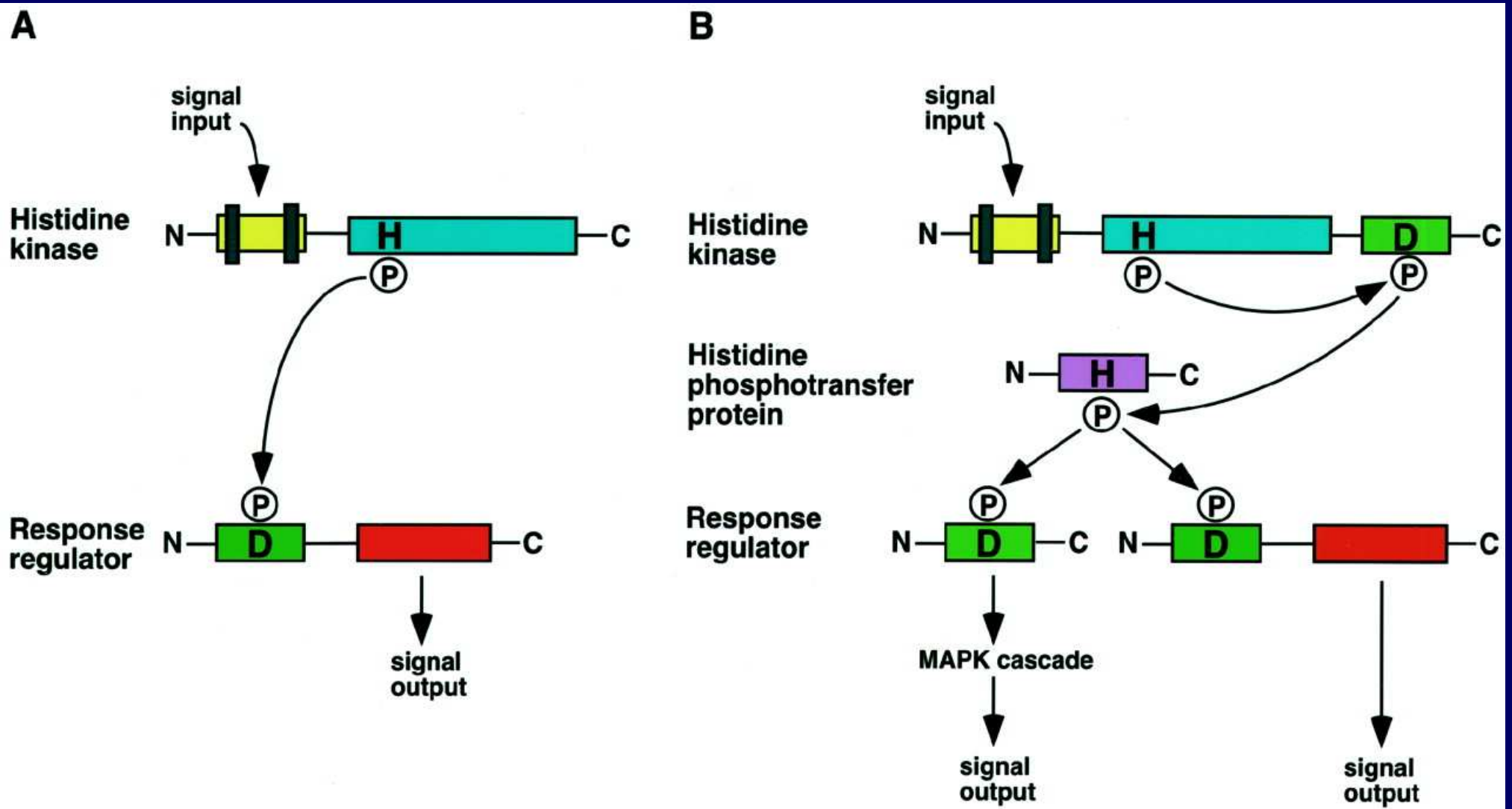
CK responsive genes - ARR1



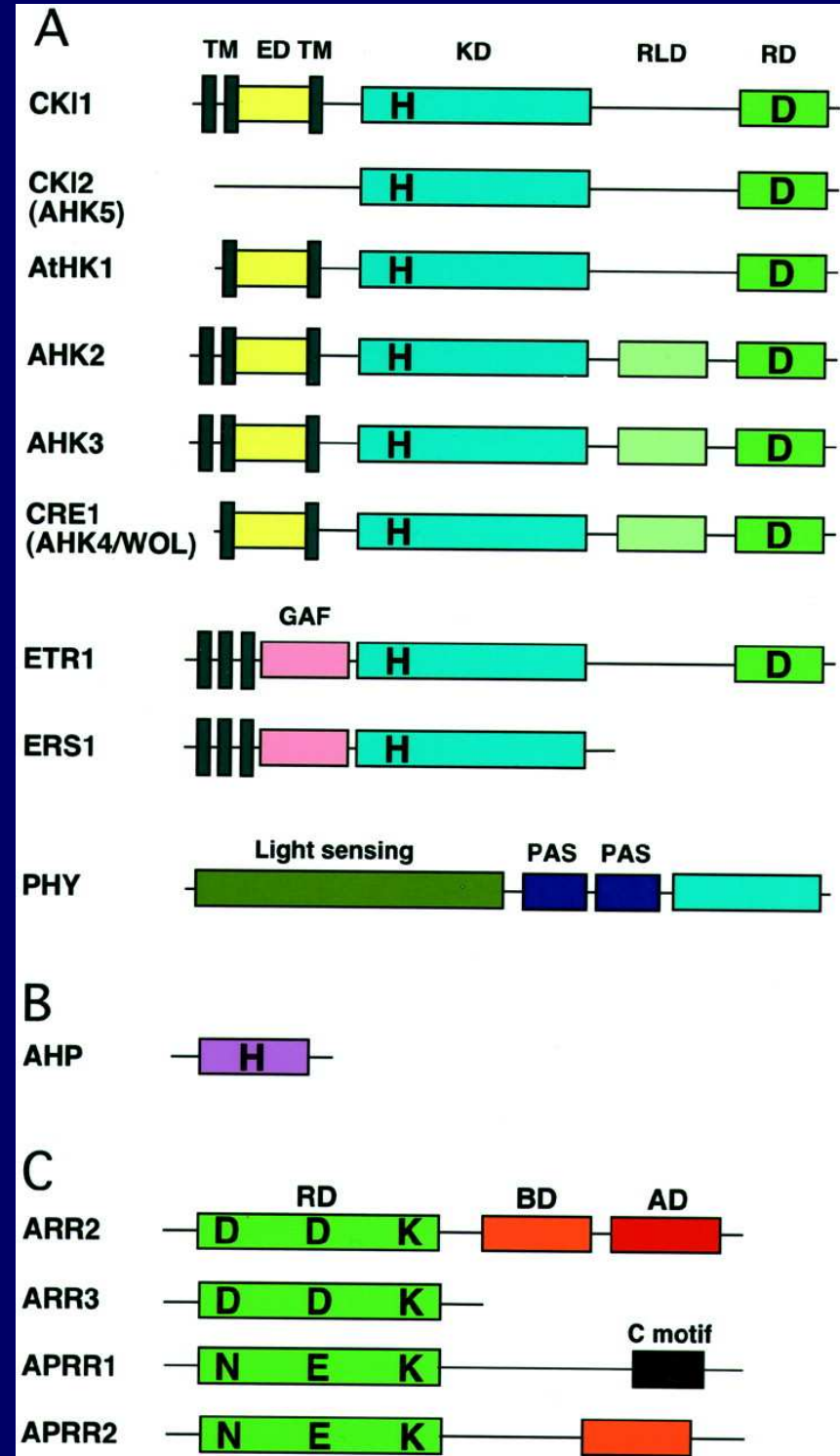
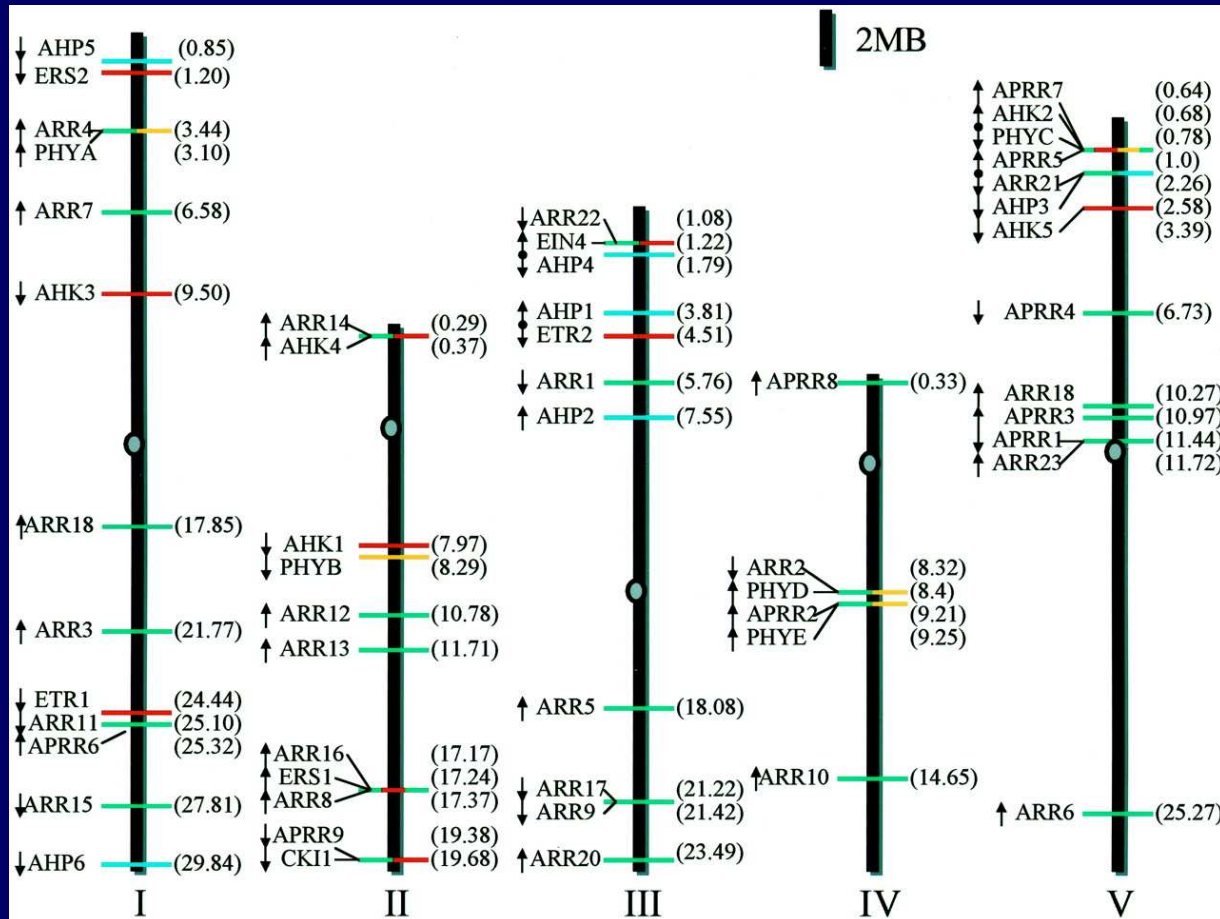
Phenotypes of *arrs*



His kinase transduction pathway

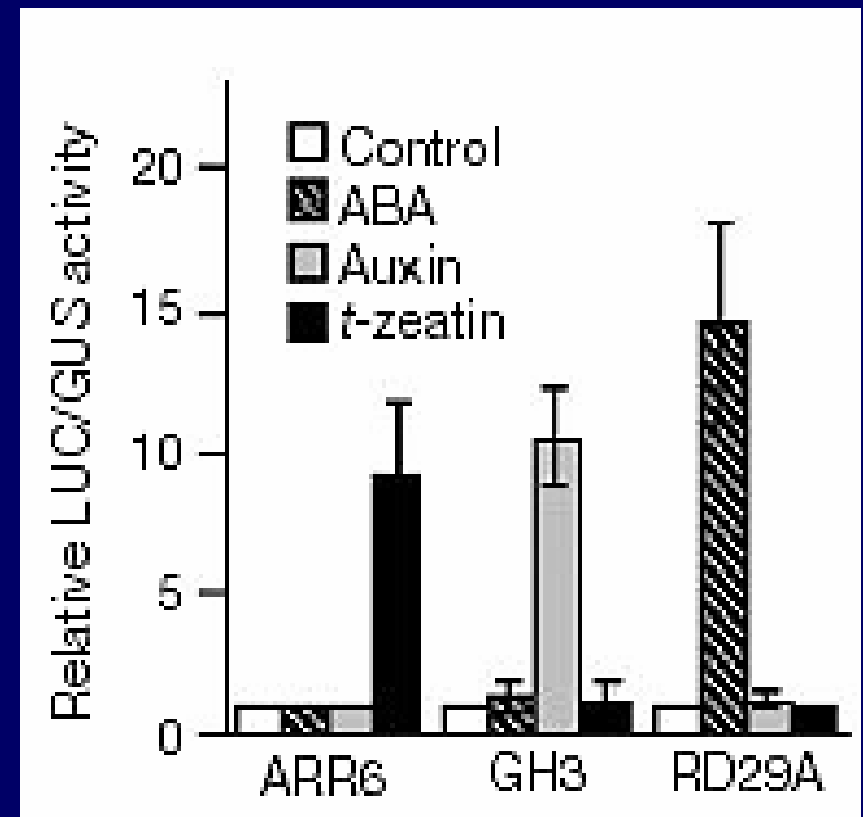
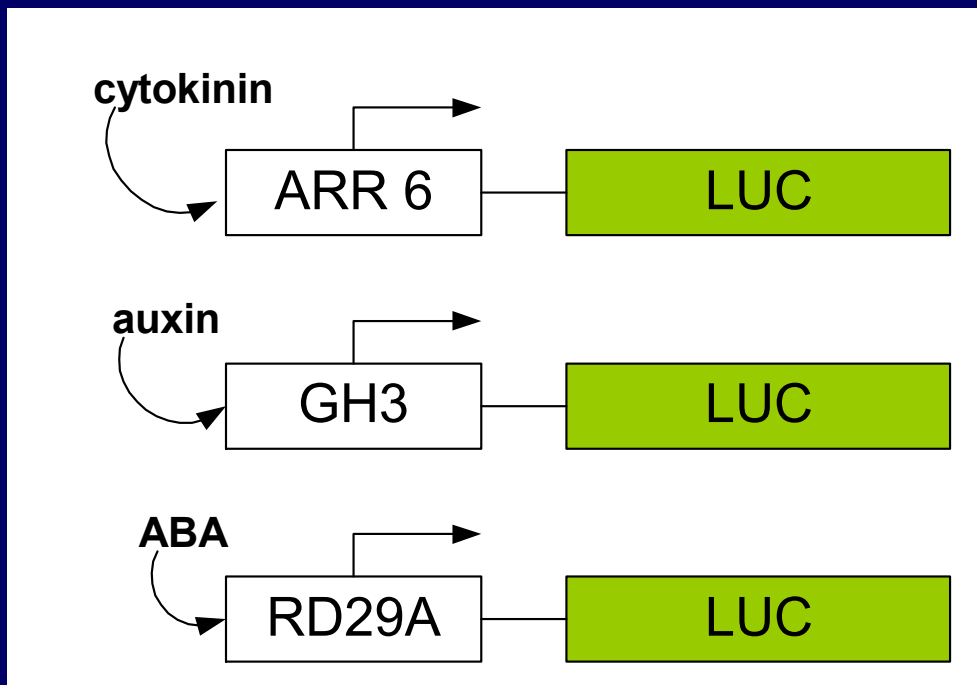


His kinase pathway components in Arabidopsis

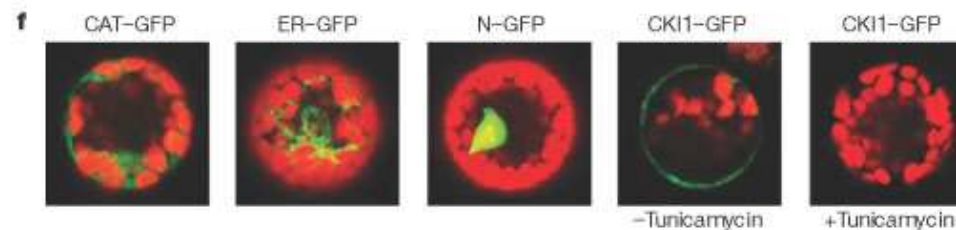
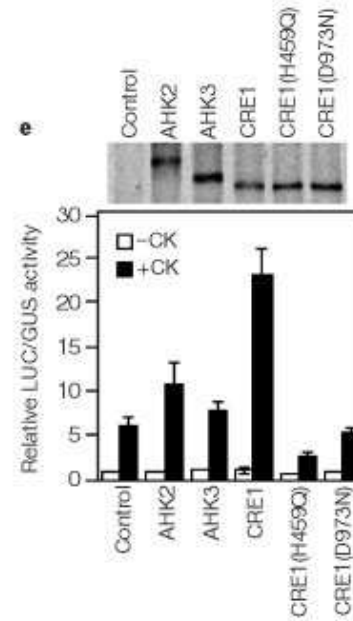
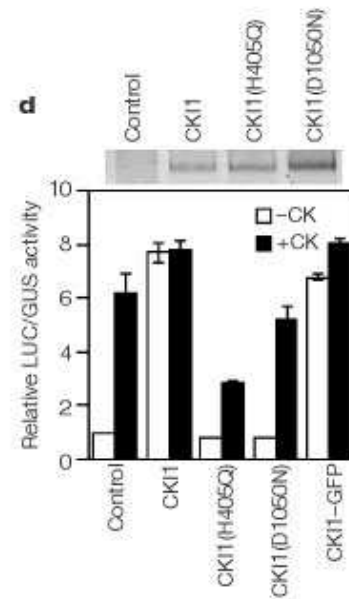
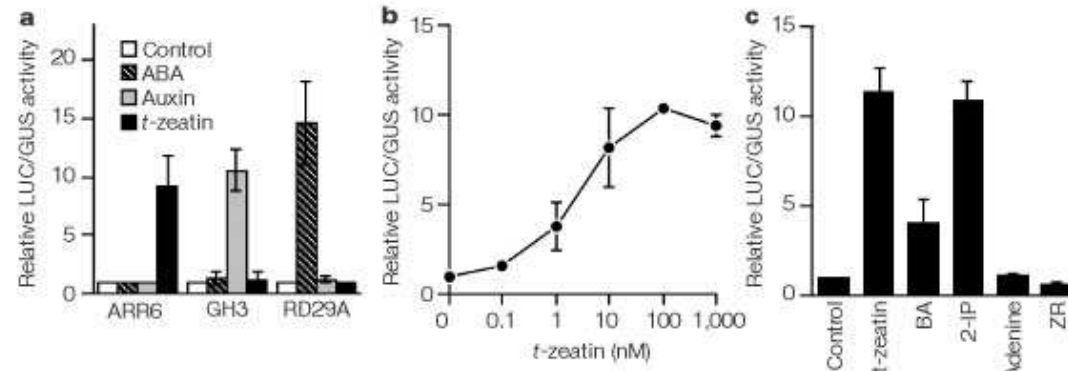


Games with protoplasts

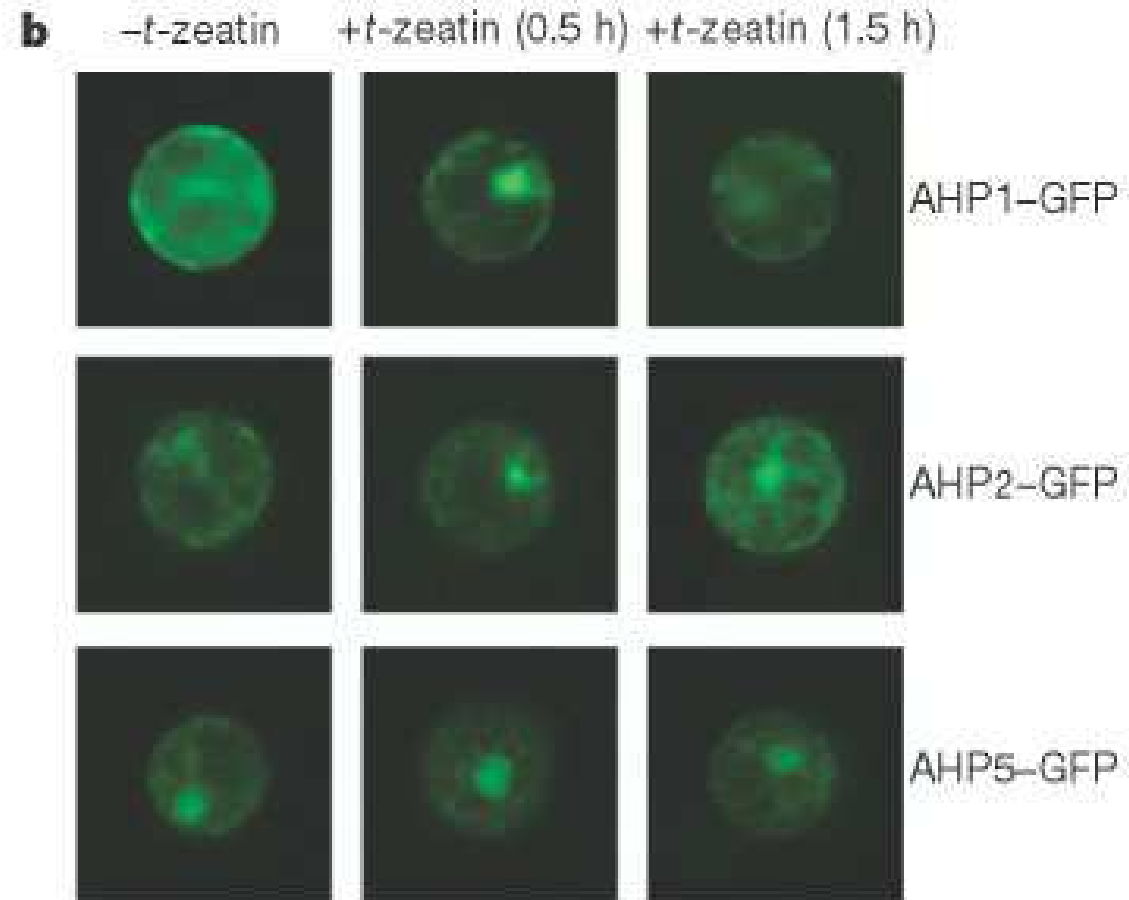
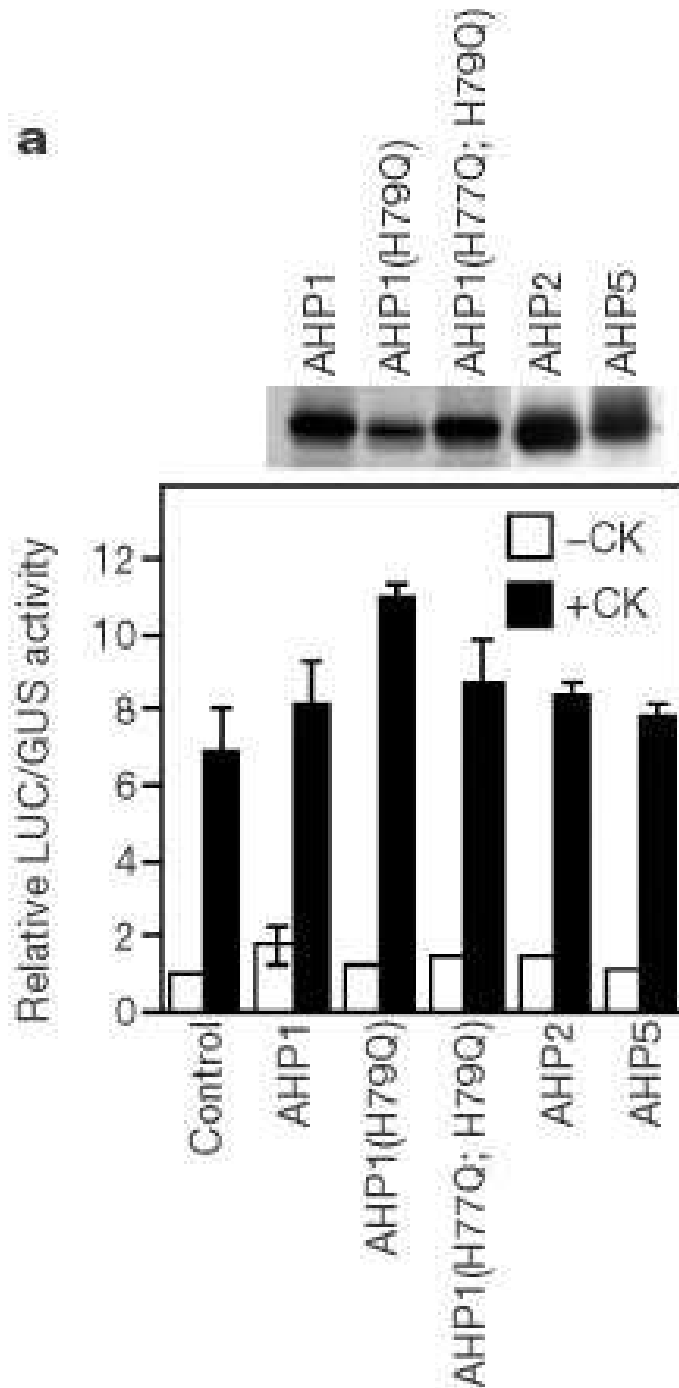
- Reporters for different pathways



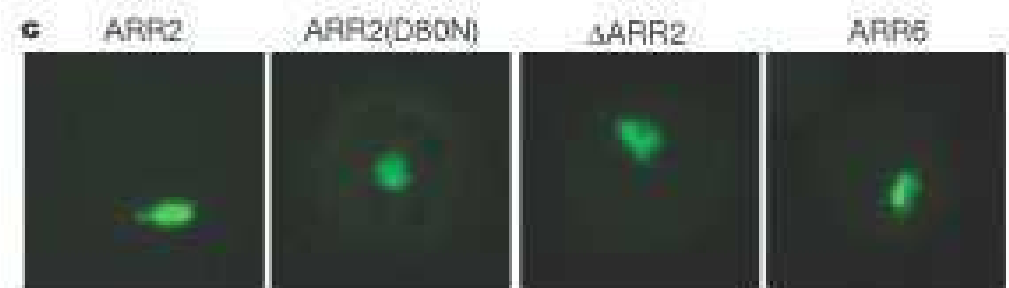
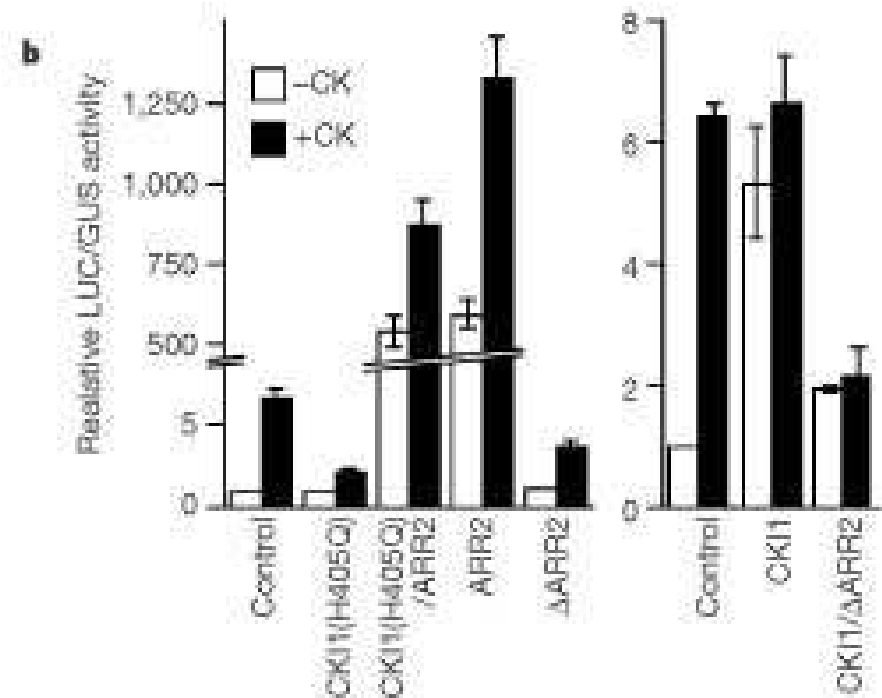
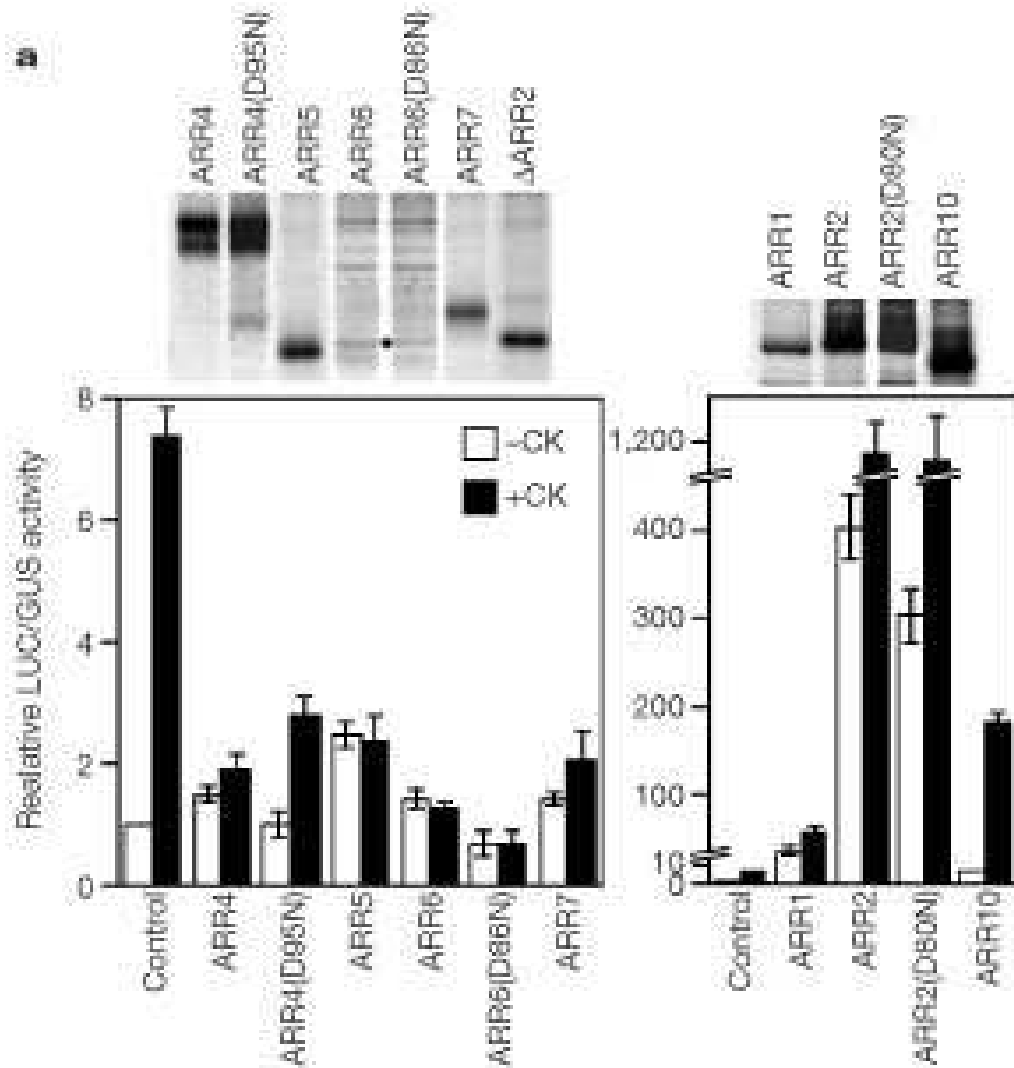
Games with protoplasts



