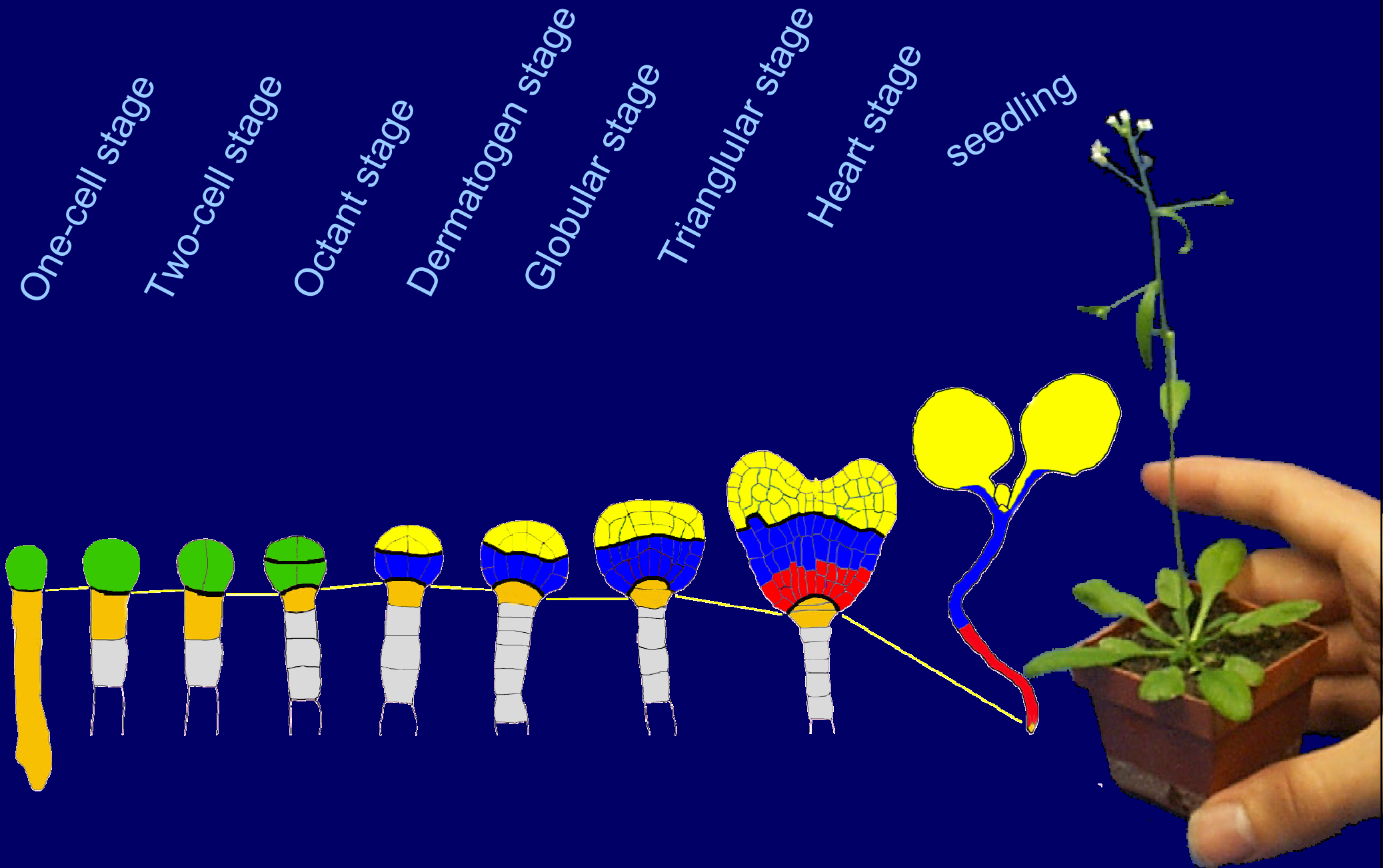
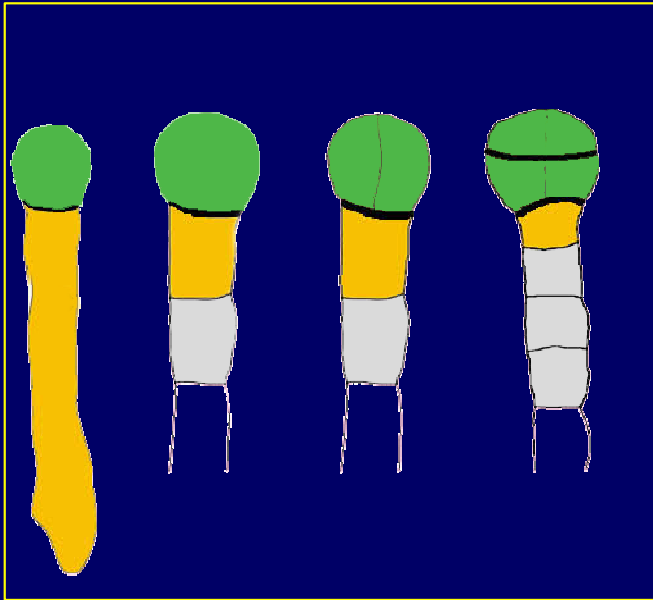


# Embryogenesis

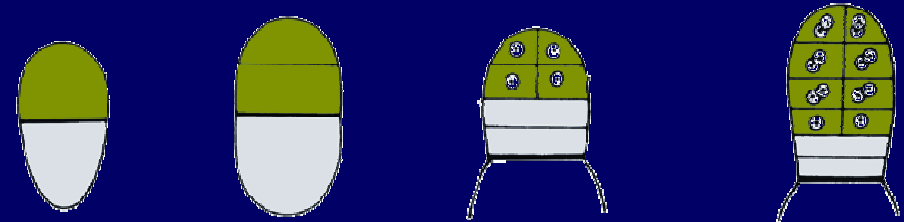
# Arabidopsis Embryogenesis