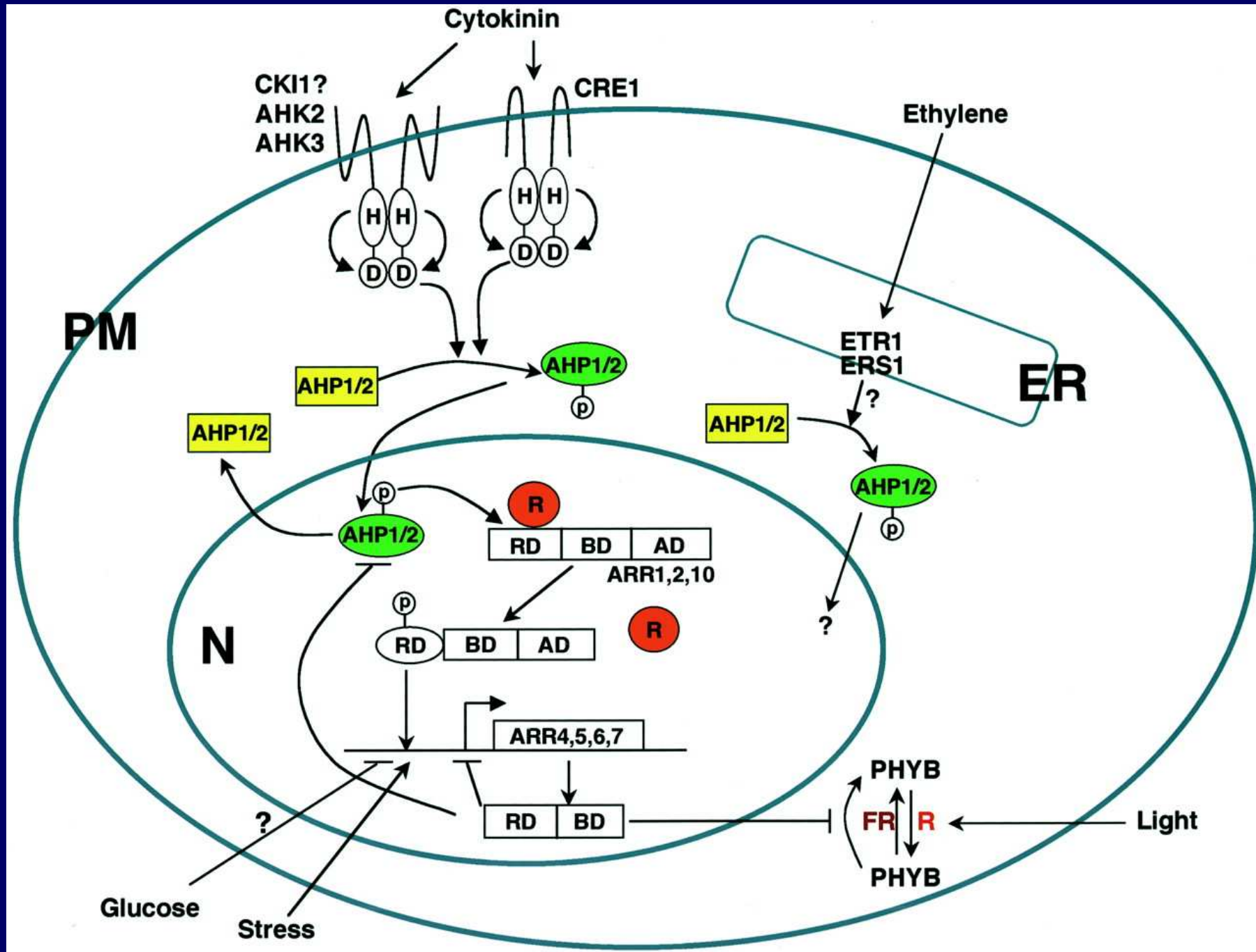
AHPs – shuttle to nucleus



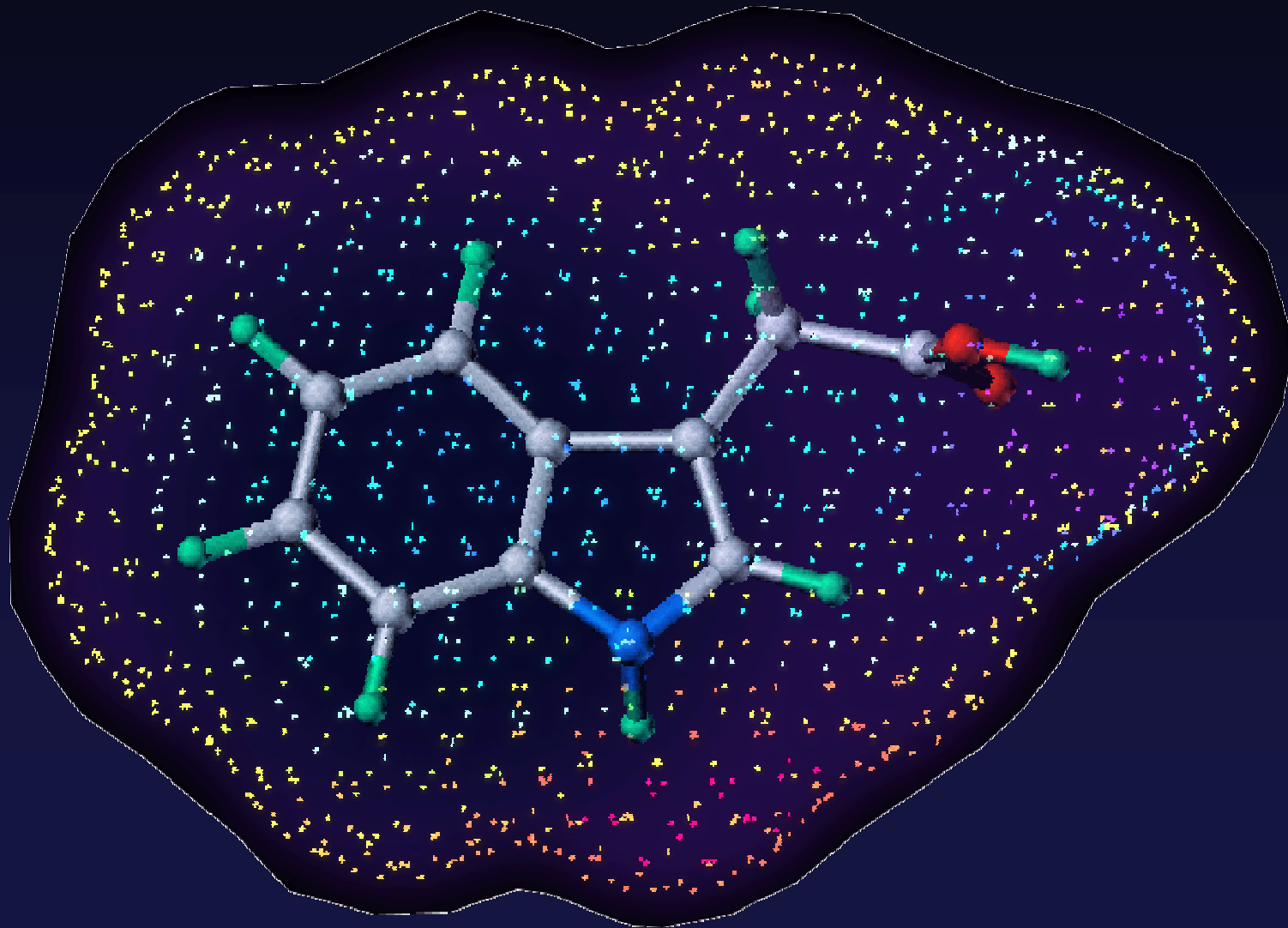
Opposite effects of two classes of ARR2s on CK signalling



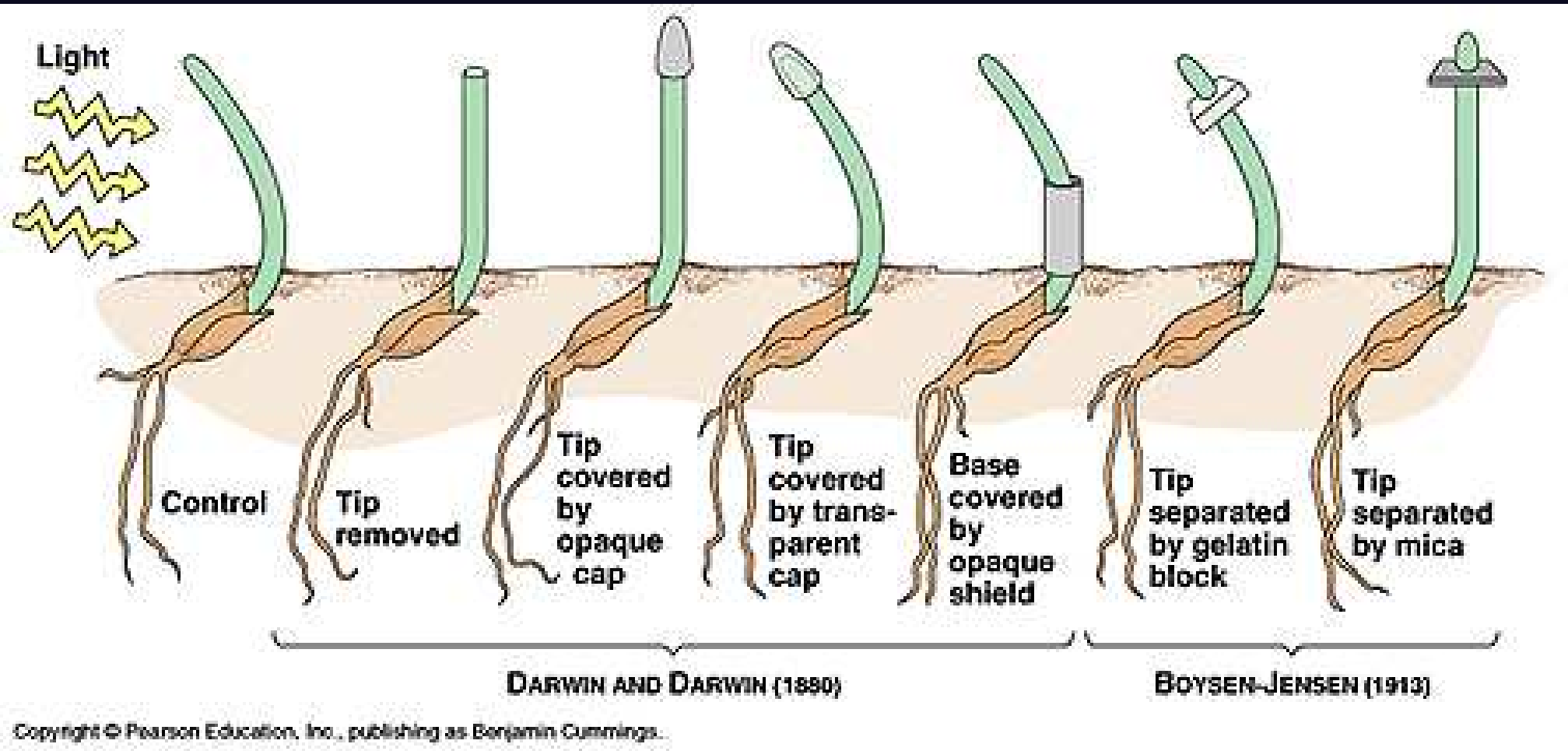
Games with protoplasts



Auxin Signaling and Transport

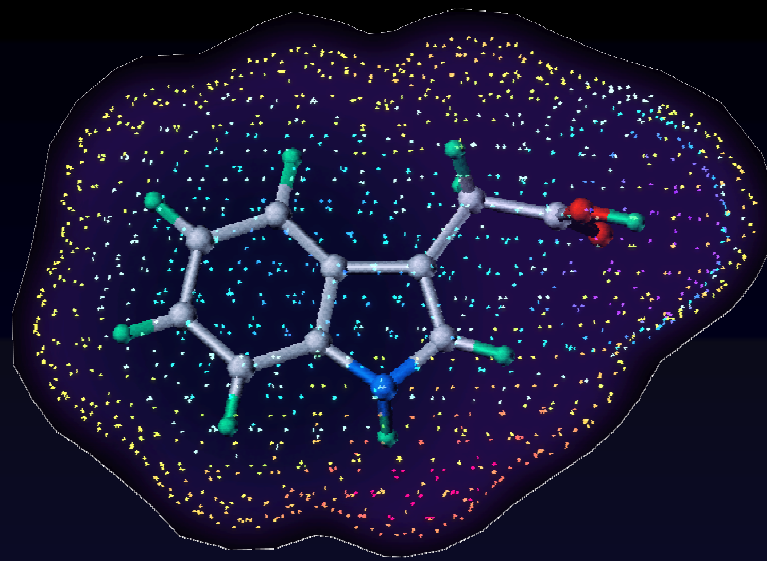


Discovery of the First Plant Signaling Molecule – Auxin and its Transport

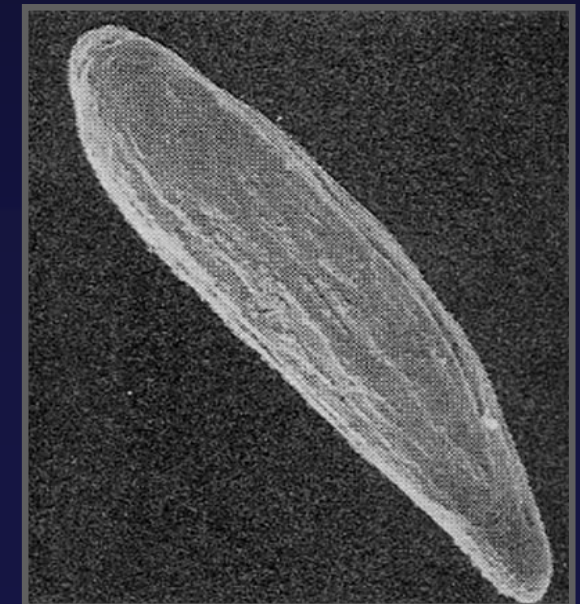


AUXIN

mediates

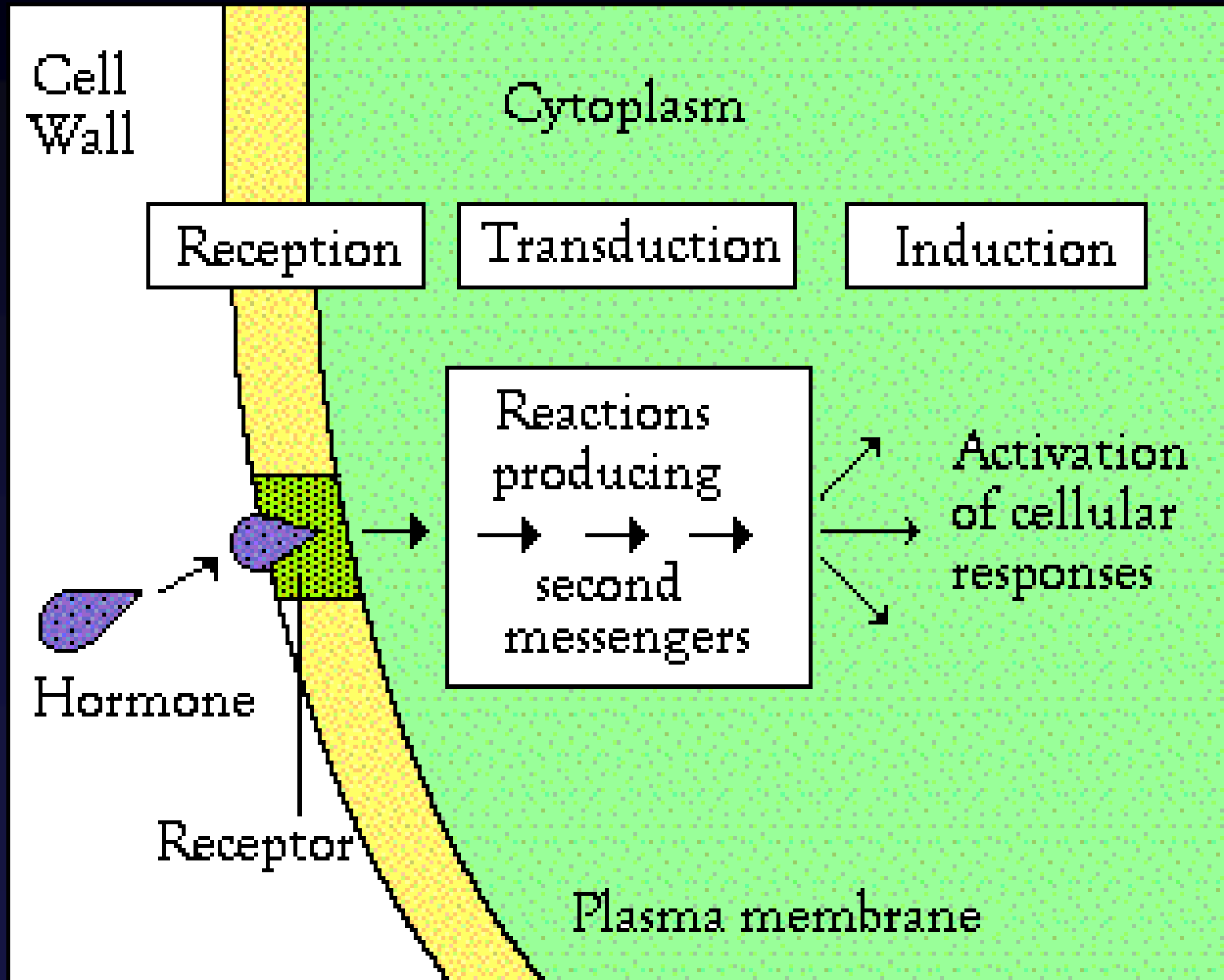


embryos



- 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



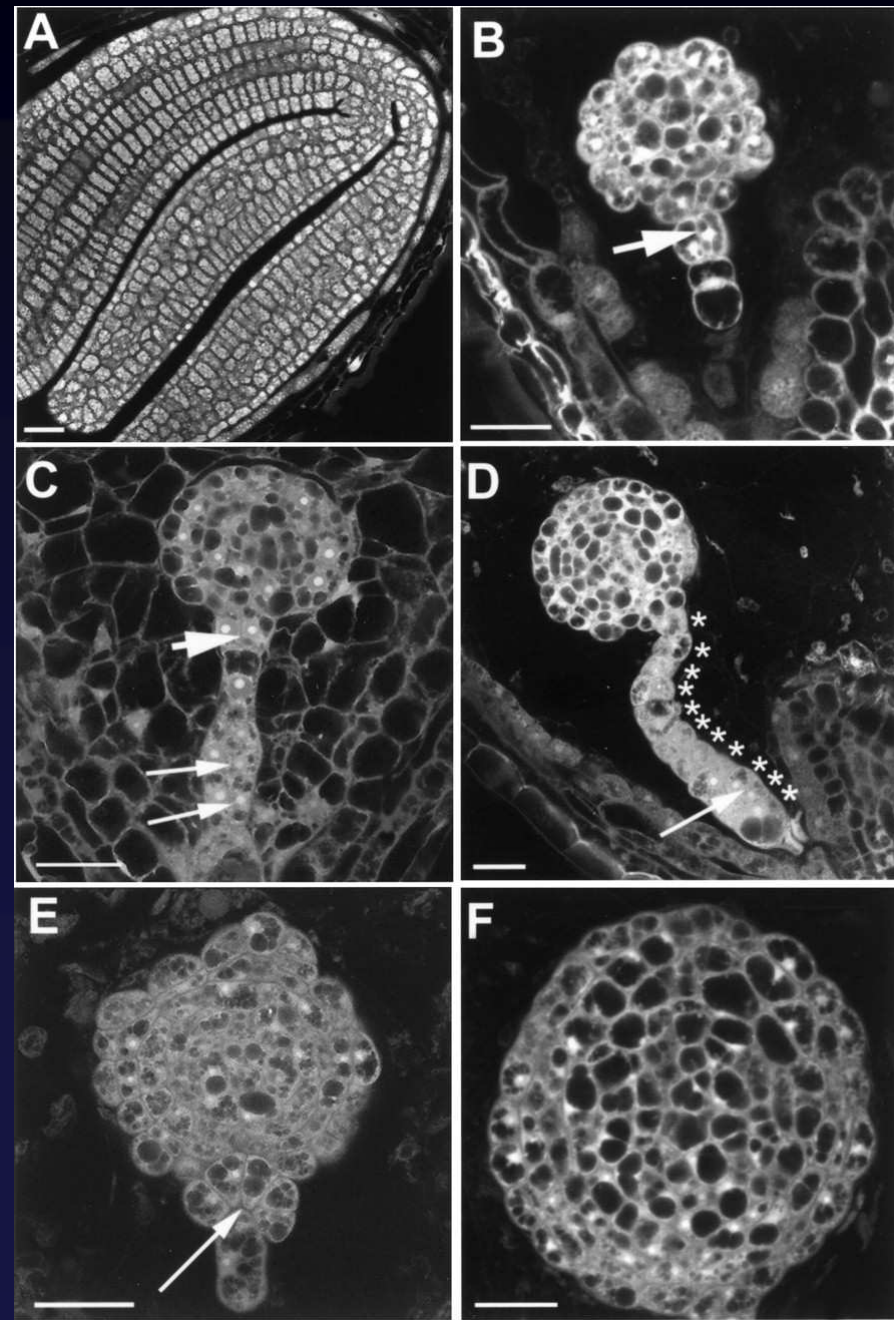
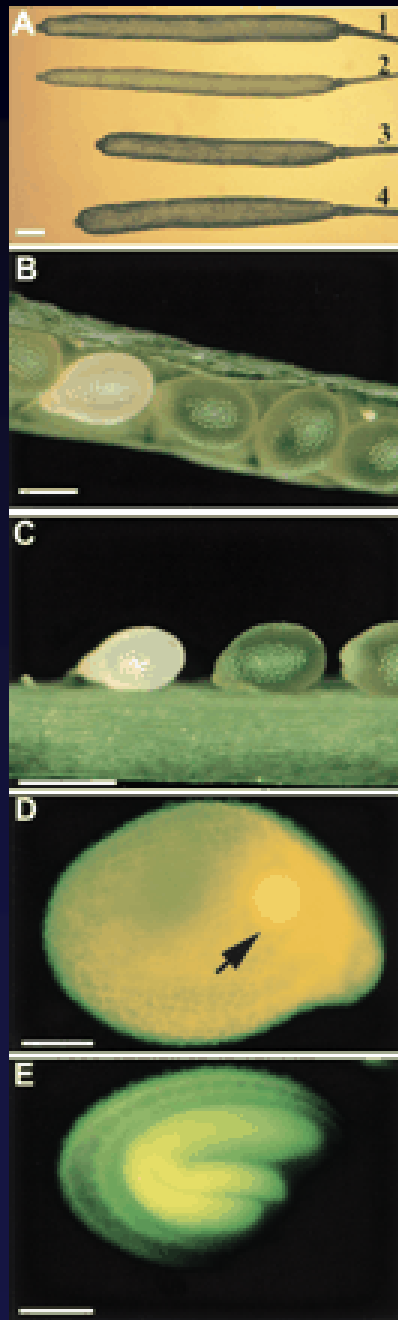
Isolation of auxin binding proteins

- Azidolabeling
- Affinity chromatography

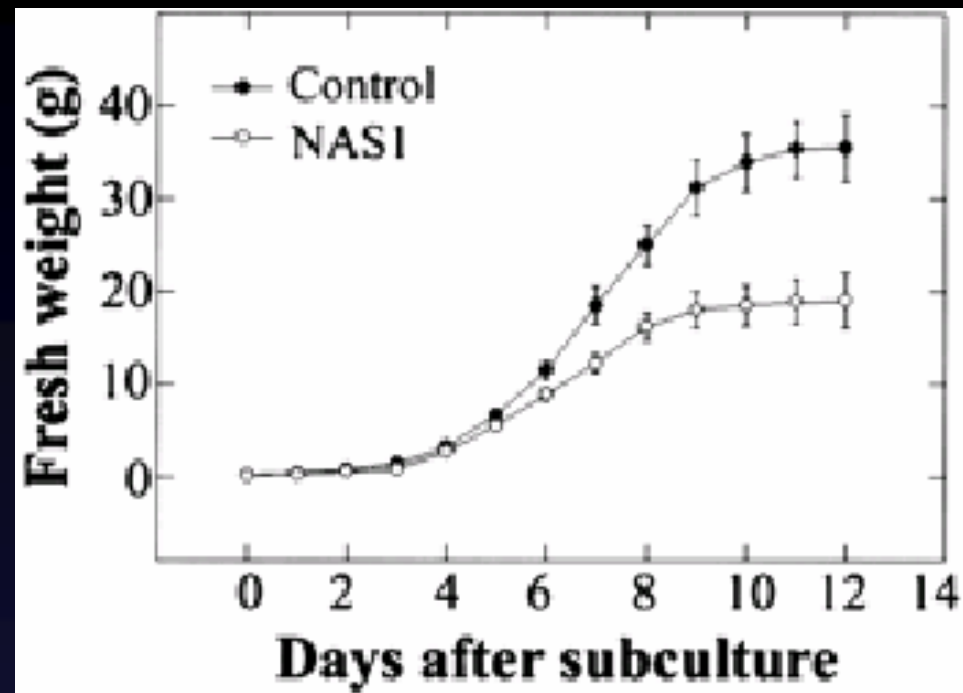
Protein sequencing, cDNA screening, gene identification

=> Auxin Binding Protein (ABP1)

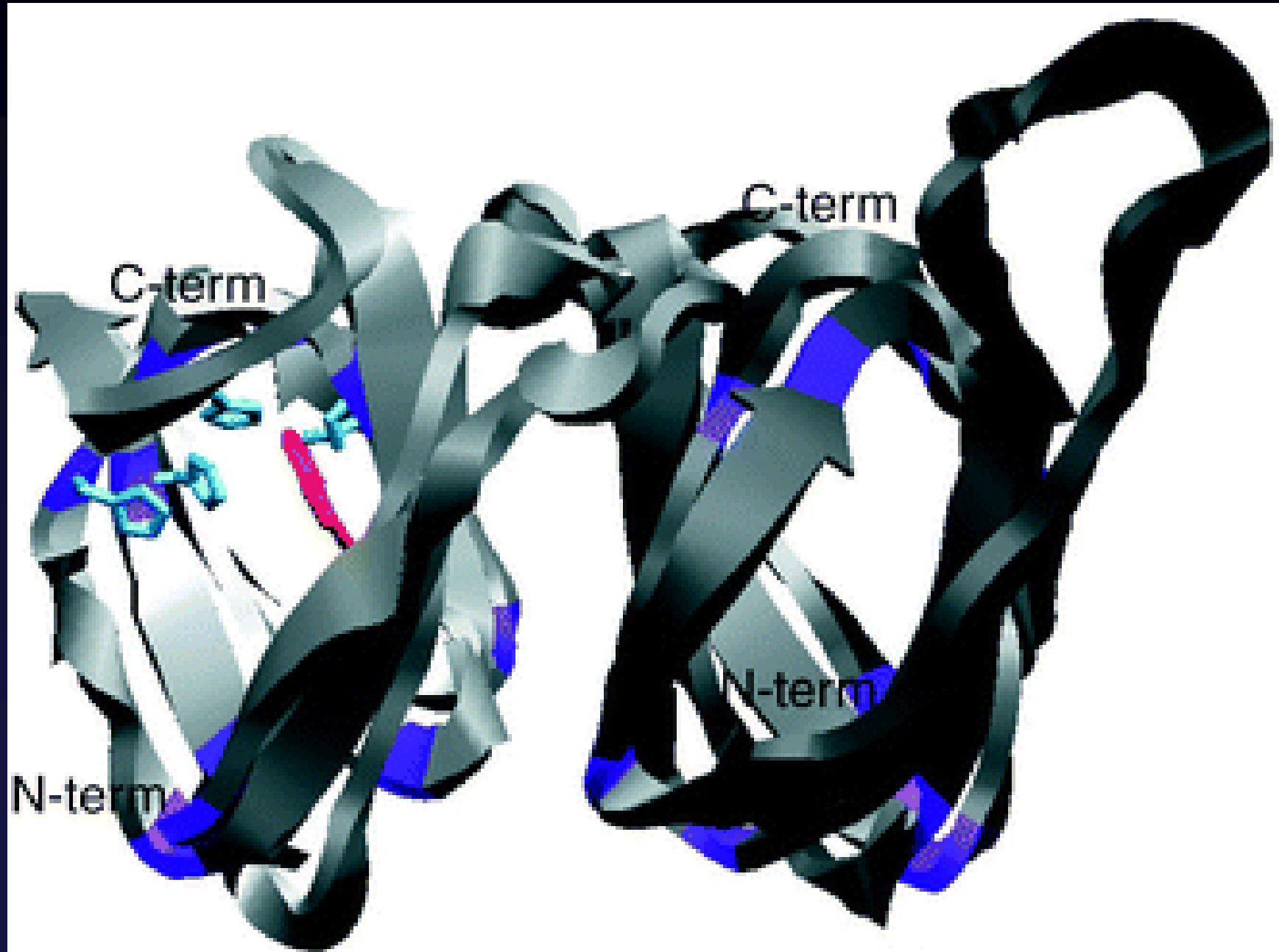
Reverse Genetic – Embryo Lethal *abp1* Mutant



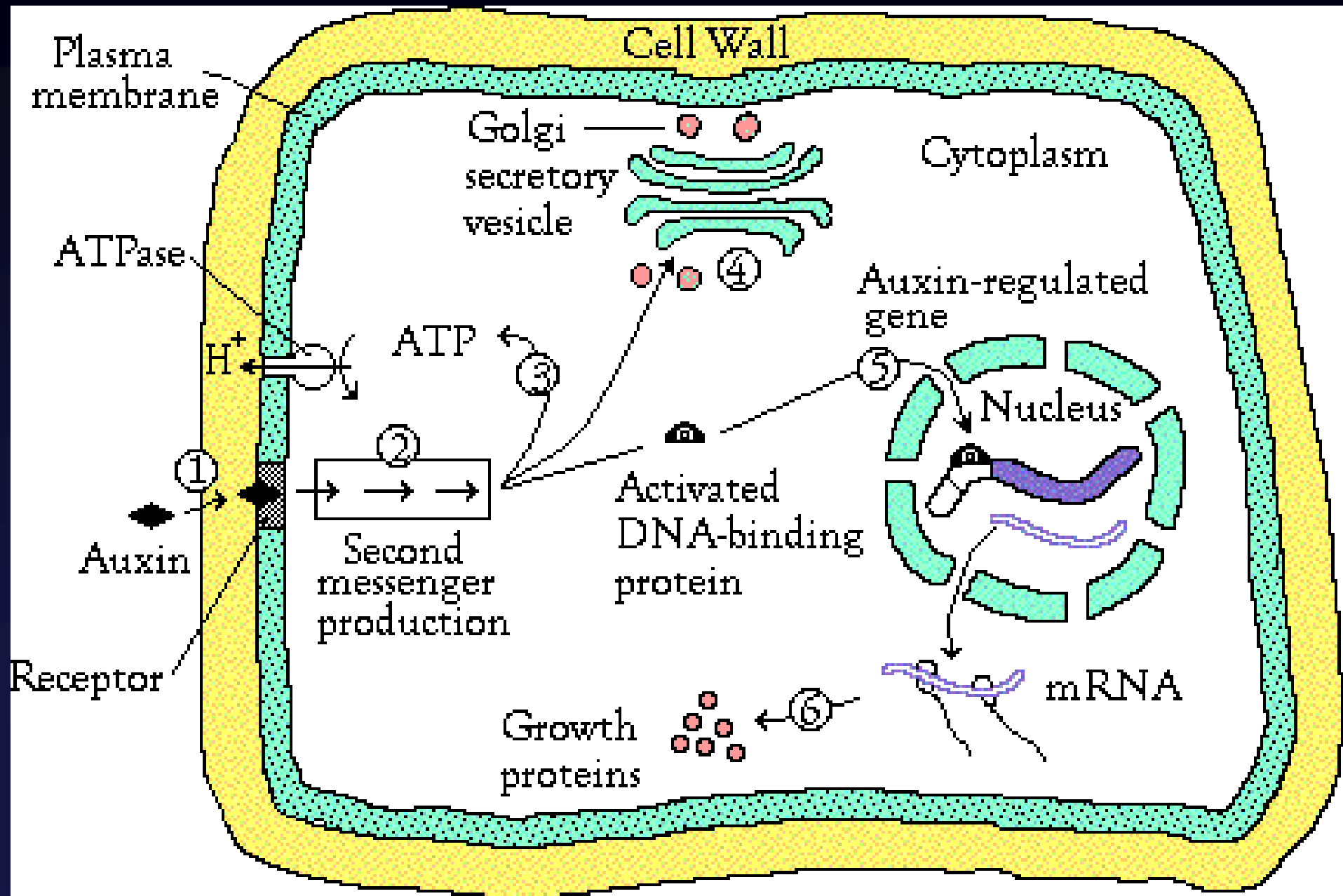
ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation



ABP1 – Structure



Optimistic Model for ABP1 Action



Genetic Approach to Identify Auxin Receptor



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

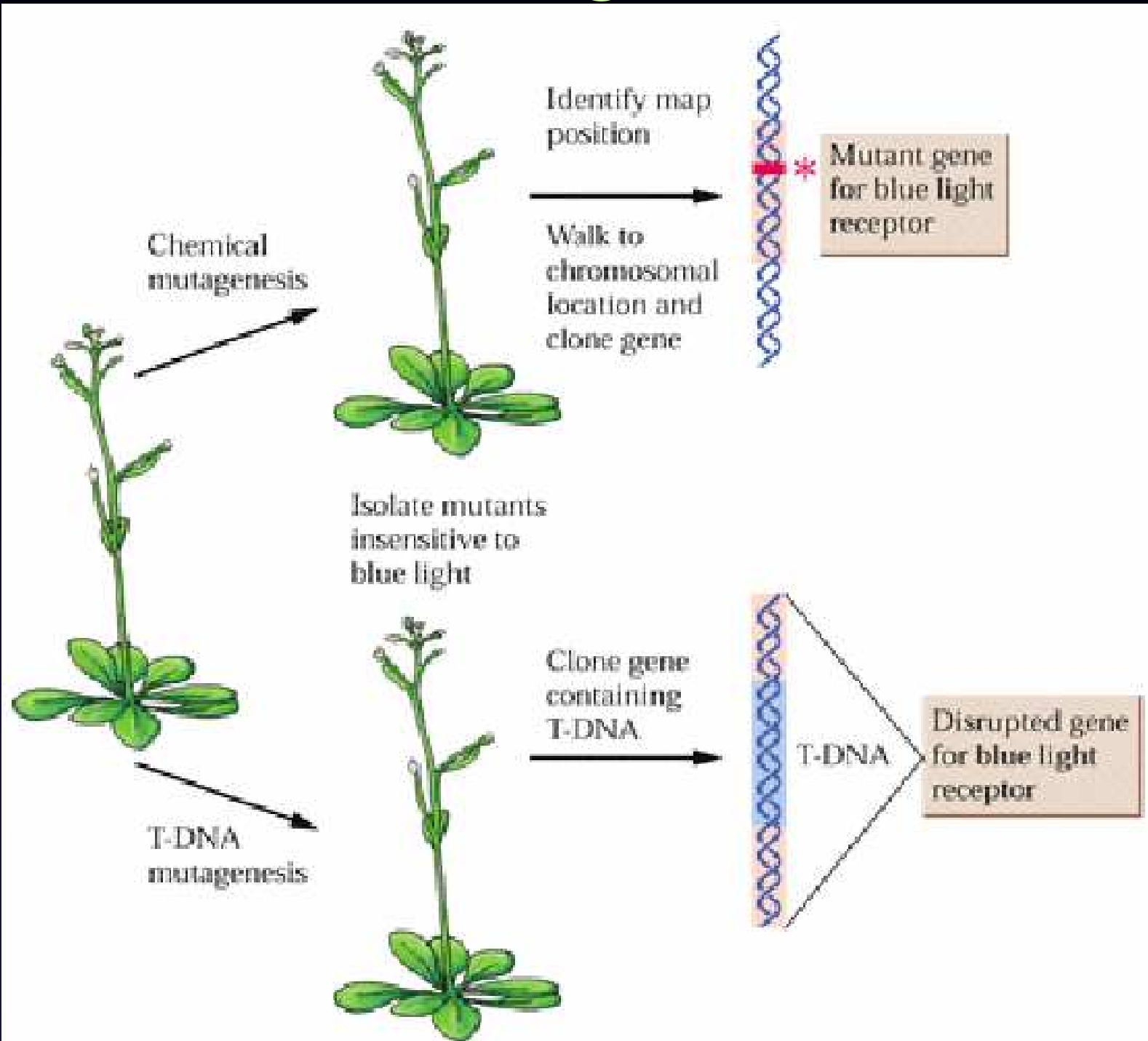
- Transport inhibitor response (tir):
tir1 - *tir7*

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

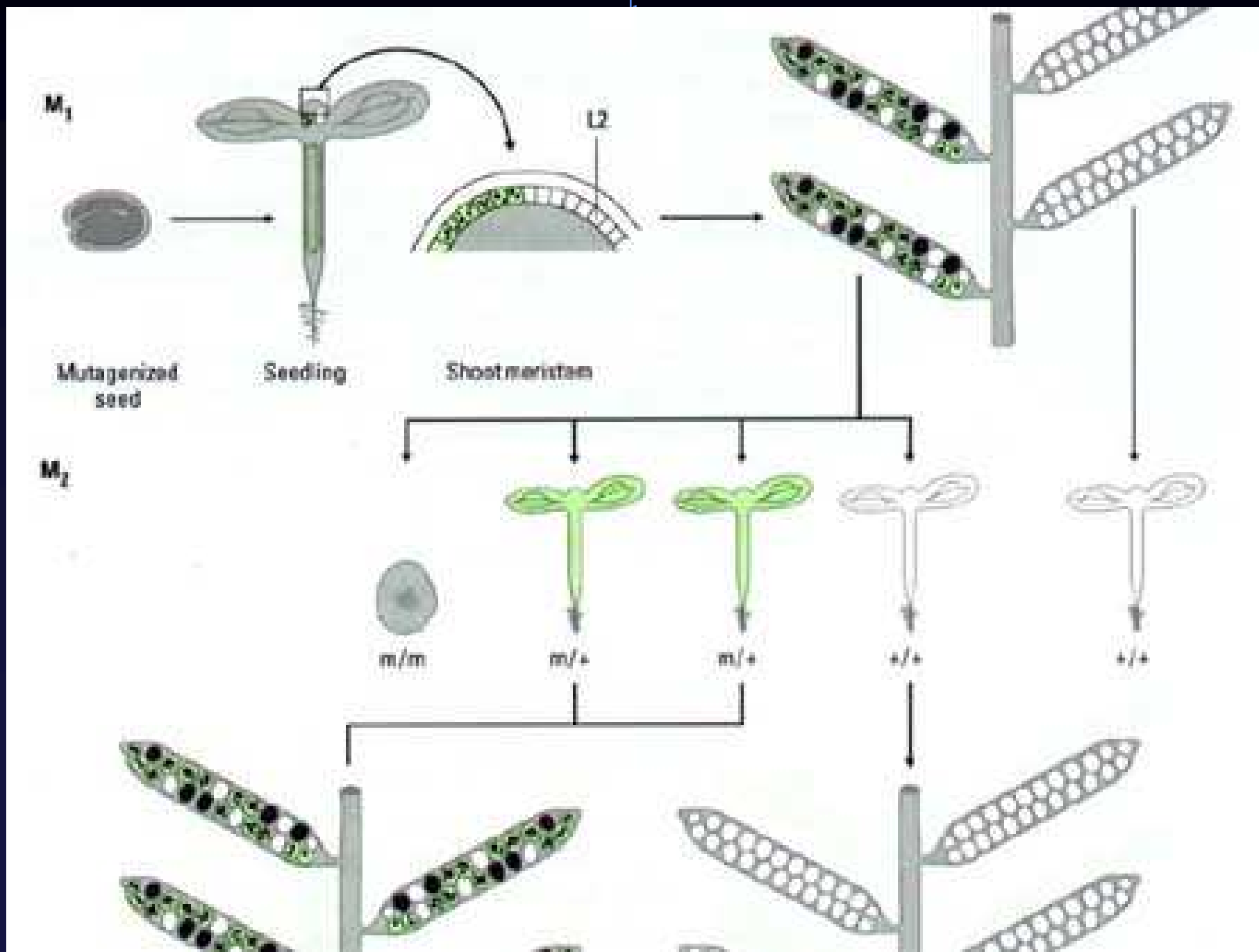
=> Role of regulated protein degradation and
transcriptional regulation in auxin
signaling

None of the identified gene looks like a
receptor

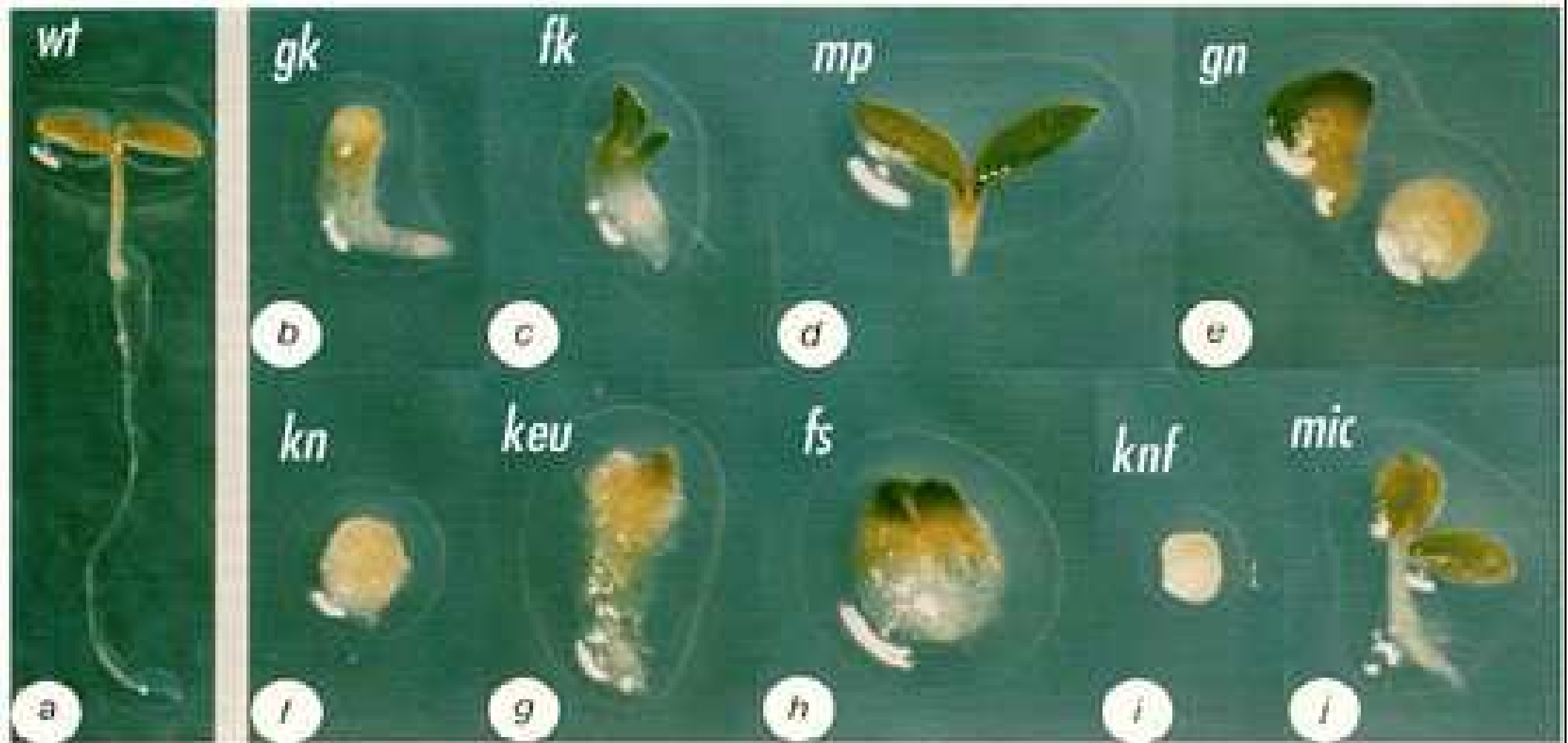
Forward genetics



EMS Mutagenesis



Mutant Screen at Seedling Level



Molecular Biology Approach to Elucidate Auxin Signaling



Does auxin regulate gene expression?

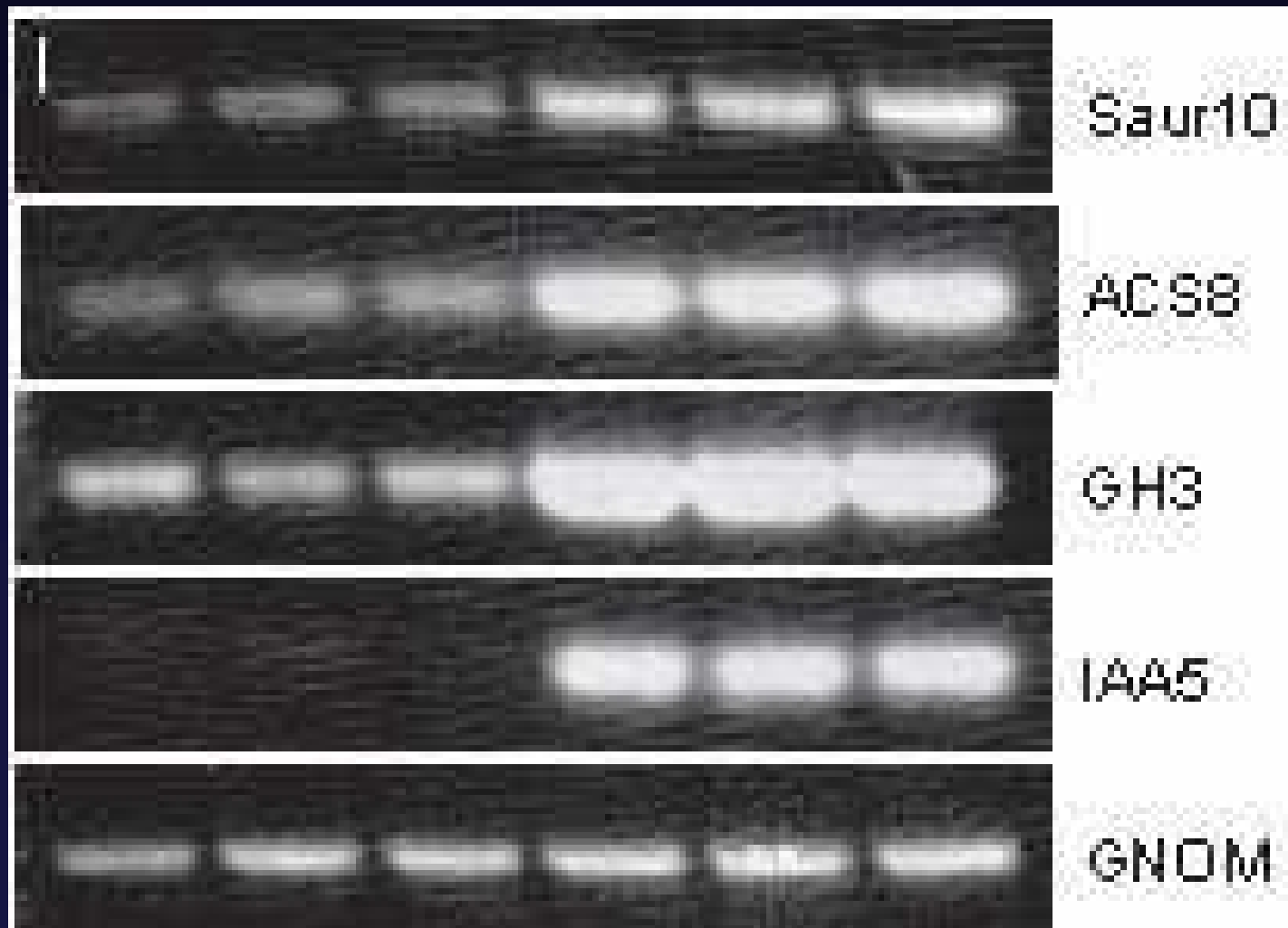
- Rapidly upregulated mRNAs
(*GH3*, *SAUR*, *AUX/IAA* genes)
- One hybrid screen with Auxin Response Elements
=> Auxin Response Factors (ARF)
- Two hybrid => *AUX/IAAs* interact with ARFs

Molecular Biology Approach to Elucidate Auxin Signaling

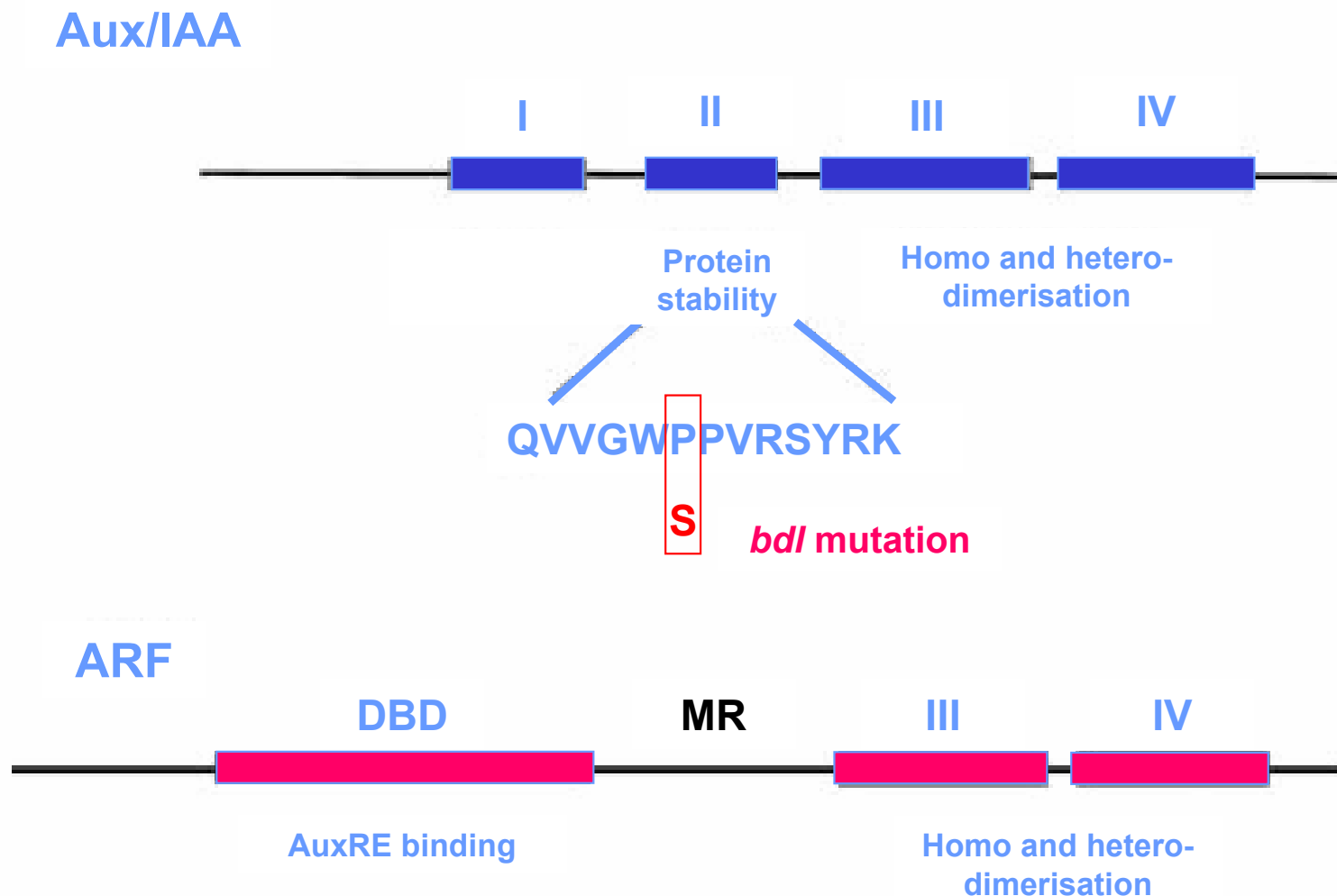
RT-PCR

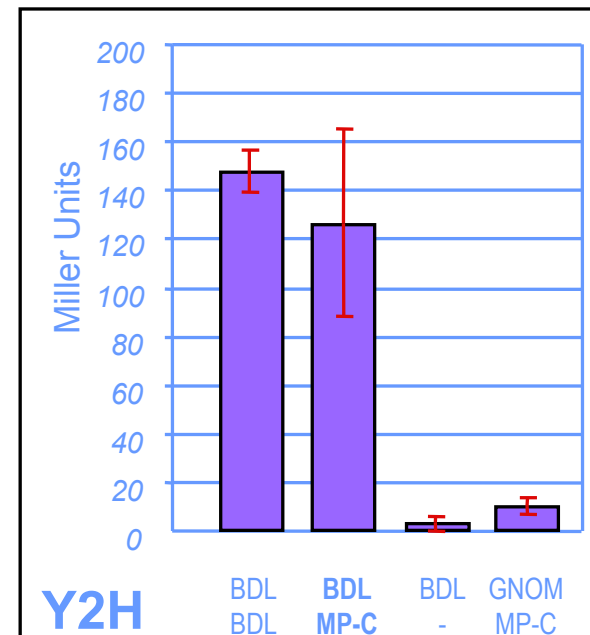
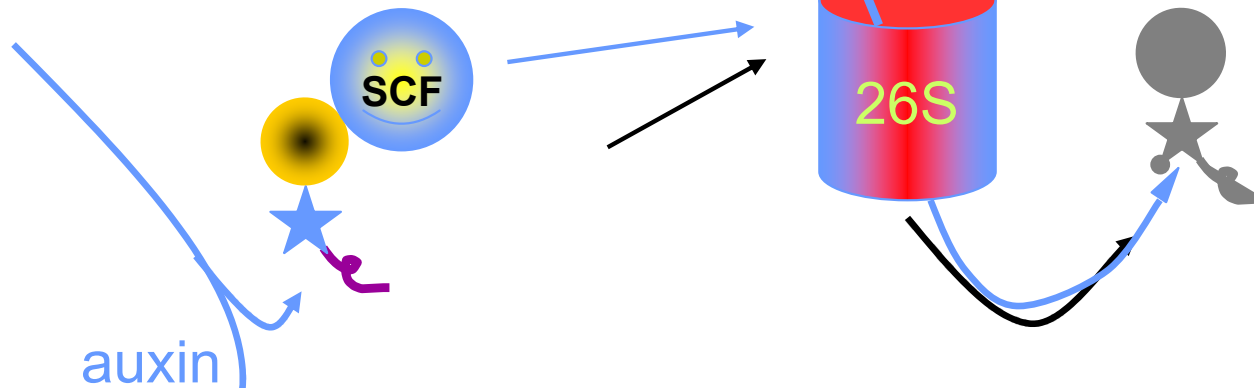
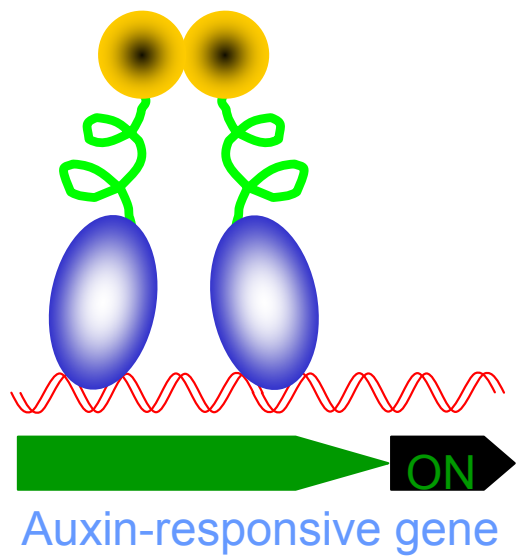
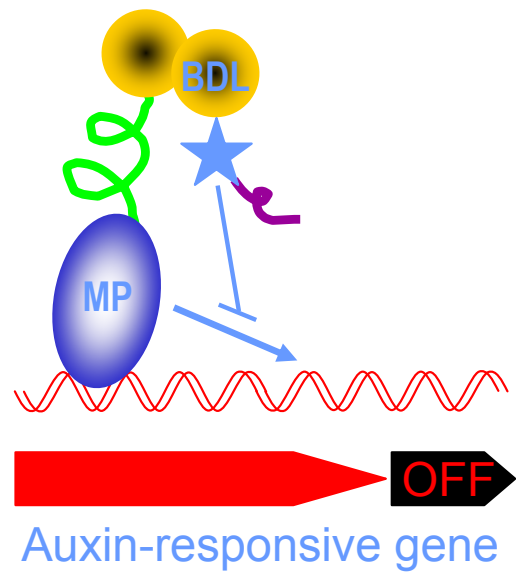
- IAA

+ IAA

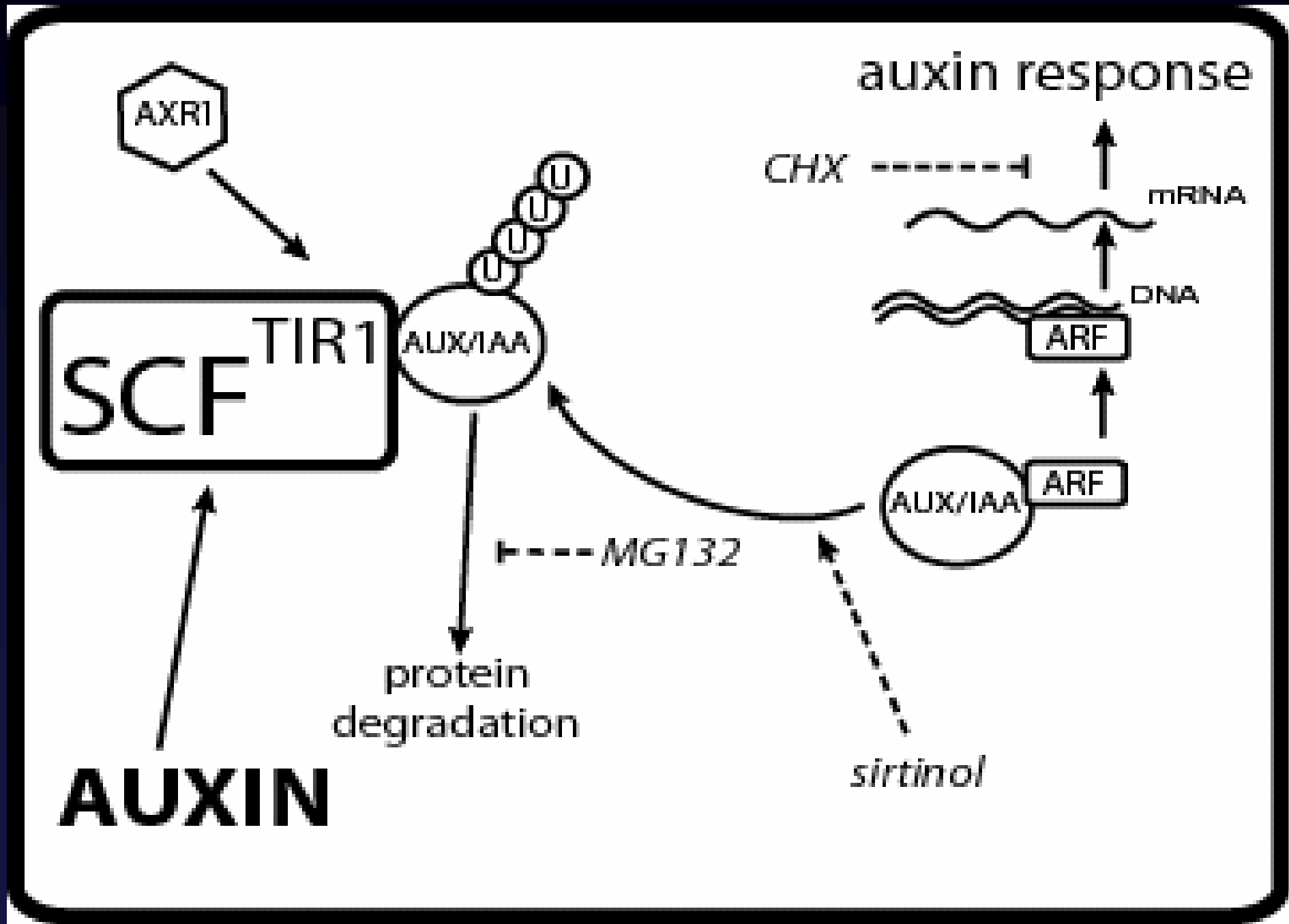


Some ARFs are Activators, whereas Aux/IAA Repressors of Auxin Response





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*)

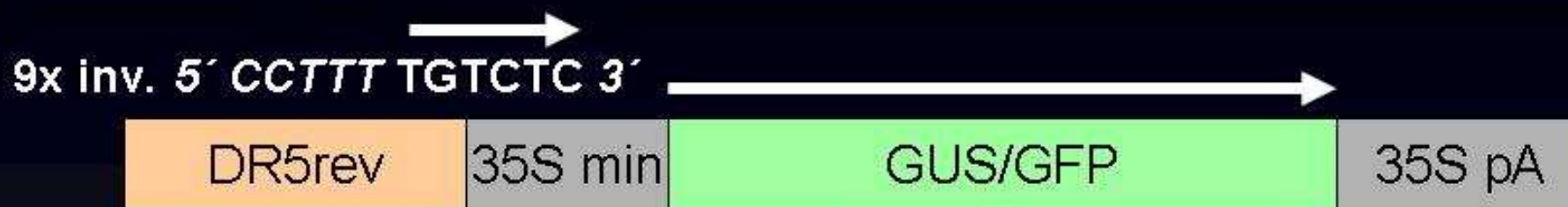
Molecular approach - auxin regulates expression

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



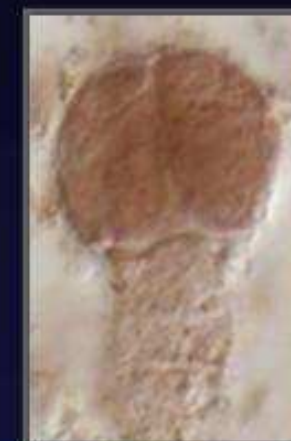
DR5



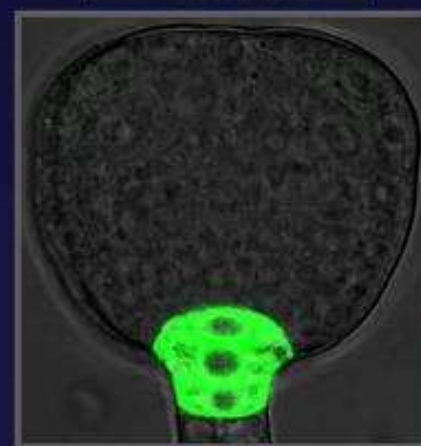
anti-IAA



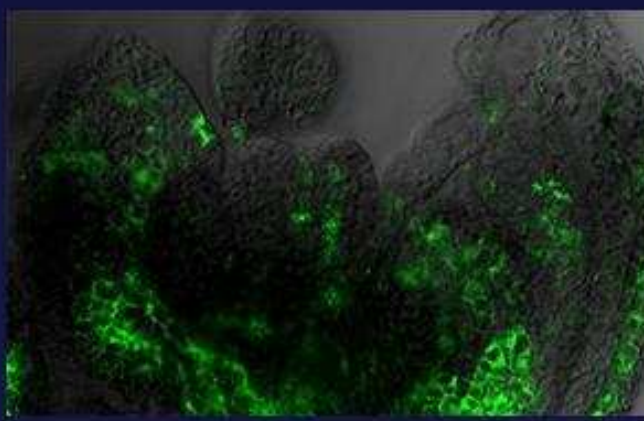
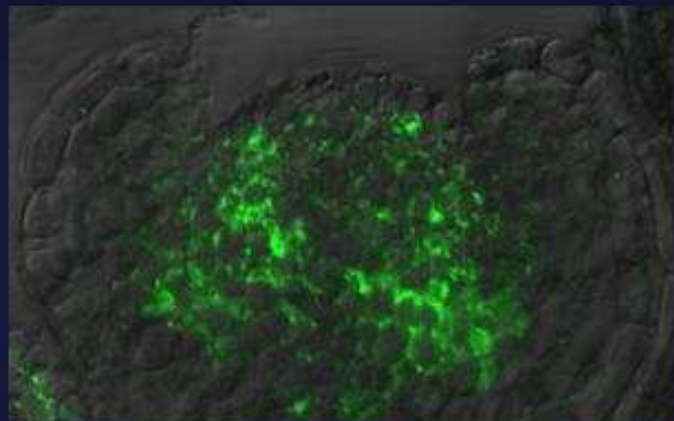
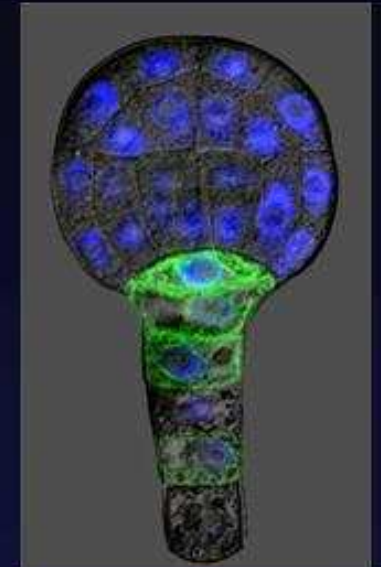
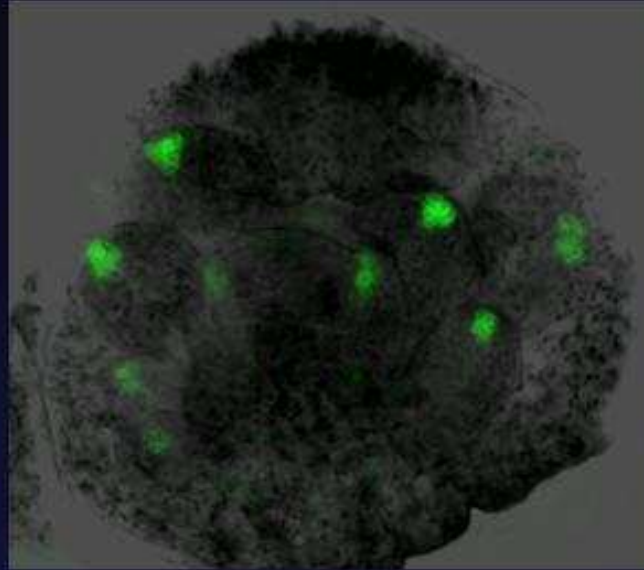
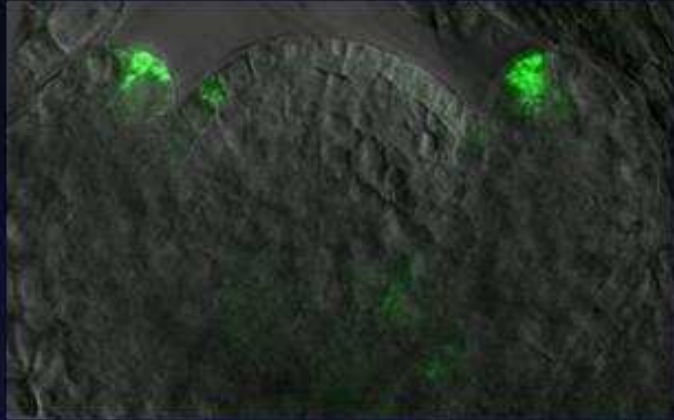
DR5



anti-IAA



Local Auxin Gradients Require Active Polar Auxin Transport



Auxin Transport

Proteins involved in auxin transport

- PIN proteins (efflux)

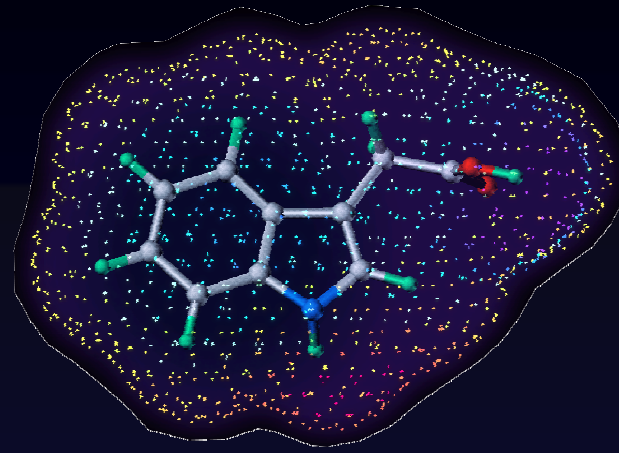
- AUX1 proteins (influx)

Role of GNOM dependent vesicle
trafficking

PIN proteins cycling and its role

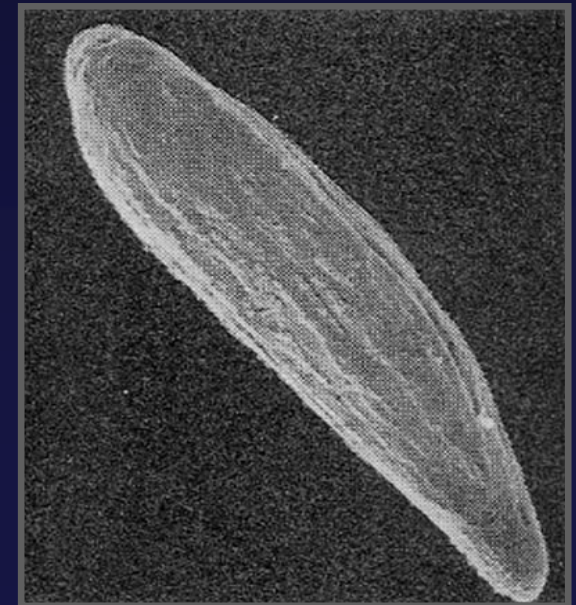
AUXIN TRANSPORT

mediates



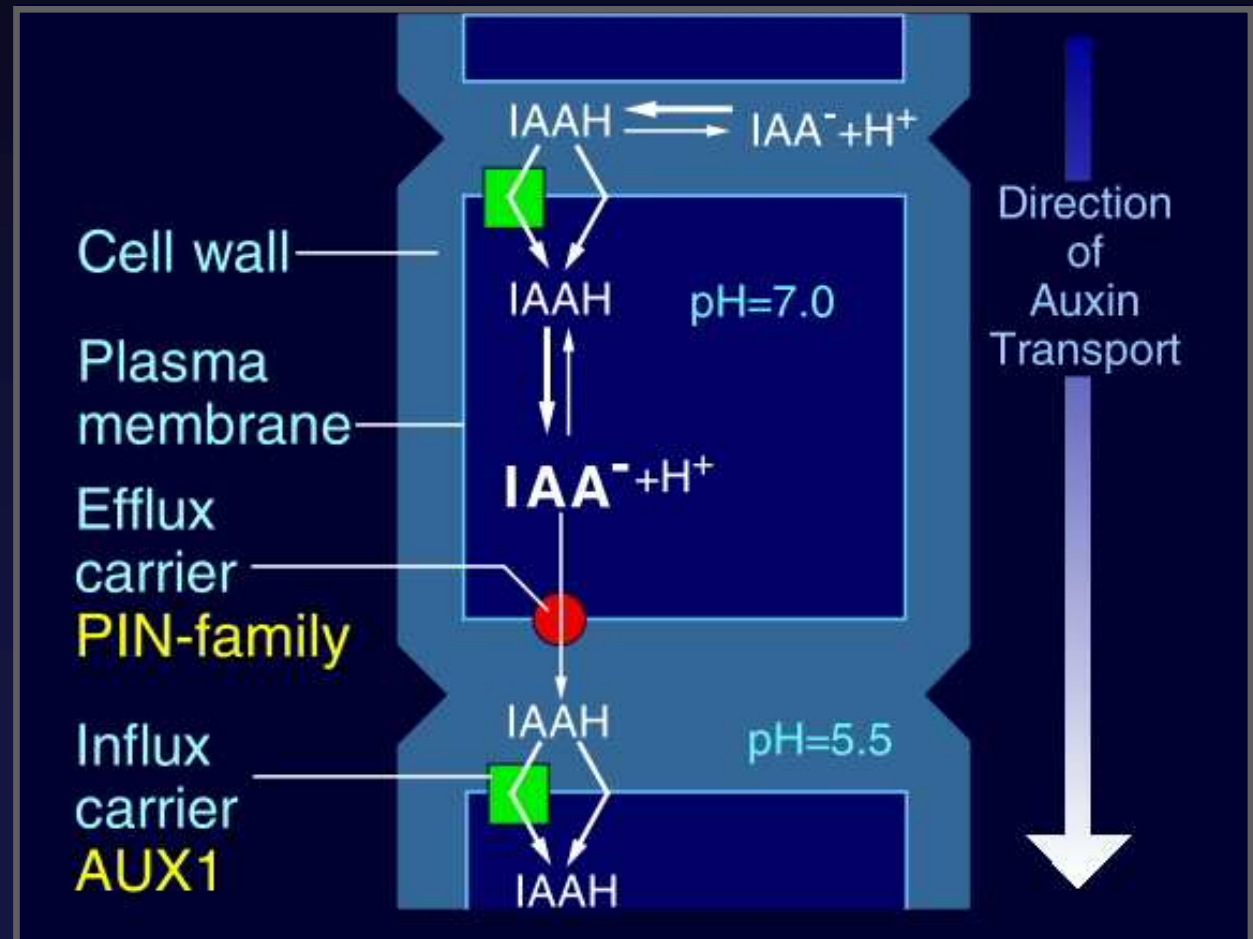
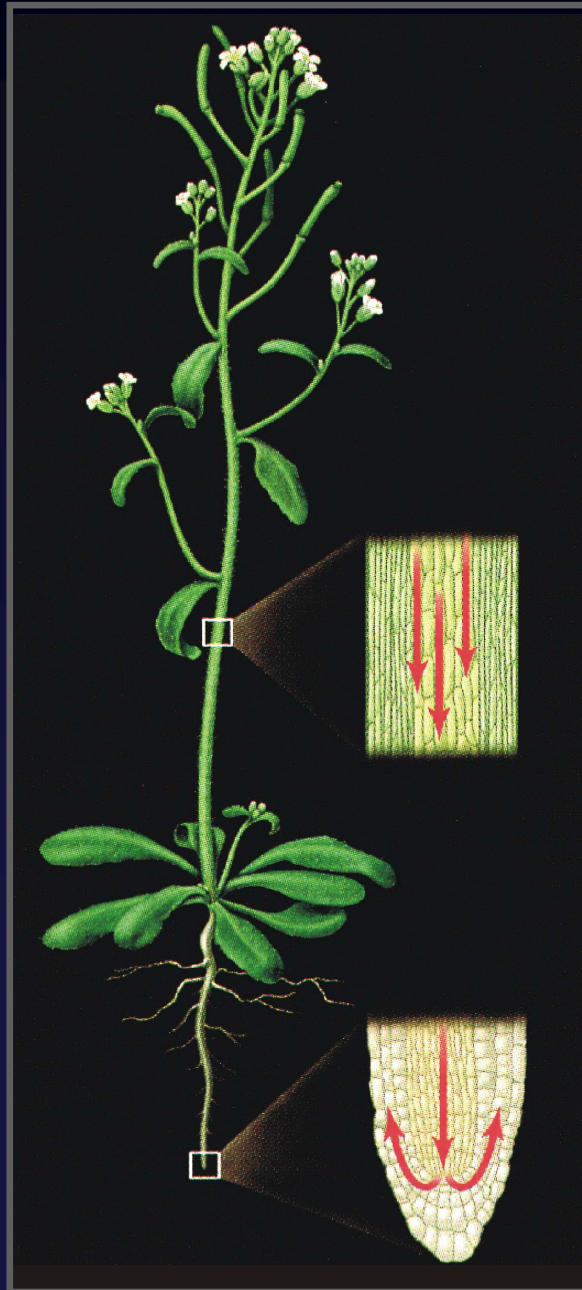
- Embryo development
- Organ initiation and positioning
- Vascular tissue differentiation
- Shoot and root elongation
- Growth responses to light and gravity
- Apical hook formation

embryos



Physiology of Auxin Transport

Chemiosmotic hypothesis

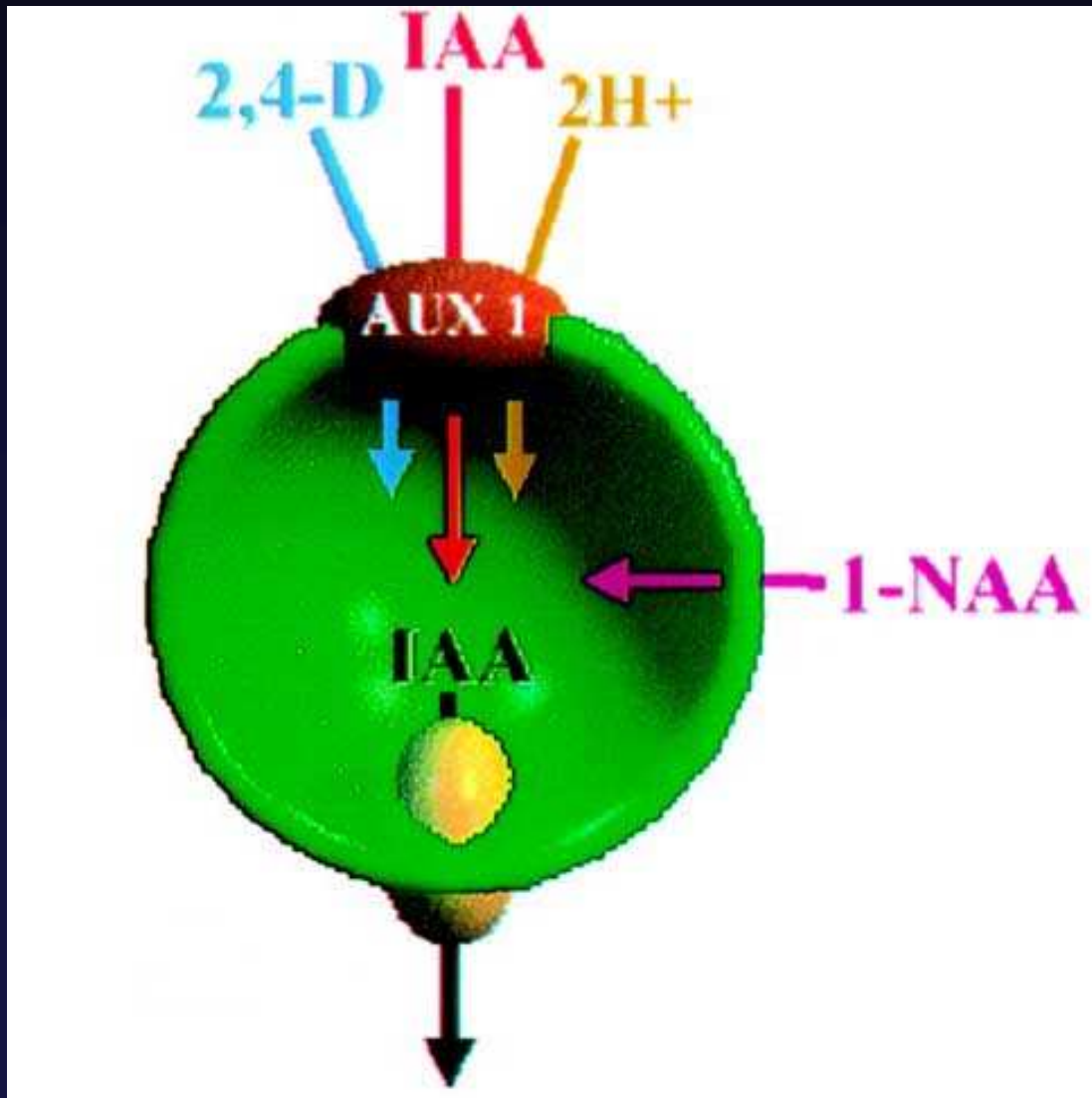


Auxin Influx

aux1 is Resistant to Auxin

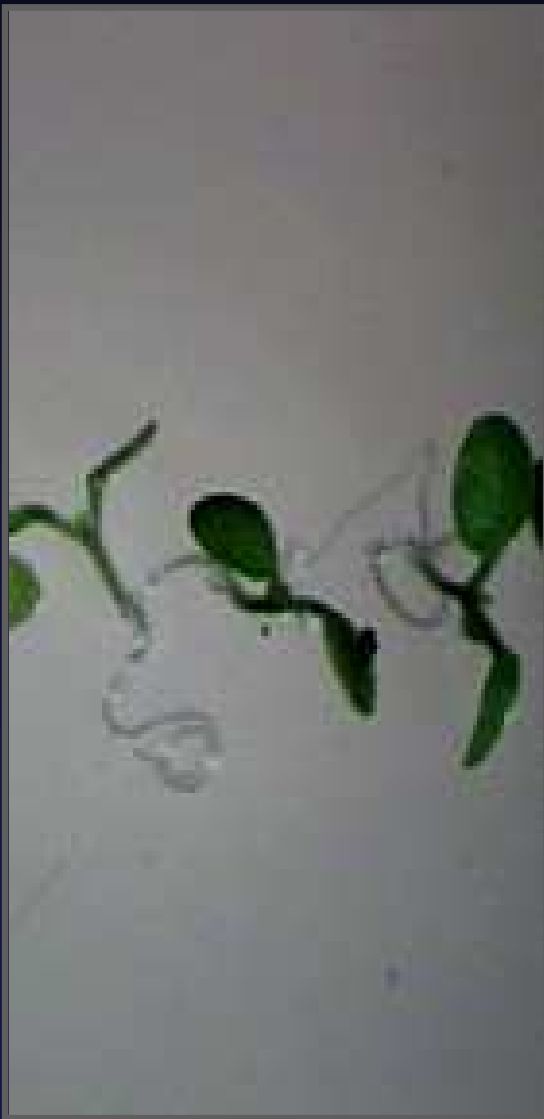
aux1 phenotype

Transport properties of different auxins



NAA Rescues *aux1* Phenotype

- NAA



+ NAA



AUX1 – Expression and Localization

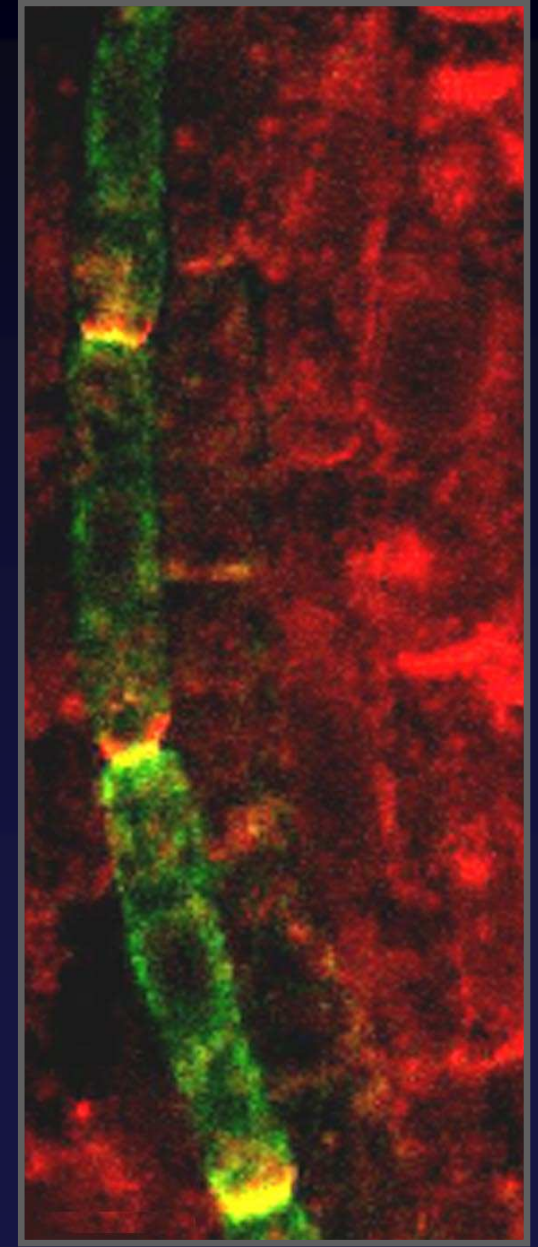
AUX1::GUS



AUX1 protein



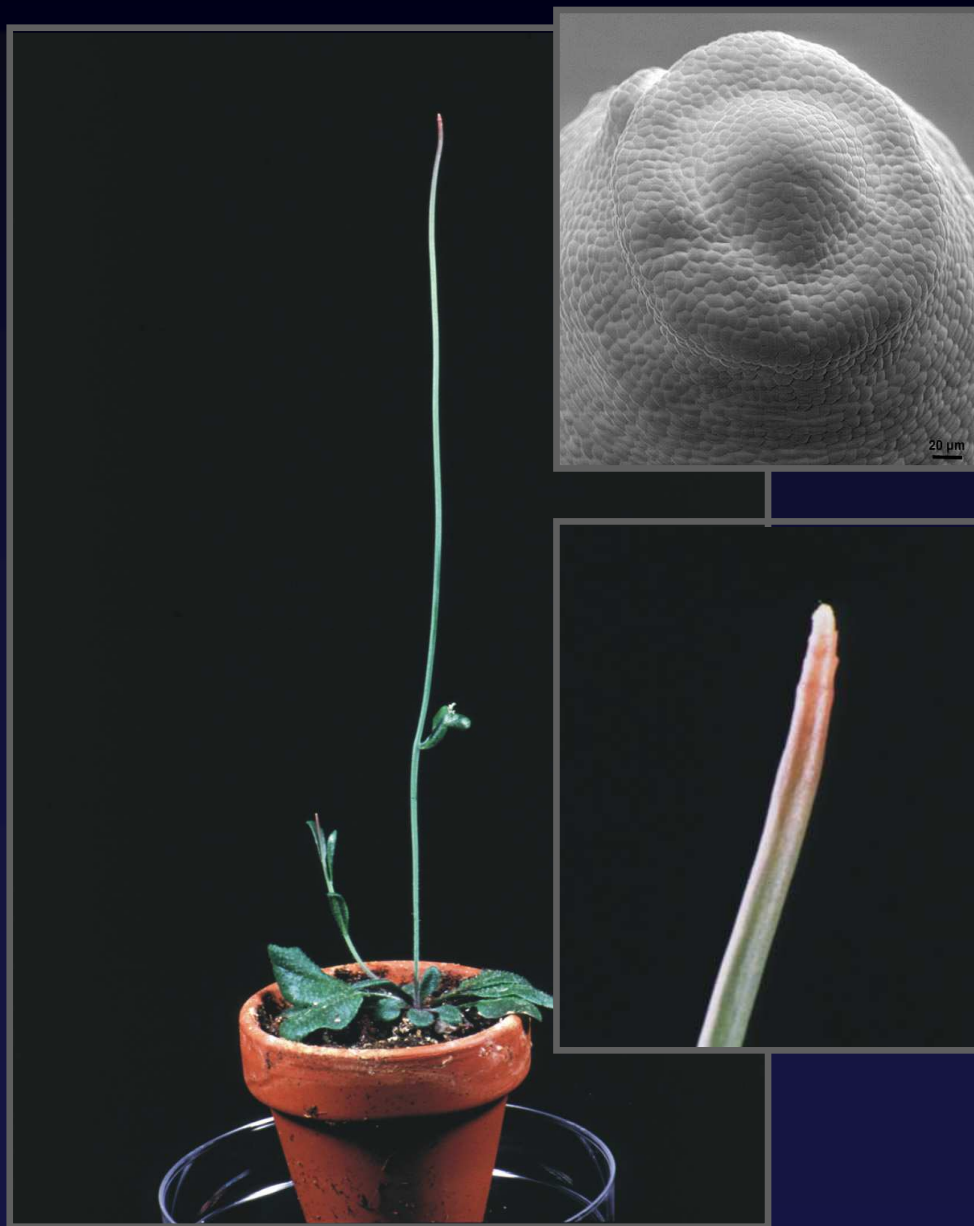
PIN1/AUX1



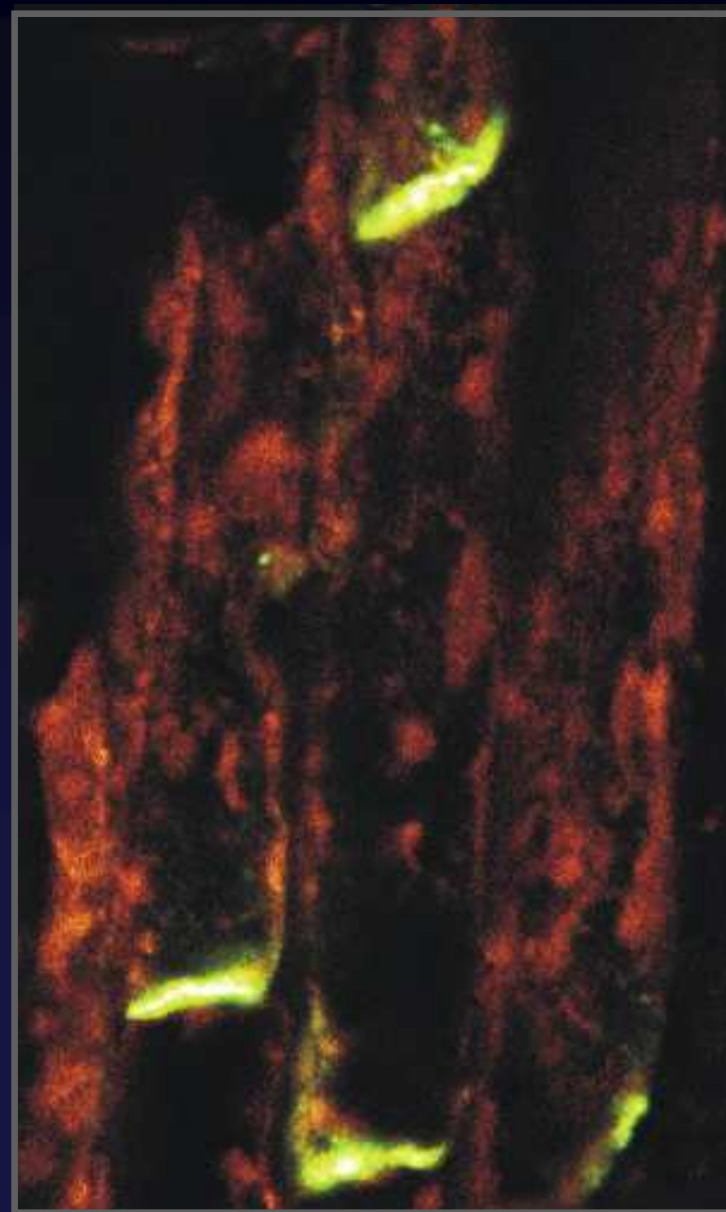
Auxin Efflux

PIN1 – the Auxin Efflux Carrier?

pin1 mutant



PIN1 protein

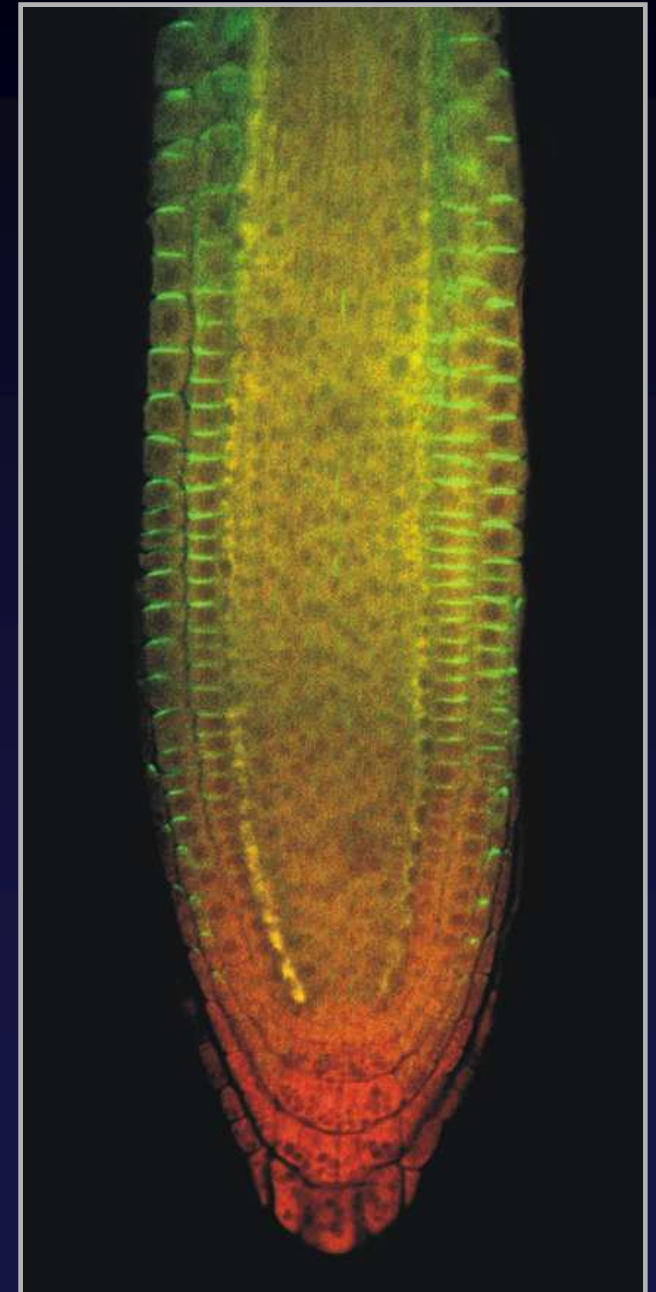
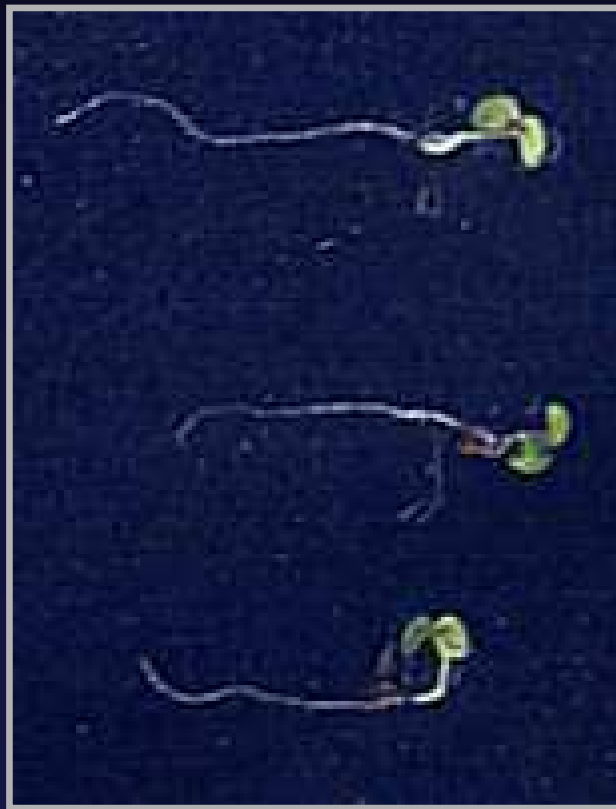
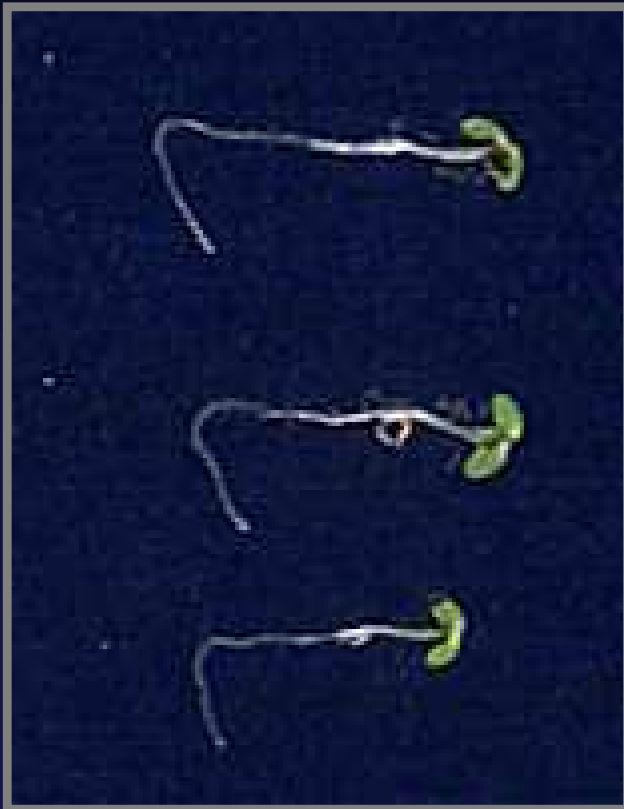


PIN2 – Root Gravotropism

PIN2 protein

Col-0

pin2



The Arabidopsis PIN Gene Family



Comparison of Arabidopsis PIN proteins

| | | |
|--------|---|---|
| AtPIN1 | 1 | MITAAEFVHVEAMVPLVAMILAVGSVRWKKLFTFDQCSGINRFVALFVPLLSFPHIAANNPANNLFLAADSLOKVEVESEFH...WCKLSENBS EDWITLFLSLSTLPLNTLV |
| AtPIN2 | 1 | MITGKMDVDVAAAMPVPLVAMILAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...GOAFSRRGS LEWMITIFSLSTLPLNTLV |
| AtPIN3 | 1 | MISWHOLYVTLTAVIPLVAMILAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...WANLTRSGS LEWMITIFSLSTLPLNTLV |
| AtPIN4 | 1 | MITWHOLYVTLTAVIPLVAMILAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...WANLTRSGS LEWMITIFSLSTLPLNTLV |
| AtPIN5 | 1 | MINGCQVVKVIEAMVPLVAMILAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...WAKYSNKGYSYCS WSTISFSLCLLTSLSLV |
| AtPIN6 | 1 | MITGNEFYTVCMAMPVLFVPMFVAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...WAVFFKAG IDLHLITLFLSTLPLNTLV |
| AtPIN7 | 1 | MITWHOLYVTLTAVIPLVAMILAVGSVRWKKLFTFDQCSGINRFVAVFVPLLSFPHIANNPANNLFLAADSLOKVEVESEFH...WANLTRSGS LEWMITIFSLSTLPLNTLV |
| AtPIN8 | 1 | MISMLIHHVVSATVFLVSMTHGFLAARHLKLESEFDGAGINKEVARSISPELLSEFQISENNPKSKPLLSLSDILQKFLVVVVAMVLRVHPTGGGRGKIGMIVITGLSIEVLENTTI |

| | | |
|--------|-----|---|
| AtPIN1 | 116 | MGIPLEKGMKGNFSDLMVQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.DTAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN2 | 116 | MGIPLEIRAMYGDFSNLWQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSITSERVSDSDVISLNGREPLOTDAEIGDDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN3 | 116 | MGIPLEIRAMYGDFSNLWQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN4 | 116 | MGIPLEIRAMYGDFSNLWQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN5 | 116 | VGVPLAKAMYQQAVDVEVQSSVFGAIVLLELLEFLVLEERK...VAVFFKAG IDLHLITLFLSTLPLNTLV |
| AtPIN6 | 116 | MGIPLEIRAMYGDFSNLWQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN7 | 116 | MGIPLEIRAMYGDFSNLWQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |
| AtPIN8 | 121 | LGMEIISNHYGDEAASILEQIVVLLQCIWYIIMLFLFEEYRGAKLLISEQFE.ETAGSIVSIHWDDIMSISLDGQRPLETEAEIKEDGKLVVTRRSNASK...SDIYSRRSQLS..ATF |

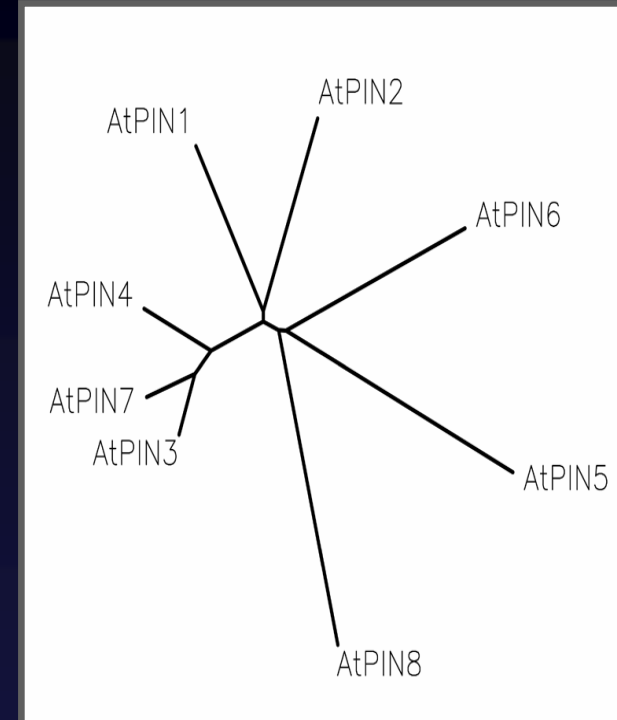
| | | |
|--------|-----|---|
| AtPIN1 | 229 | RPSNLTNAEIIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN2 | 235 | RASNLTGAEIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN3 | 224 | RPSNLTGAEIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN4 | 221 | RPSNLTGAEIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN5 | 158 | GFSSNNSISVQVDNINI...ESKRETVVVEKSE..LE |
| AtPIN6 | 228 | RASNLTGAEIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN7 | 227 | RPSNLTGAEIYSQSSRNPTPRGSSFNHT.DEYS...MMASG...GG...RNSNFGP.GE.AVFGSKGPTPSPRESNYEEDGGPAKPTAAGTAAGAGRPHQSGGSGGGG |
| AtPIN8 | 169 | GALEHGGNDQ.EEANT...EDEKKEEEDVAVIVTRTSVGTMK |

| | | |
|--------|-----|---|
| AtPIN1 | 325 | GAHYPAFNPQMFSPNTGGGGGTAAGCNAPV...VGRKQDGNRDLHMFVWSSASPVSDVFE...GGGNNHADYSTANDHOVDVKISVQGNSS...DNQVVER |
| AtPIN2 | 342 | SYEPFNPMTG...STSGASGVKKKESGGGGGGGGVGVGGQN...KEMNMFVWSSASPVSE...ANAKNMTRGSSTVSDPKYSIIPHD.NLATAMQNLINEMSPER |
| AtPIN3 | 305 | AGSYPAFNPQMFSPNTGGGGGTAAGCNAPV...VGRKQDGNRDLHMFVWSSASPVSDVFE...GGGNNHADYSTANDHOVDVKISVQGNSS...DNQVVER |
| AtPIN4 | 303 | AGSYPAFNPQMFSPNTGGGGGTAAGCNAPV...VGRKQDGNRDLHMFVWSSASPVSDVFE...GGGNNHADYSTANDHOVDVKISVQGNSS...DNQVVER |
| AtPIN5 | 192 | LDVNSGTP...VWVK...EFANGR...TYOSSPMMWESGORHARDENGSEKESIFSDALKWAPATASG |
| AtPIN6 | 313 | LDVNSGTP...VWVK...EFANGR...TYOSSPMMWESGORHARDENGSEKESIFSDALKWAPATASG |
| AtPIN7 | 308 | PGSYPAFNPQMFSPNTGGGGGTAAGCNAPV...VGRKQDGNRDLHMFVWSSASPVSDVFE...GGGNNHADYSTANDHOVDVKISVQGNSS...DNQVVER |
| AtPIN8 | 210 | STGNKTSAPKE...NHHV...KSNNSDAKELHMFVWSSASPVSDVFE...GGGNNHADYSTANDHOVDVKISVQGNSS...DNQVVER |

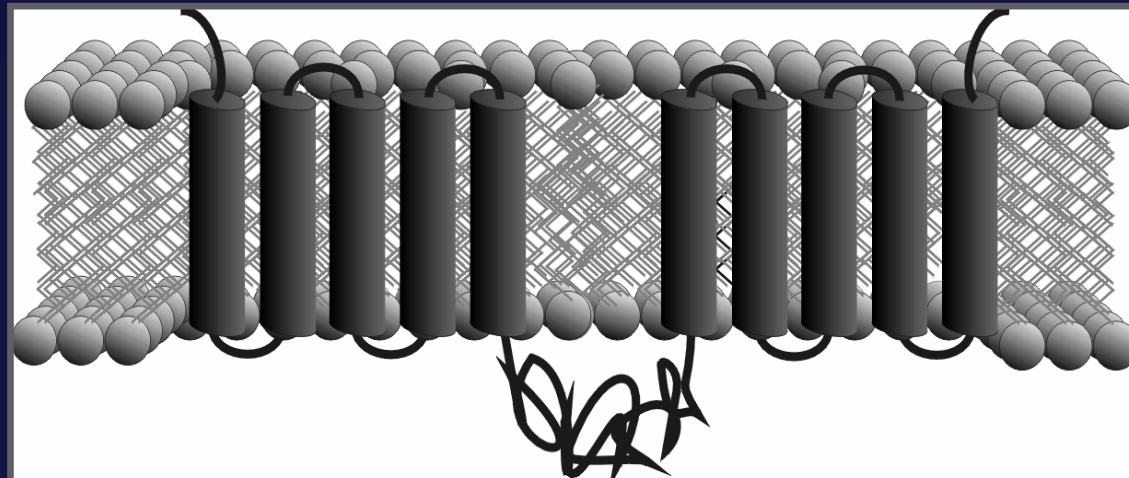
| | | |
|--------|-----|---|
| AtPIN1 | 423 | EEFSFGNKDDSDSKVL...ATDGNNSNKT...QAKVMPTSVMTLILIMVWRKLRNPNSYSSEFGITWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN2 | 443 | RVEMDQGNNG...GKSPTMGKKGSDVEDGGGPPKQMPFASVMTLILIMVWRKLRNPNSYSSEFGITWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN3 | 424 | QFSPFAGKEAERPKDRENSLKNKAPNSFALQSKFGLGAEASORKNMPPASVMTLILIMVWRKLRNPNSYSSEFGITWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN4 | 407 | LDSEGERIEK...ATASLNKMGNSALEAAGDGGGNG...THMPTSVMTLILIMVWRKLRNPNSYSSEFGITWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN5 | 192 | VMSLVKLLATNPNCMCSILGLIWAFAISNRWHLLEGLGEGSILMSKAGTAMFNMGIF |
| AtPIN6 | 381 | ASMEEGAAGKDT...TPVAIG...KQEMESIVMMLLITVGRKLSRNPNTYSLLGLVWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN7 | 411 | EEESRVRKEVPEHLKIRCNSLNLNPKAETETGTVPVKHMPPASVMTLILIMVWRKLRNPNSYSSEFGITWSEISFKWNIEMFALAKSISILSDAGLGMAMFSLGLF |
| AtPIN8 | 210 | LLKMKRKLITNPNTYATLIGIHWALHFRGLGNLEPMDRS...HLSGGLGMAMFSLGLF |

| | | |
|--------|-----|--|
| AtPIN1 | 526 | MAENFRIACGNRRFAAAMRFRVVPVAVMLASVYVLRGVELEHVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN2 | 551 | MALQPKIACGKEVAGFAMVRFLEGPVAVIATSIATIGRDLLEHVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN3 | 544 | MALQPKIACGNEVATFAMVRFLEGPVAVIATSIATIGRDLLEHVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN4 | 520 | MALQPKIACGNSVATFAMVRFLEGPVAVIATSIATIGRDLLEHVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN5 | 253 | MALQKELVCGTLETVMGVKRIASPAAMATGSEIVLGHGQDVRVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN6 | 474 | MALQKELVCGTLETVMGVKRIASPAAMATGSEIVLGHGQDVRVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN7 | 523 | MALQKELVCGTLETVMGVKRIASPAAMATGSEIVLGHGQDVRVAIIQAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |
| AtPIN8 | 271 | MASQSSIACQTKMIIIMLLKVELEPALMIASAYCERKSTEFKVAIQAAALPQGVVPPFAKYNVHEDILSTAVIFGMLIALPITLVYILLGL |

Phylogenetic tree



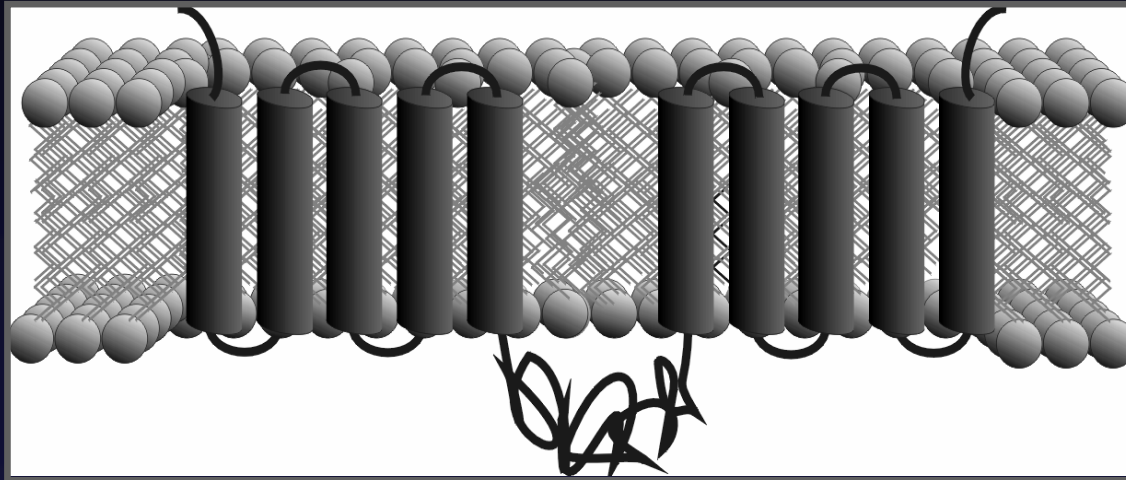
Membrane topology model



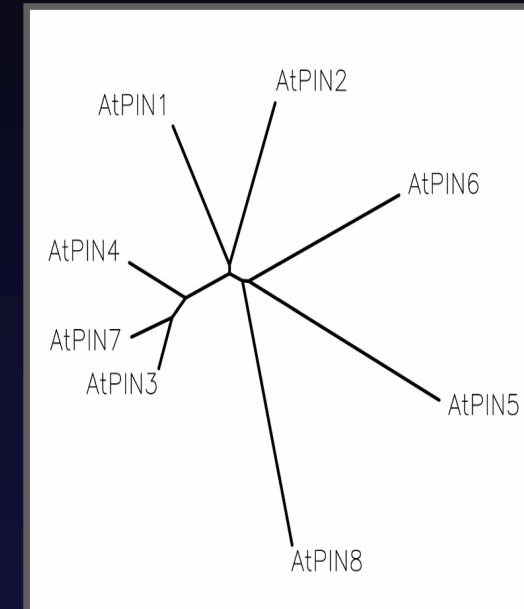
What is Molecular Role of PIN Proteins in Auxin Transport?

PINs Are Essential Components of Auxin Transport

Putative topology of PIN proteins



Phylogenetic tree

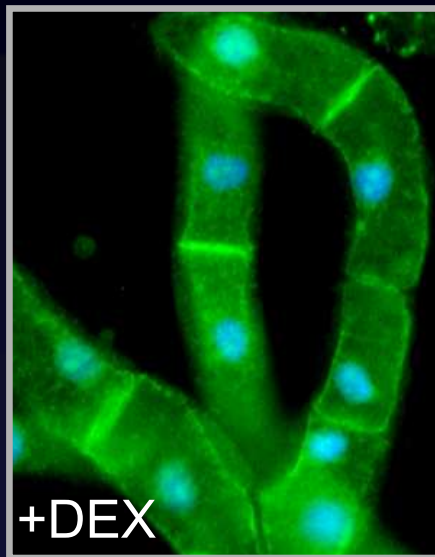
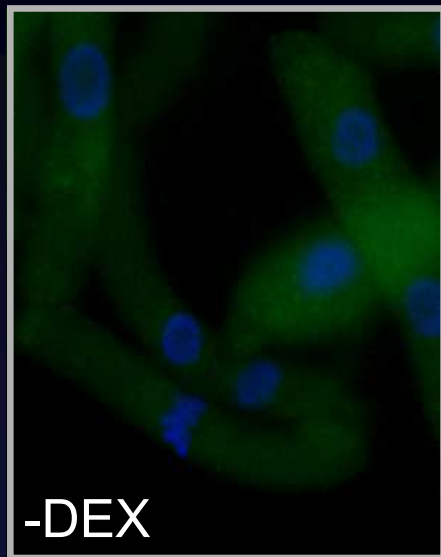


- All defects in *pin* loss-of-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors
- Local auxin distribution (gradients) are affected in *pins*
- Polar PIN localization determines direction of auxin flow

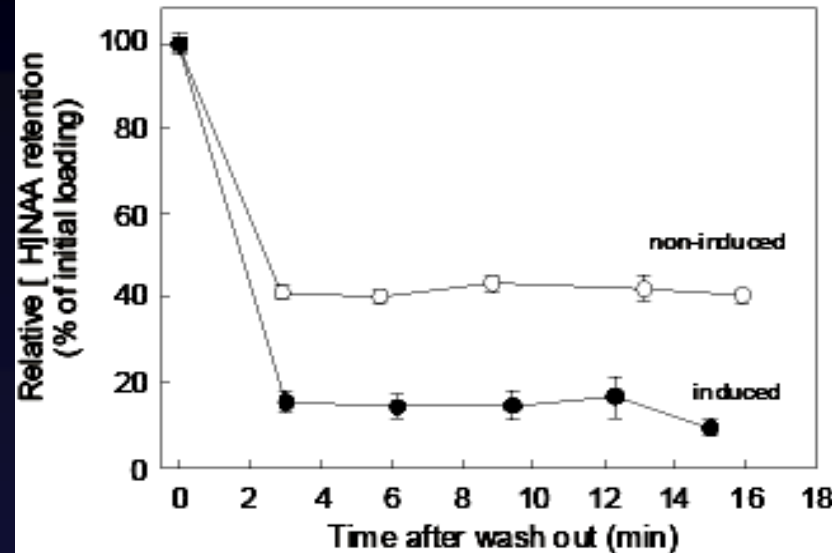
PINs Are Rate-limiting Factors in Auxin Efflux



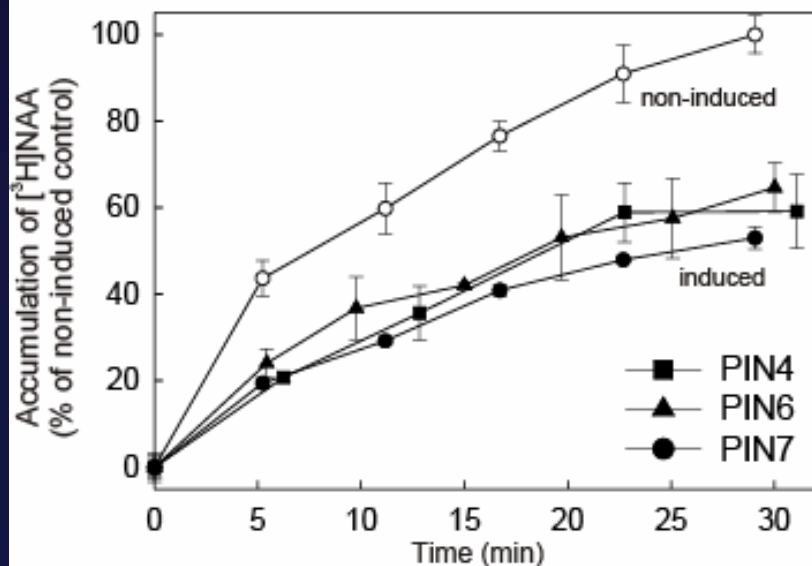
Inducible PIN1 expression



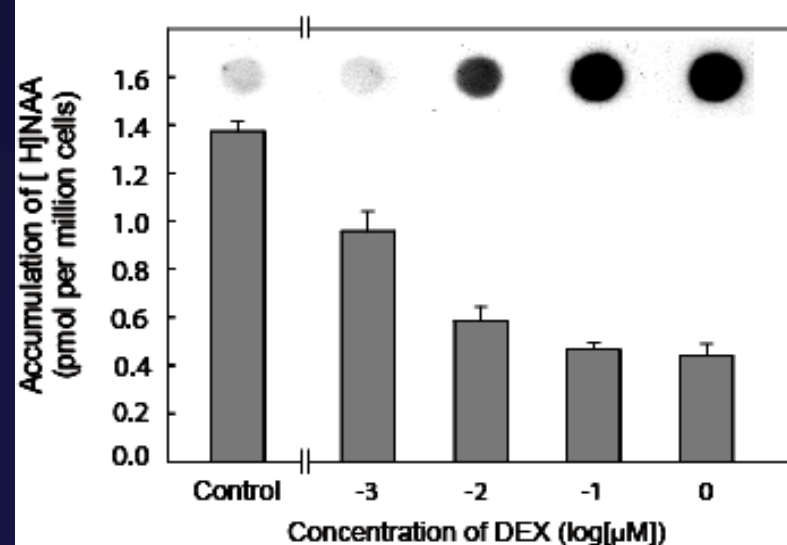
PIN-dependent auxin efflux from GVG-PIN7 tobacco cells



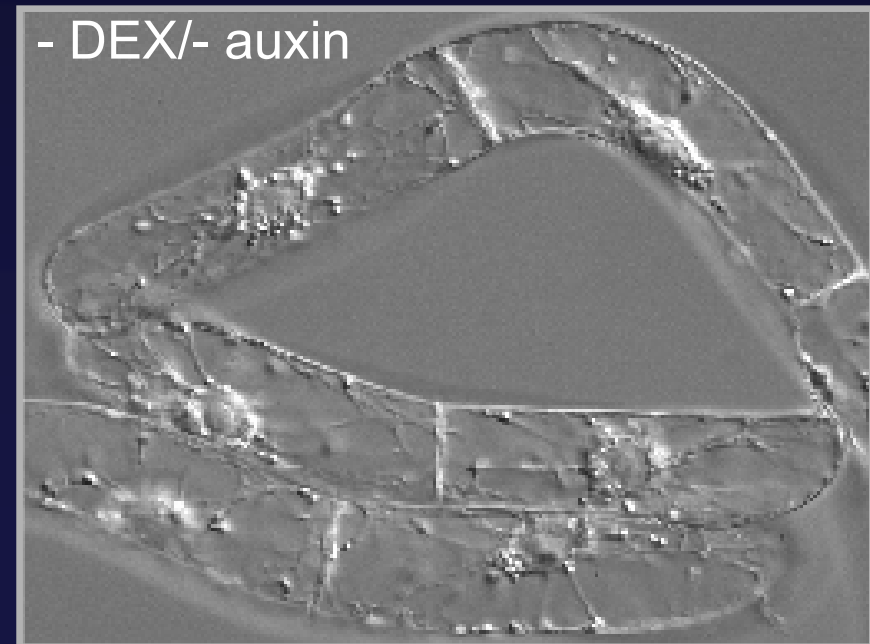
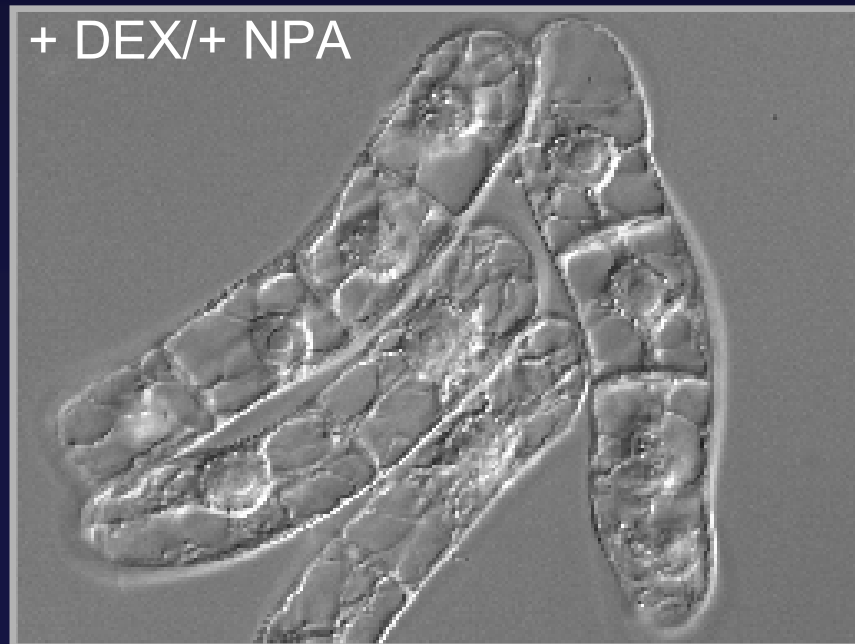
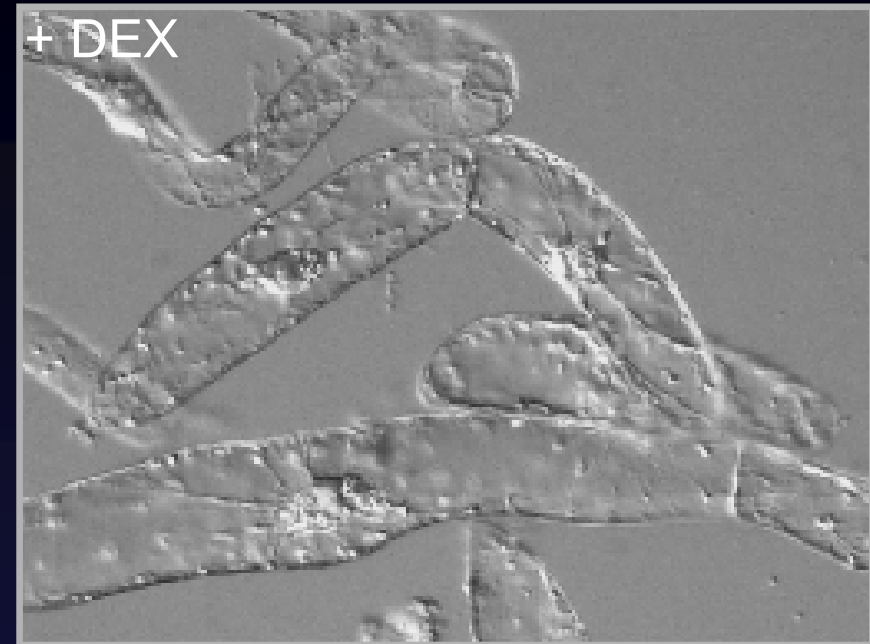
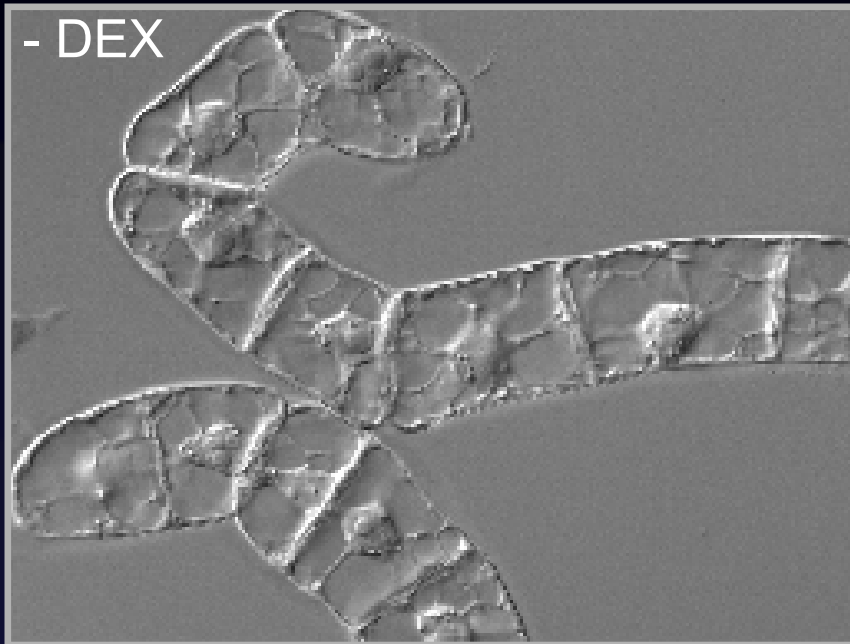
NAA accumulation kinetics



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

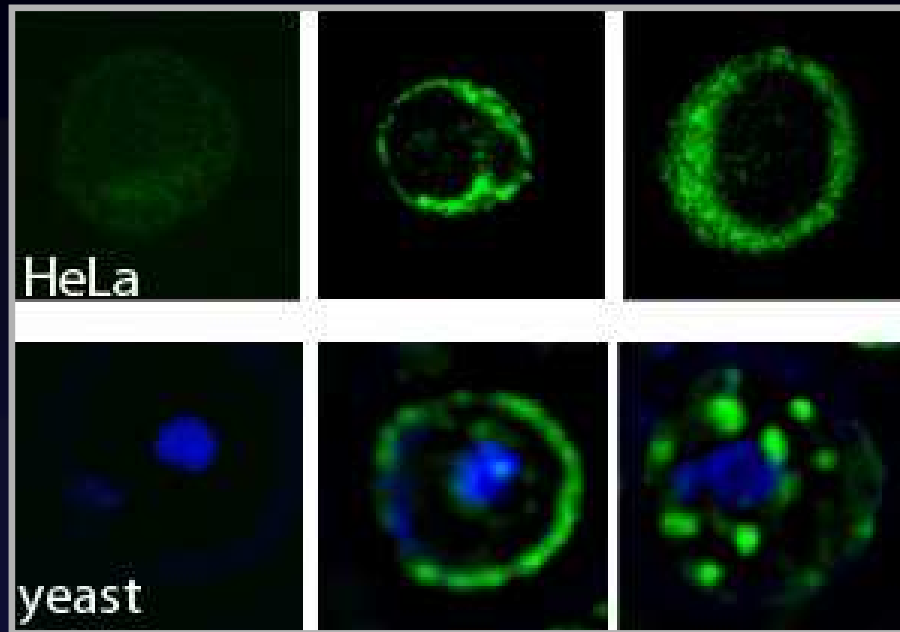


PIN-induced Phenotypes in BY-2 Cells

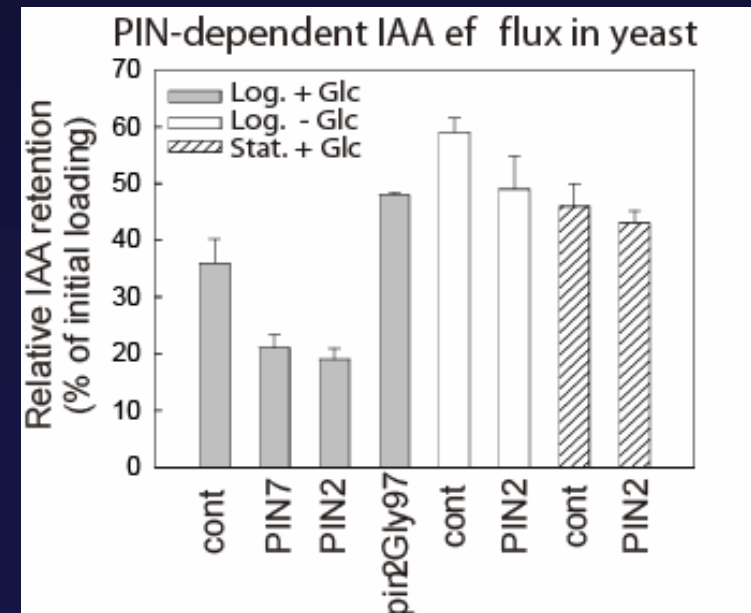
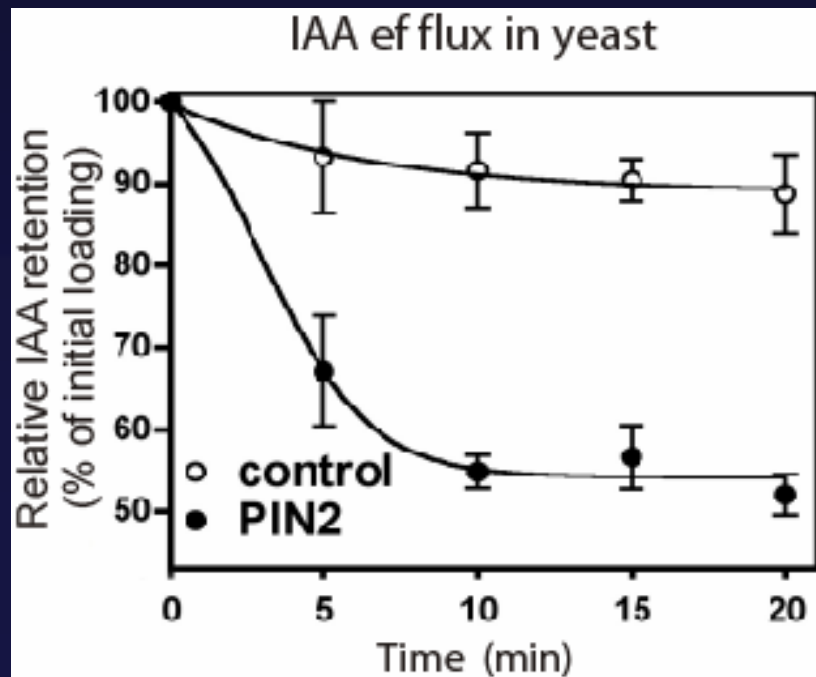
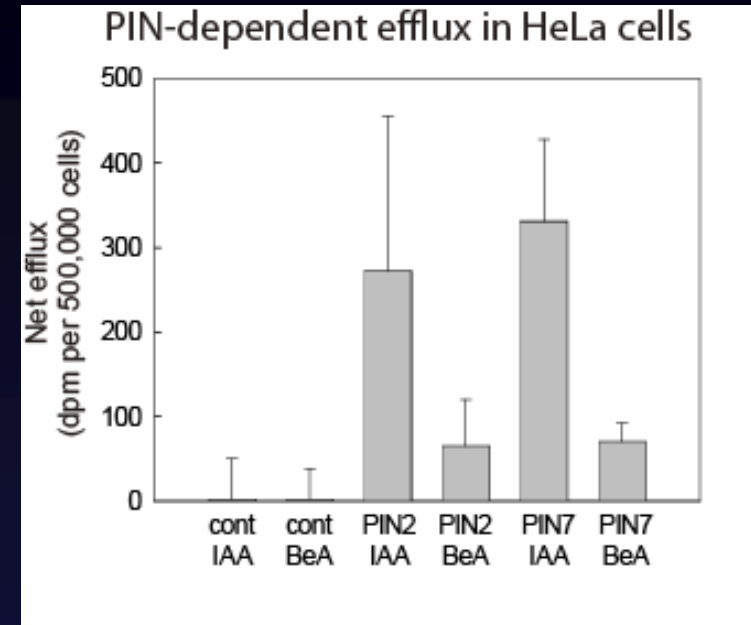


Expression of PINs in HeLa and Yeast

Heterologous PIN2 expression

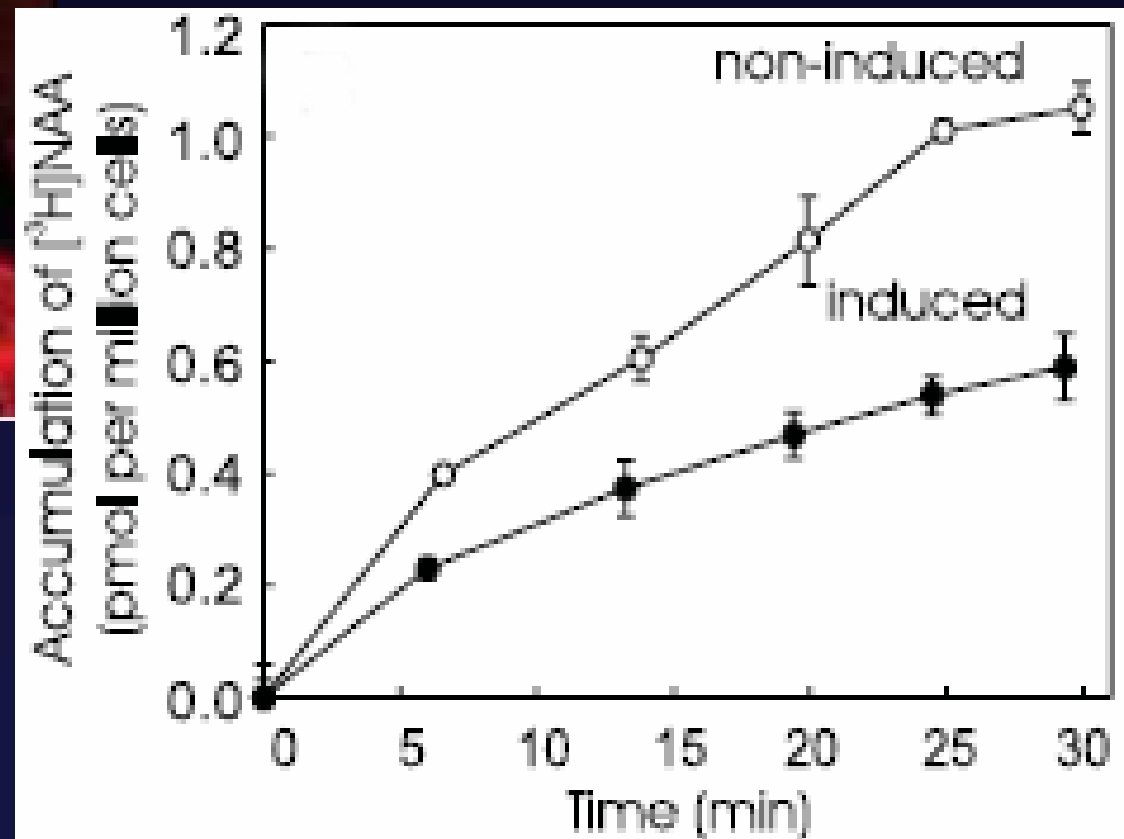
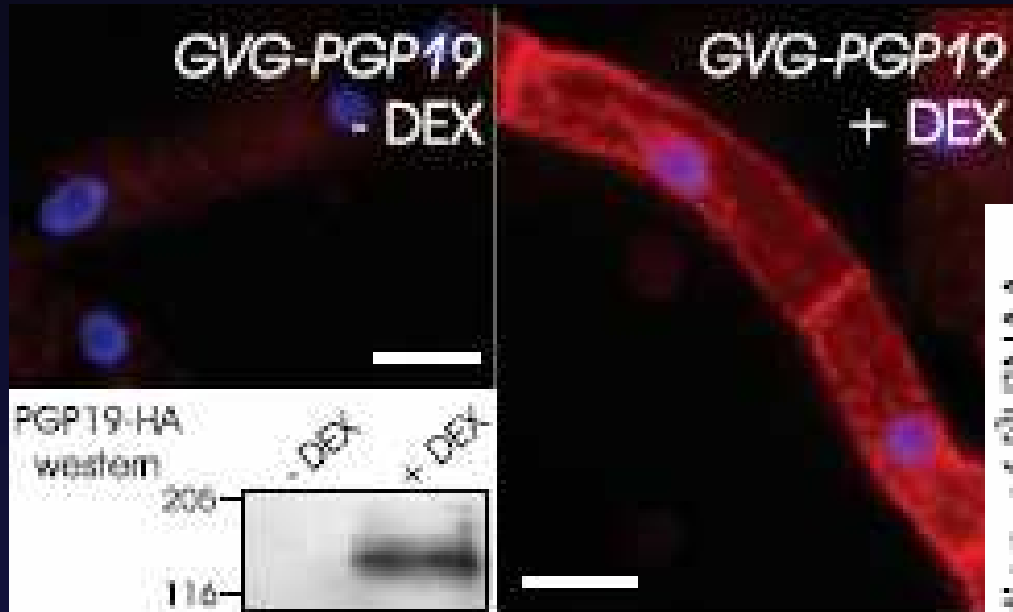


auxin efflux activity



PGP19 Mediates Auxin Efflux in BY-2 Cells

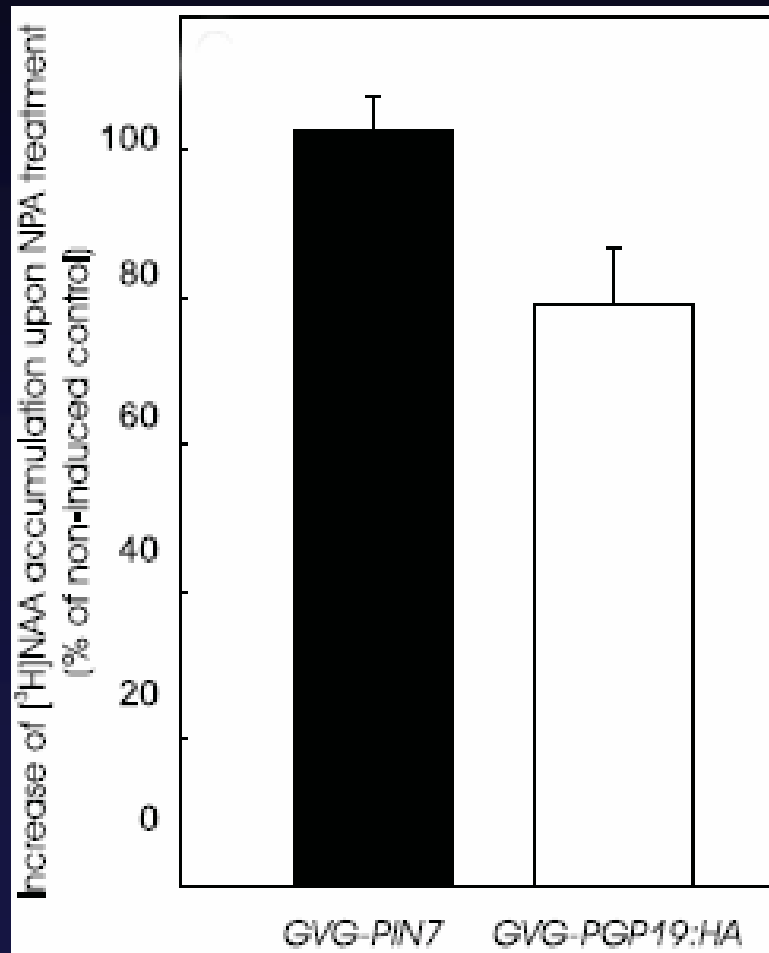
NAA Accumulation in BY-2 Cells



PGP19-induced Phenotypes in BY-2 Cells

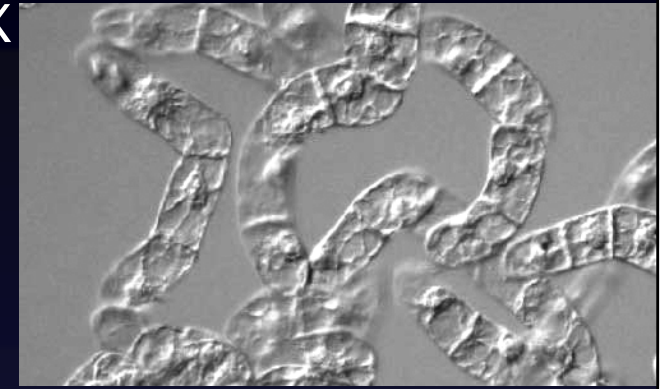


NPA Sensitivity of PIN- and PGP-mediated Auxin Efflux

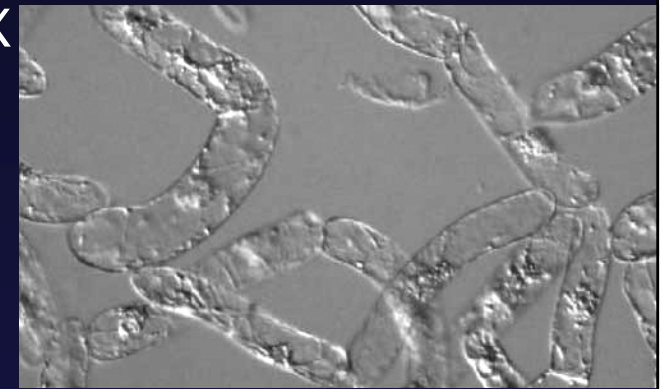


PGP19

- DEX



+ DEX

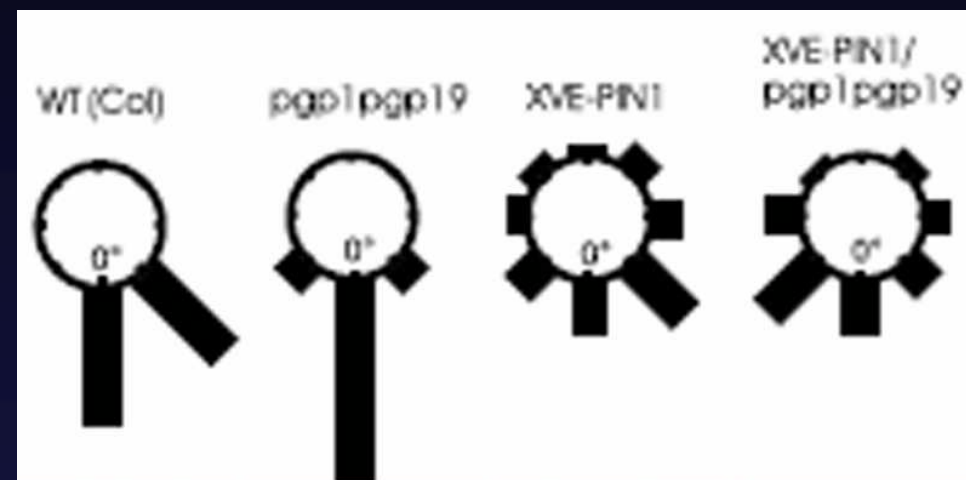
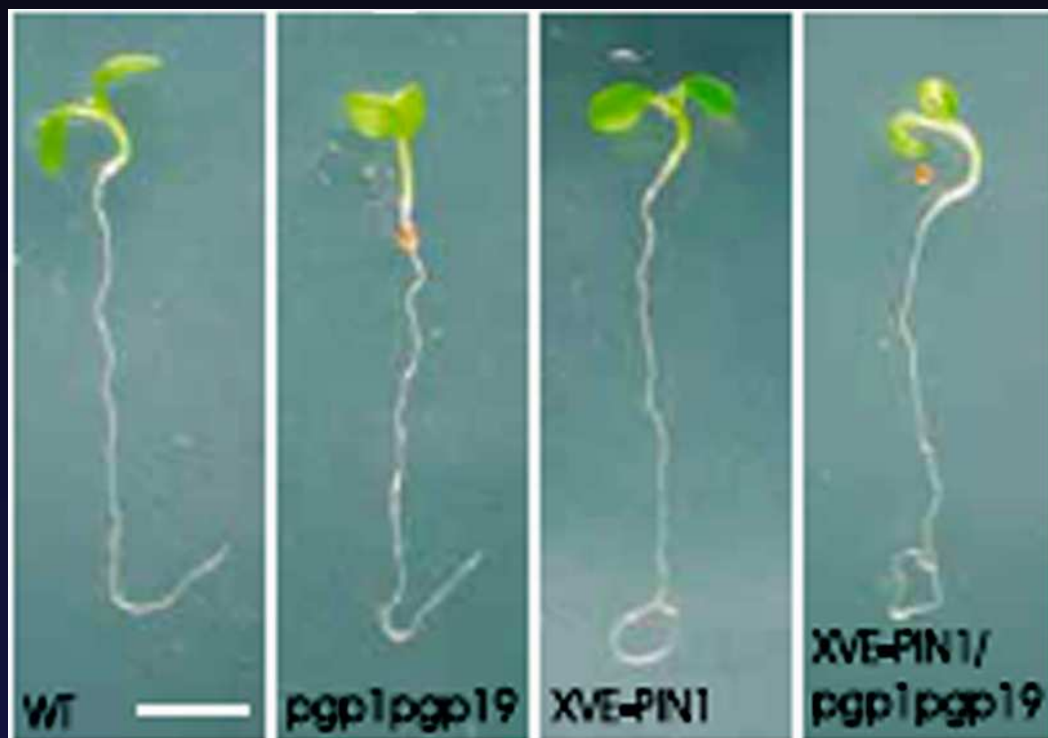


+ DEX/+ NPA



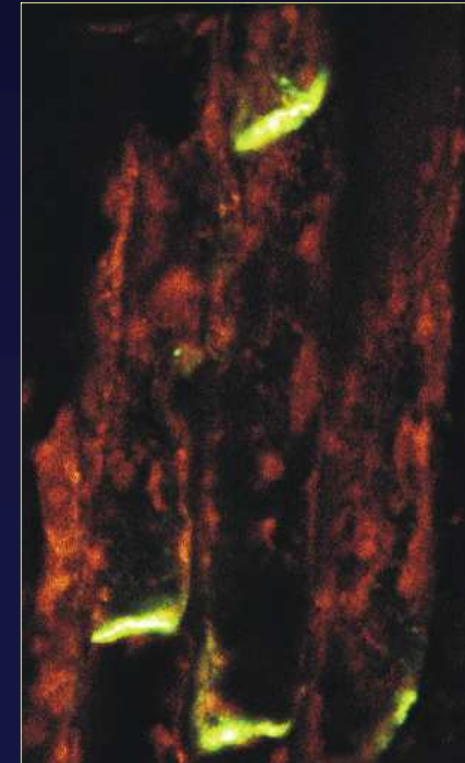
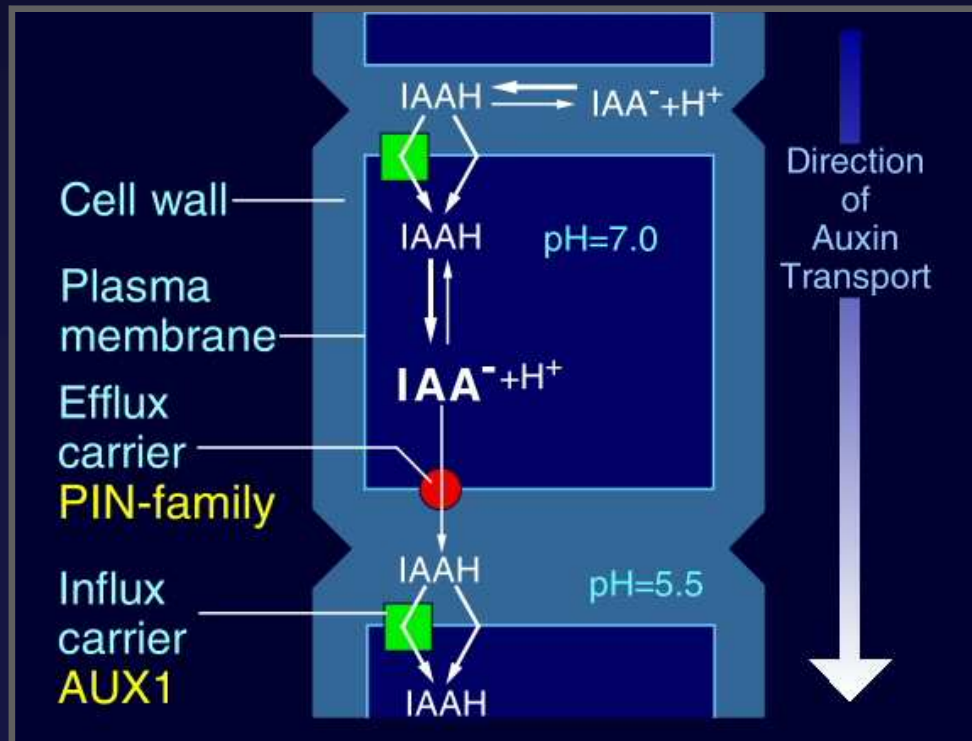
unpublished

PIN1 is Functional in Absence of PGP1 and PGP19



PINs and PGPs define distinct,
functionally independent transport systems

Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow



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

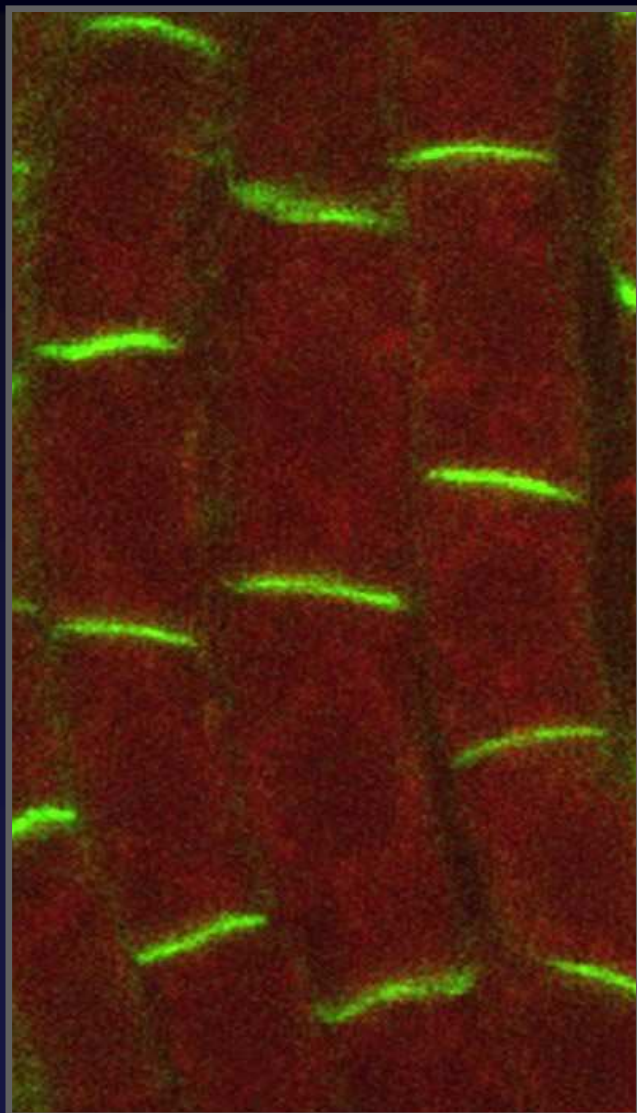
and

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

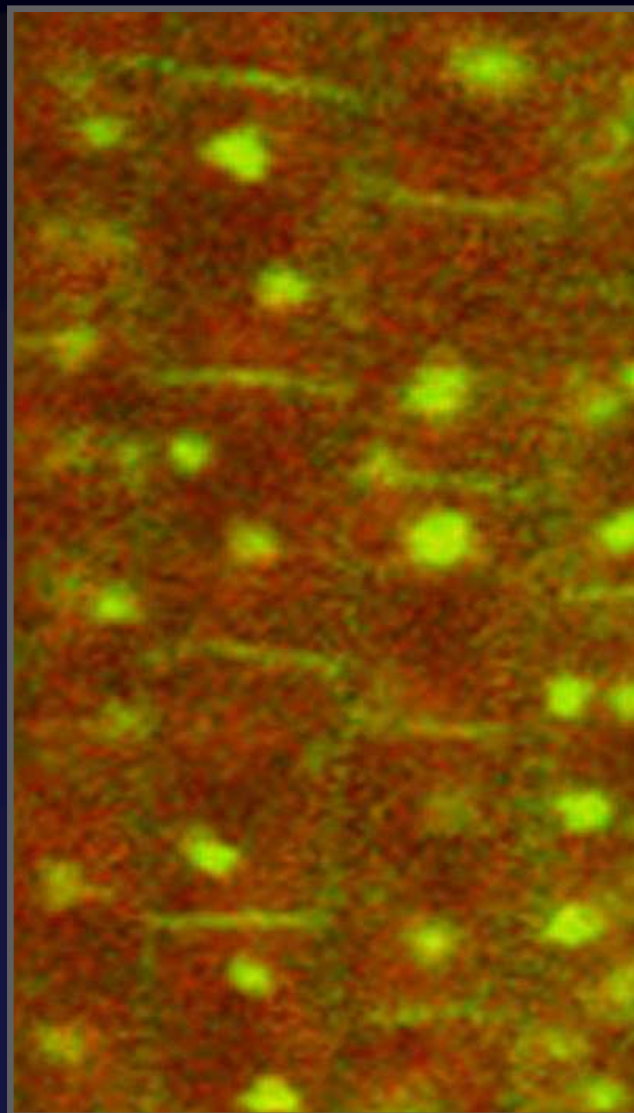
Constitutive Cycling of PINs

PIN1 Subcellular Movement

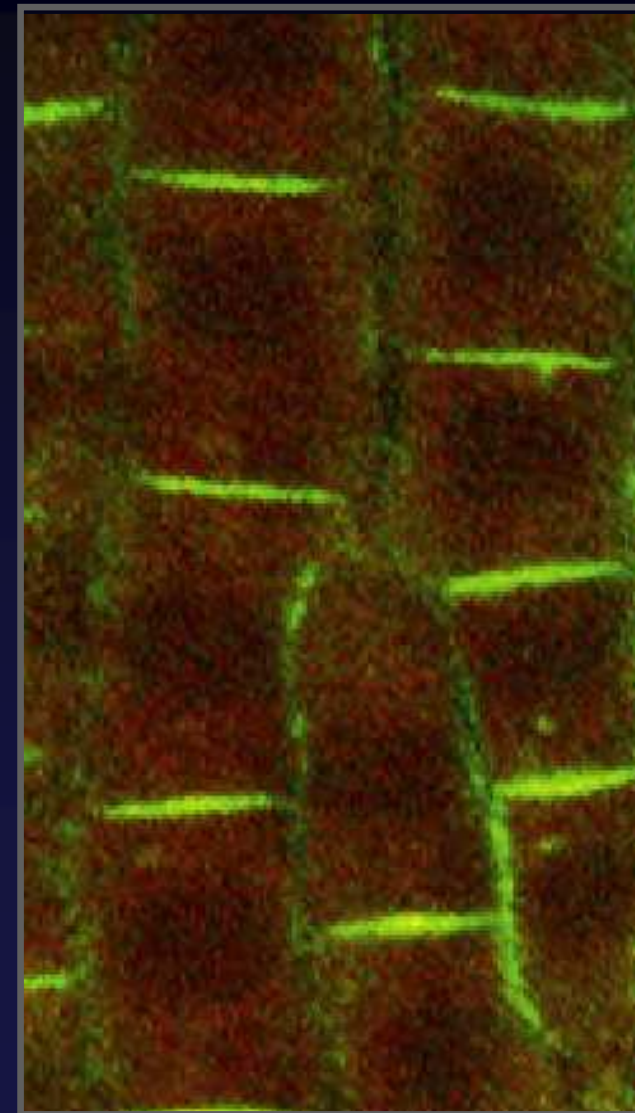
untreated



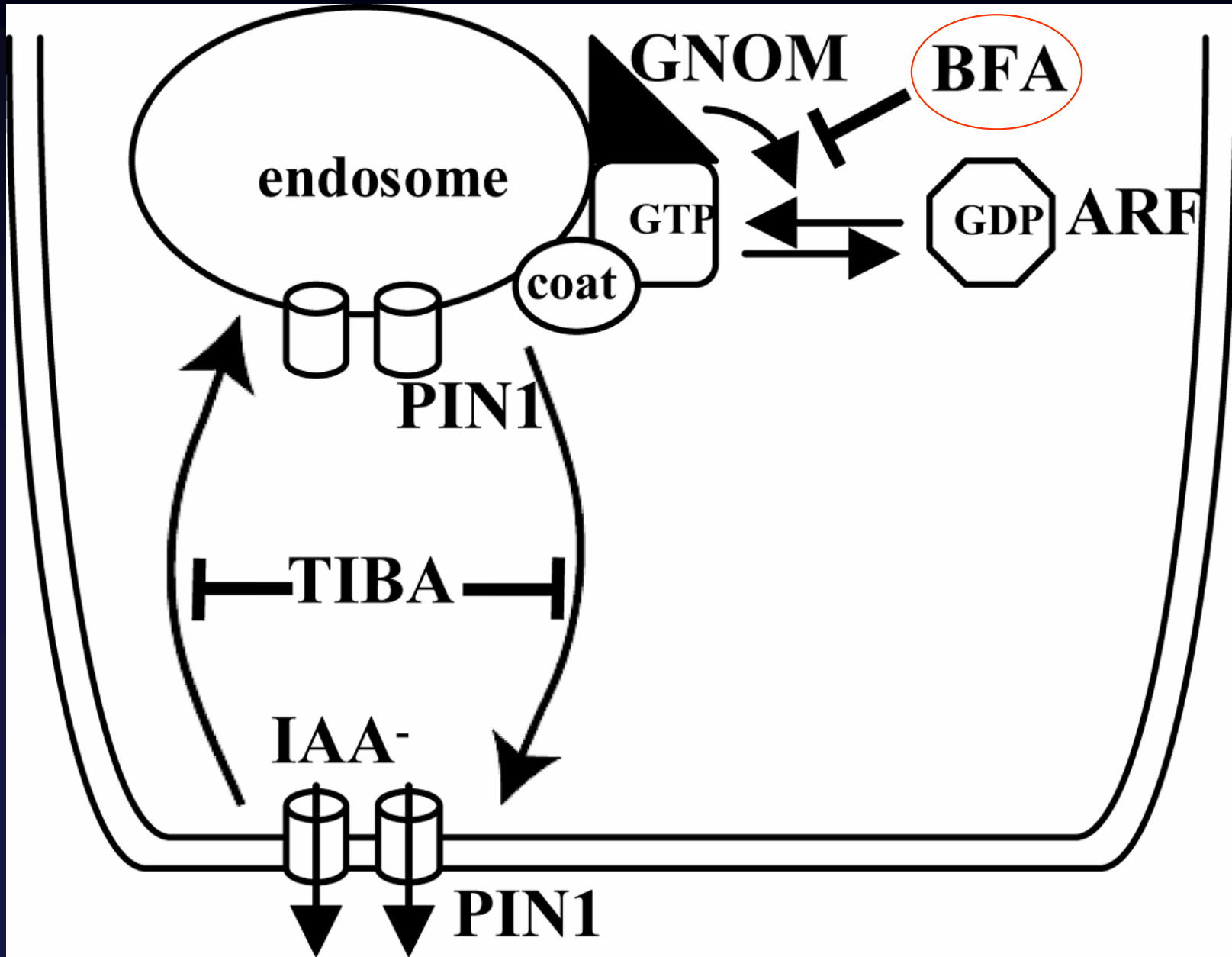
+ BFA



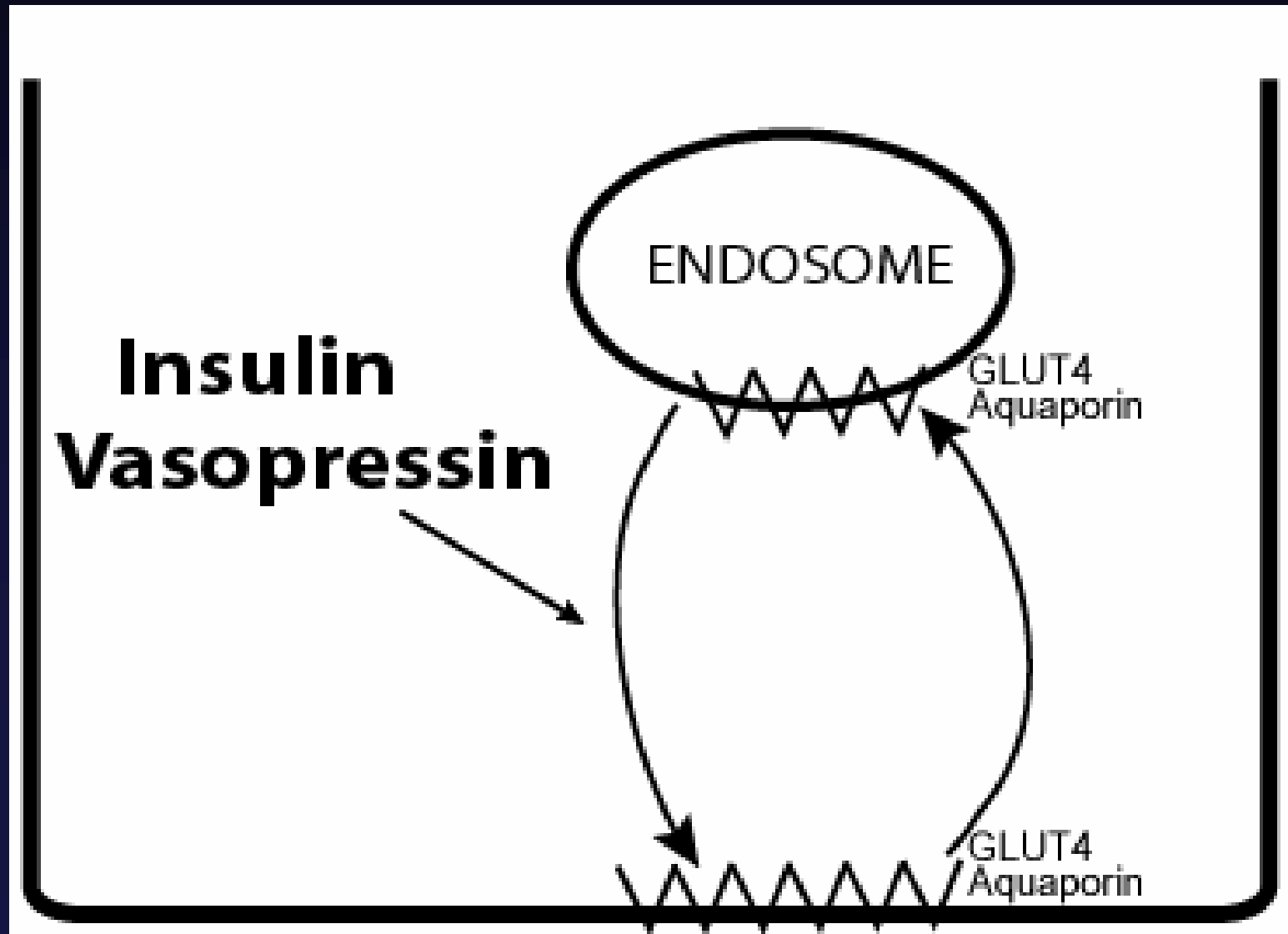
- BFA



Dynamic Movement of PIN Proteins

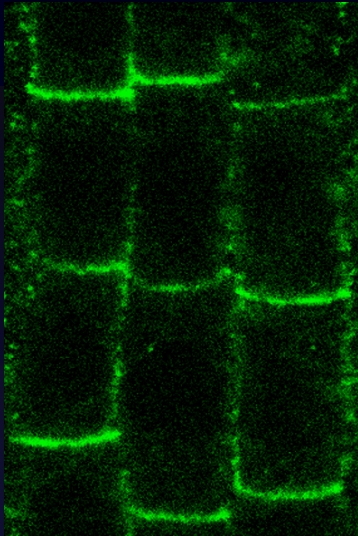


Subcellular Cycling – Means to Modulate Protein Activity?

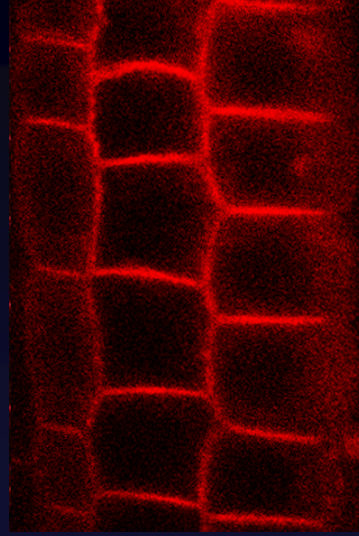


Auxin Inhibits Internalization of Plasma Membrane Proteins

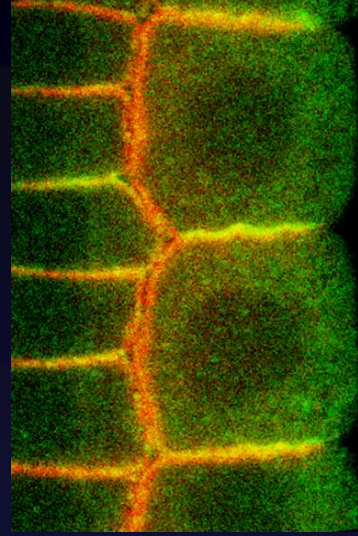
NAA
/BFA



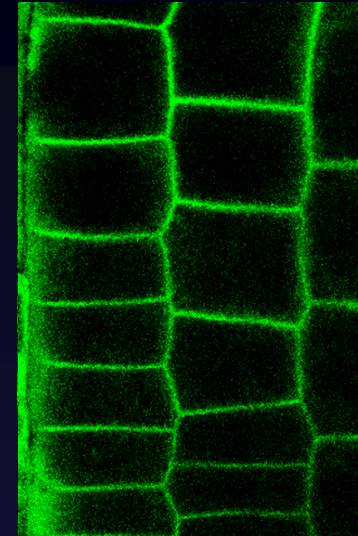
PIN1



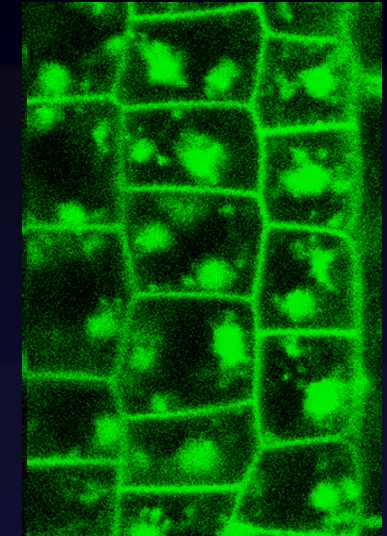
PM-ATPase



PIN2/ATPase

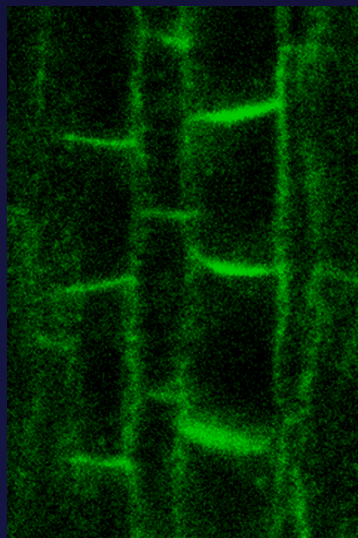


PIP2

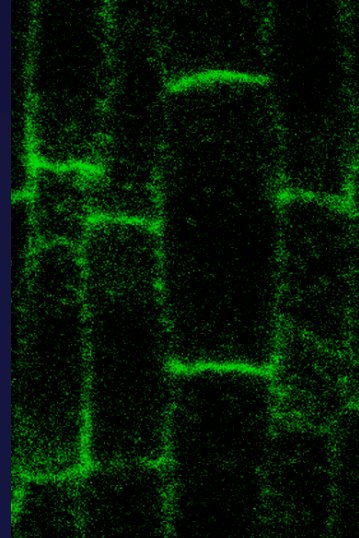


BRI1

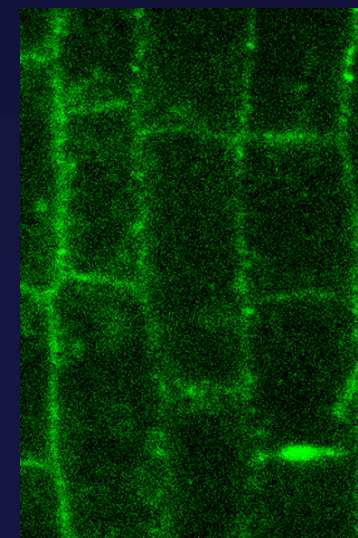
IAA/BFA



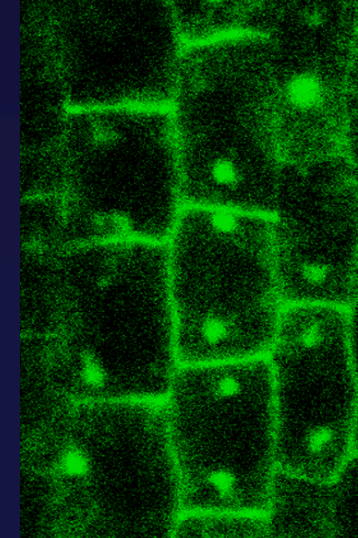
2,4-D/BFA



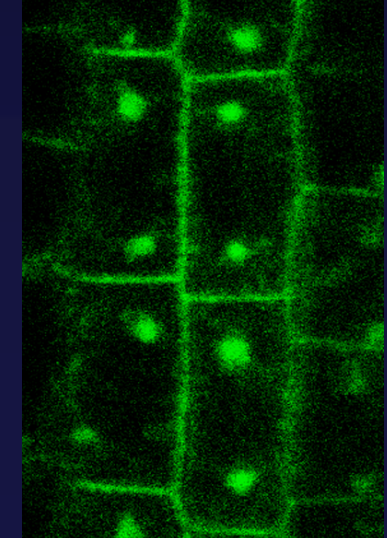
BFA in *sur2*



2-NAA/BFA



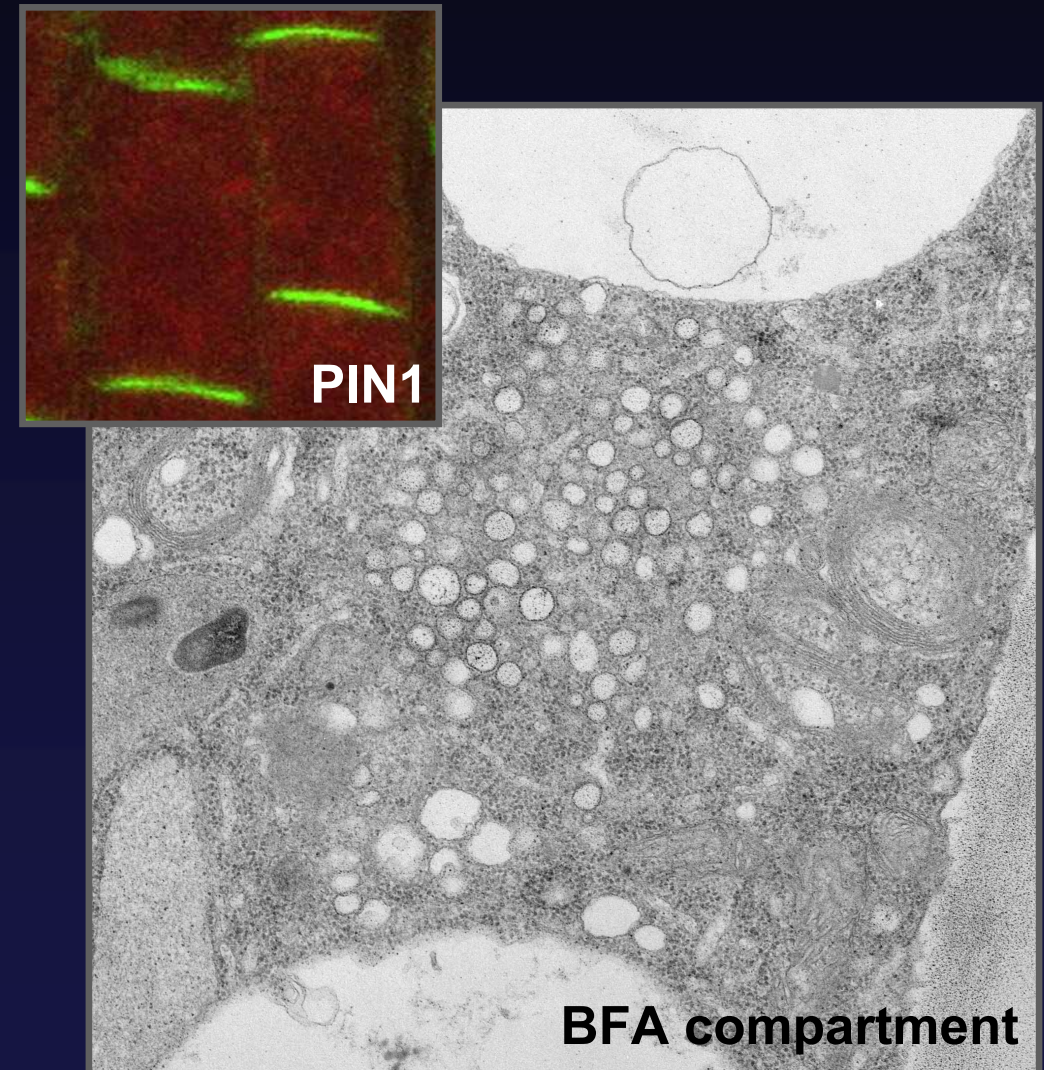
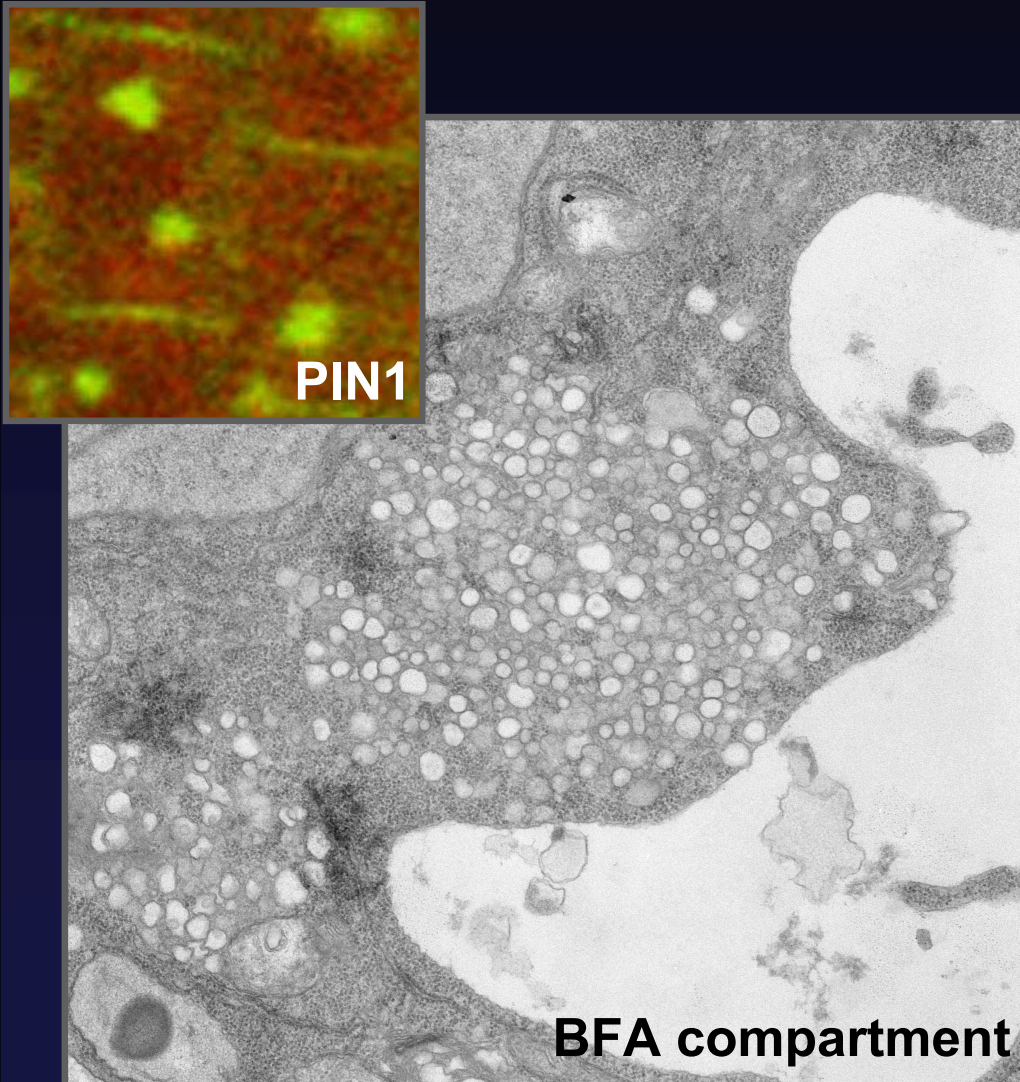
Ethylene/BFA



Place of Auxin Action in Protein Cycling

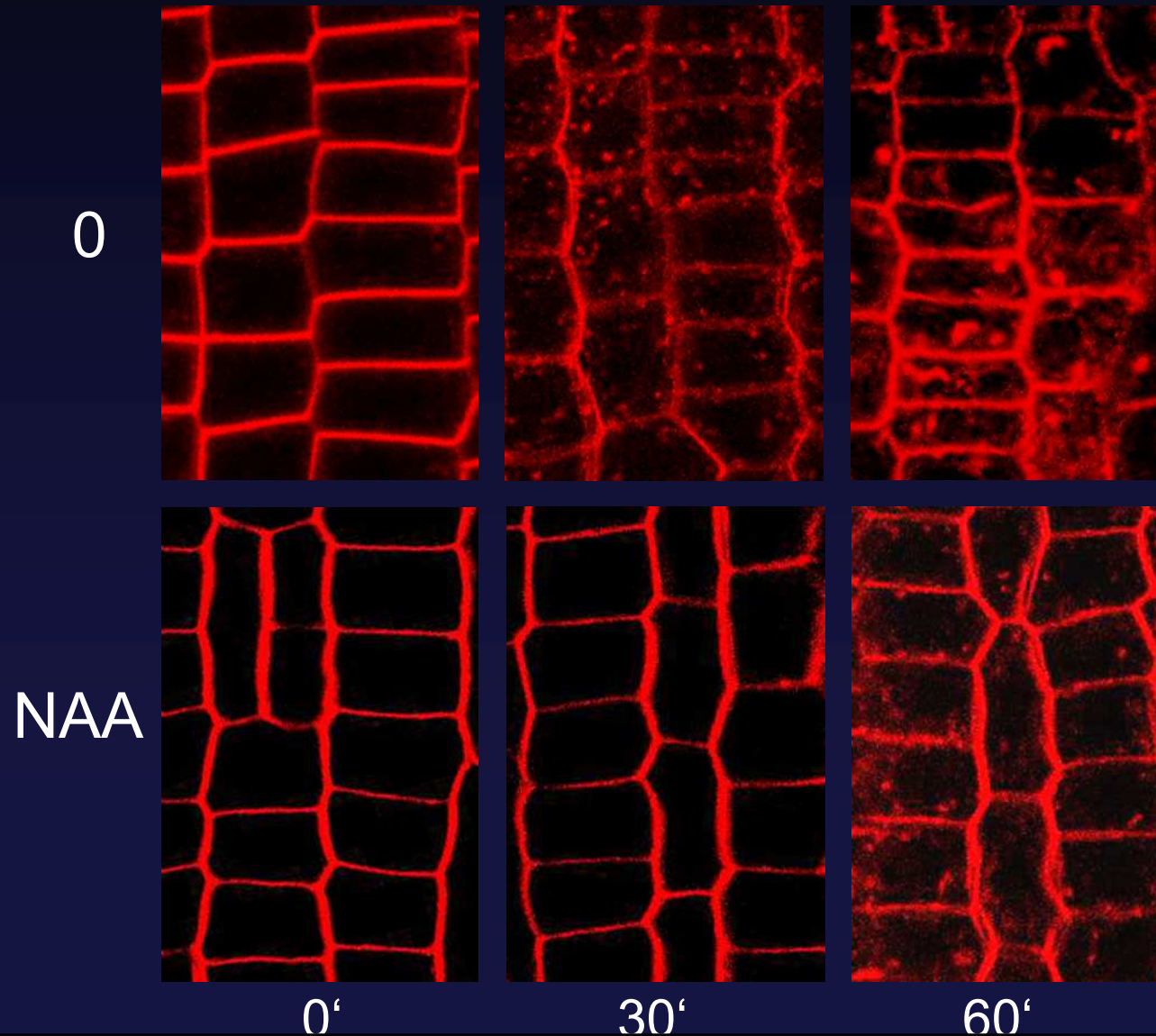
BFA

Auxin + BFA



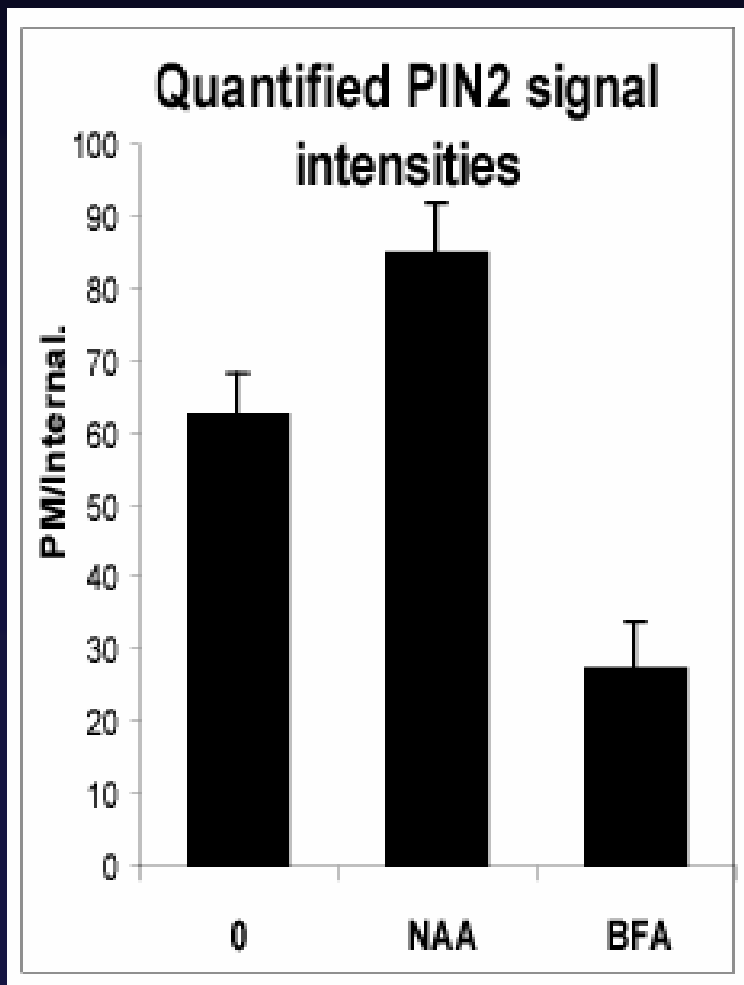
Auxin Inhibits Endocytosis

Uptake of endocytic tracer FM4-64

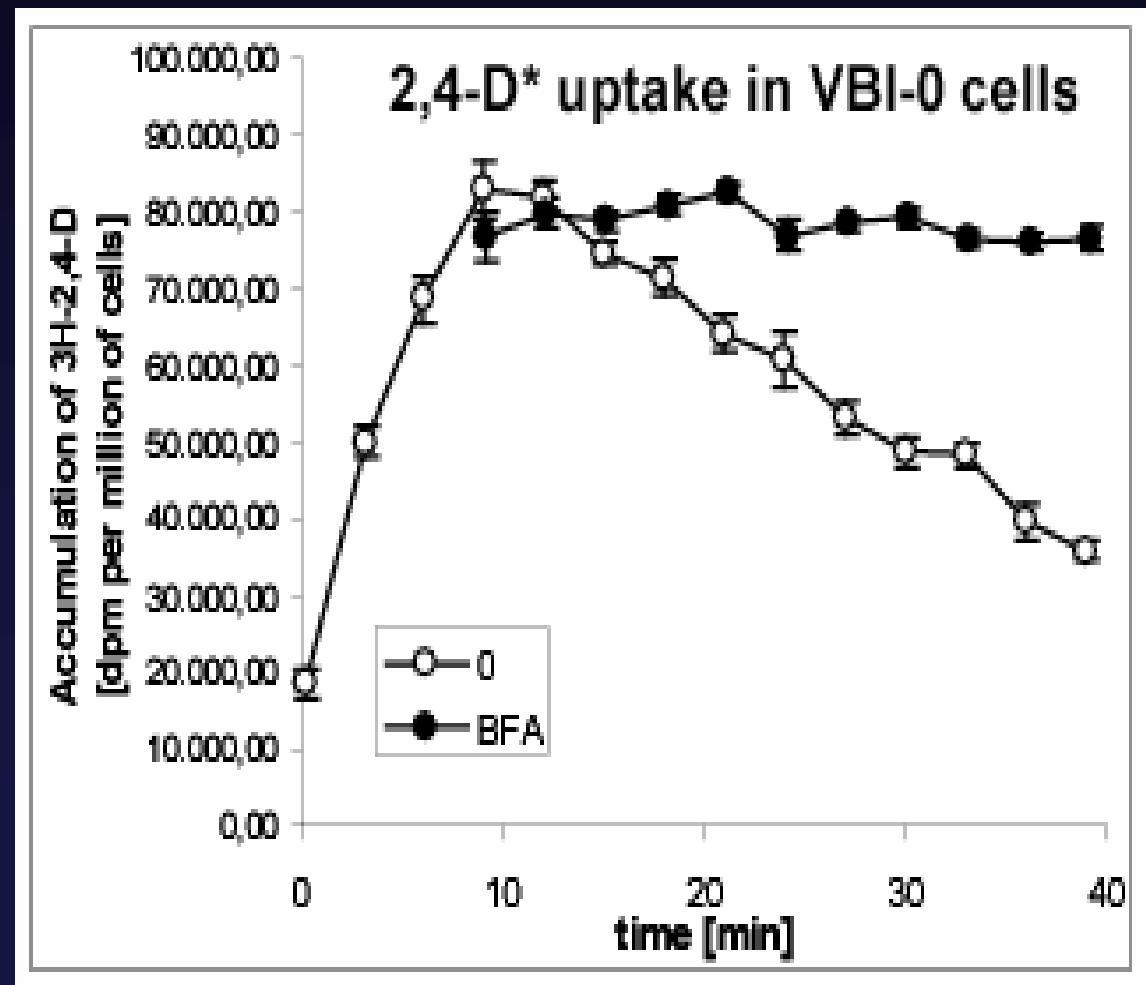


Auxin Increases PIN Levels at Cell Surface and Stimulates its own Efflux

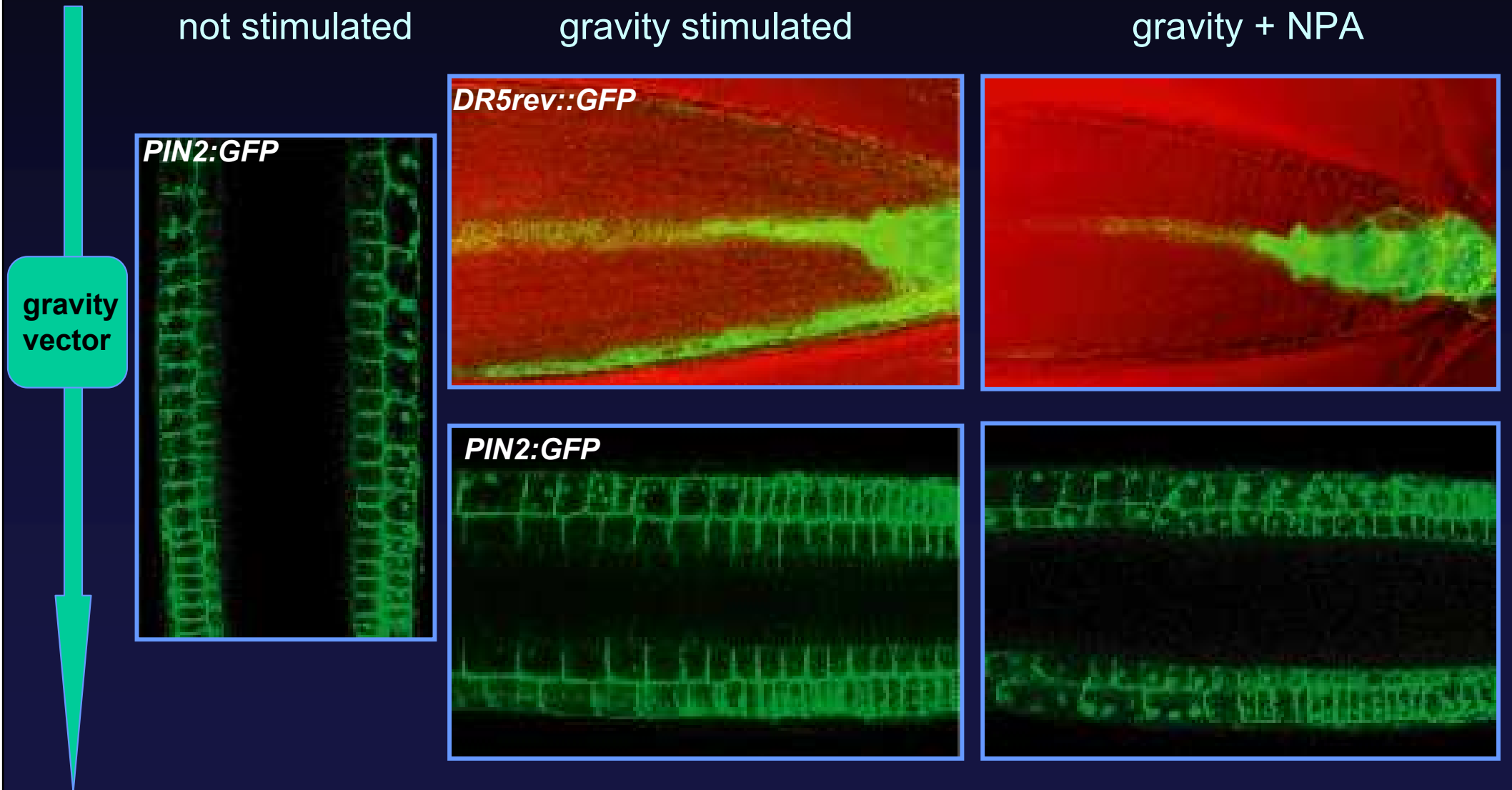
PIN2 levels at PM



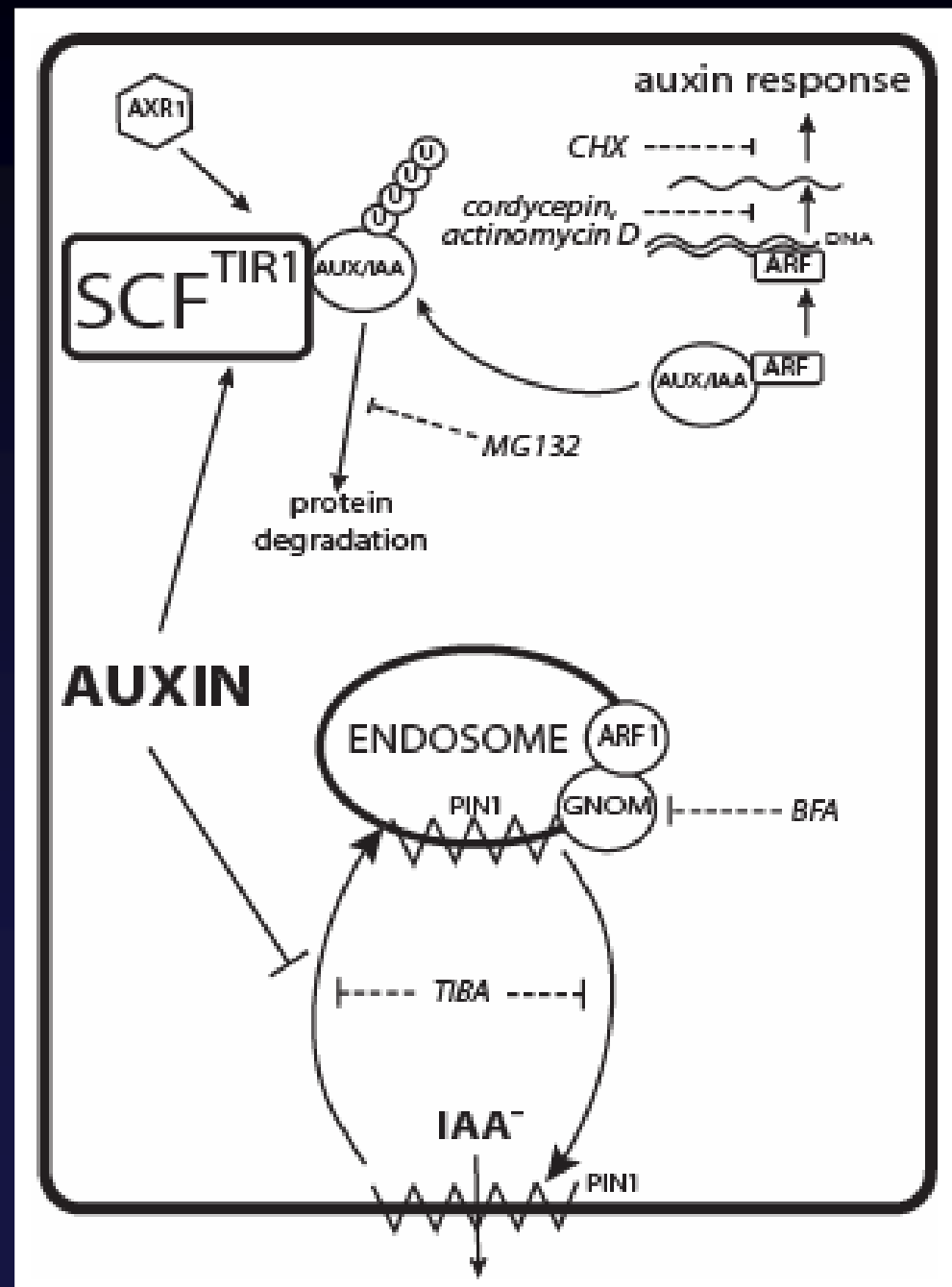
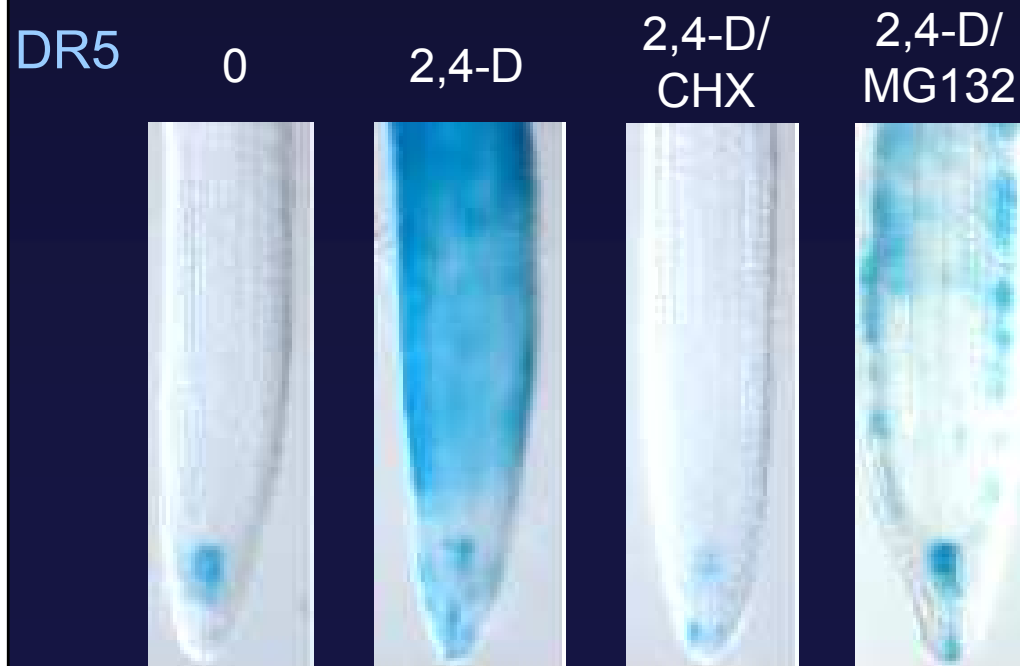
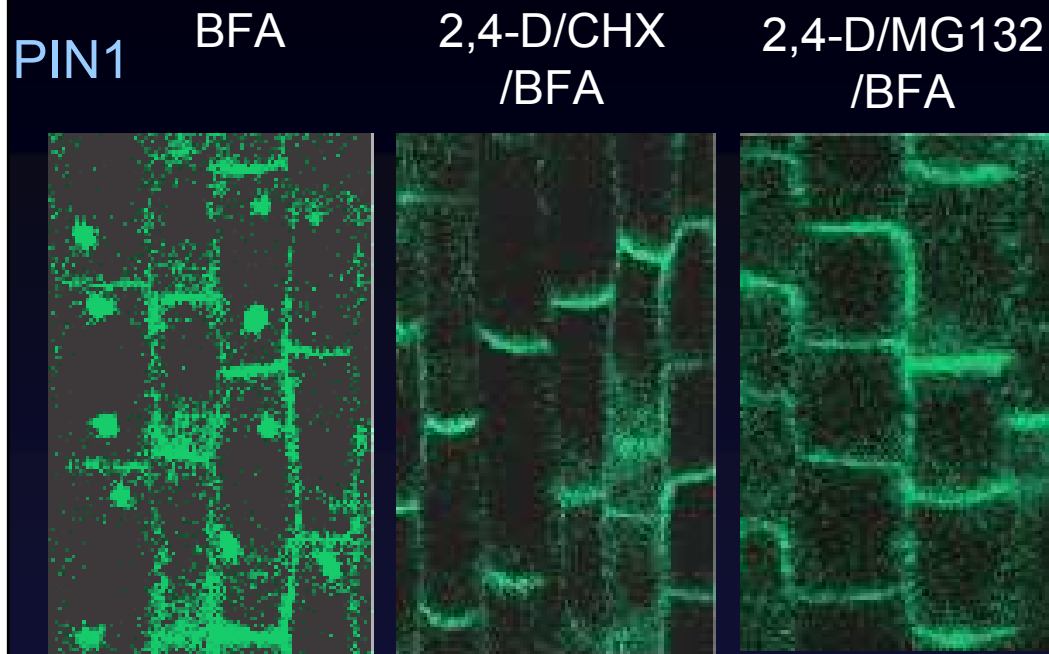
Auxin efflux in tobacco cells



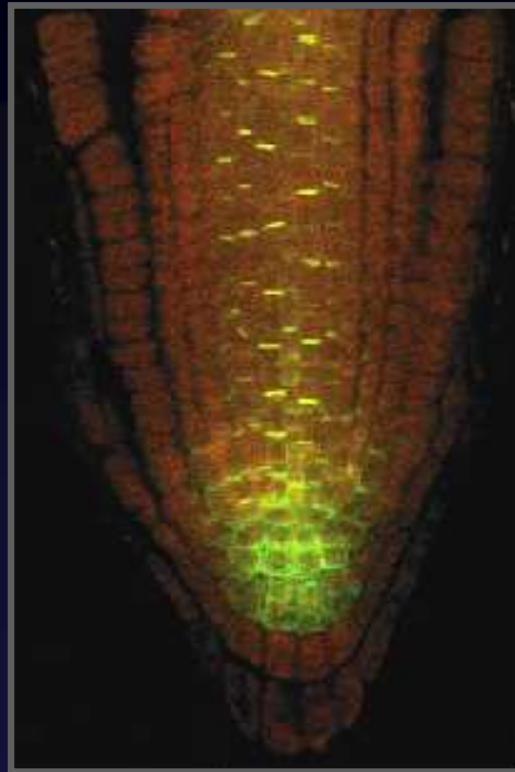
in planta Correlation between Cycling and Auxin Flow



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

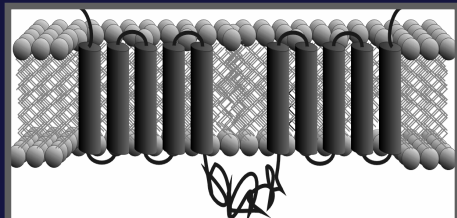
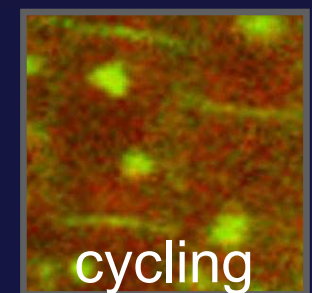
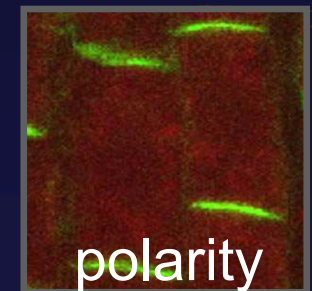
sequencing

**important
residues**

extragenic

cloning

novel genes

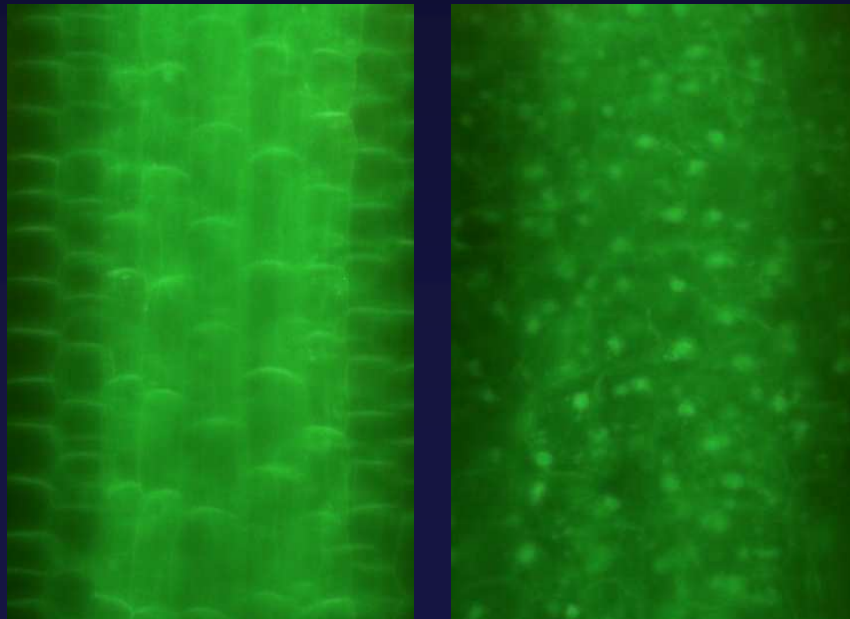


“Cell Biological”

Mutant Screens in Progress:

Auxin effect on endocytosis: 3 confirmed mutants

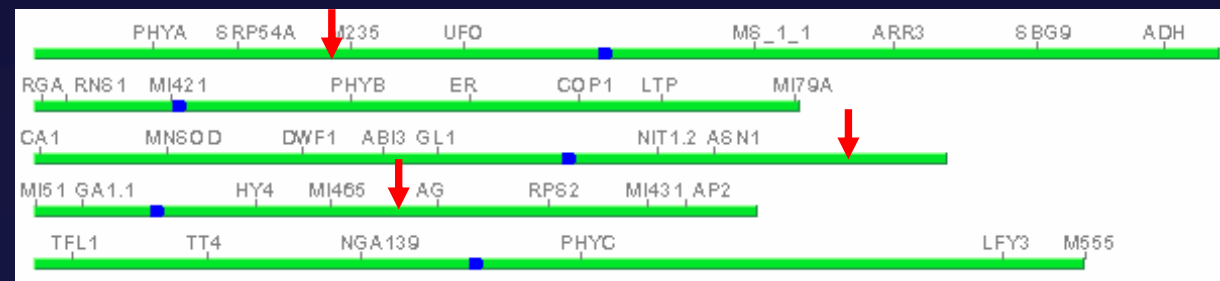
30' NAA 30 μ M/90' BFA 50 μ M



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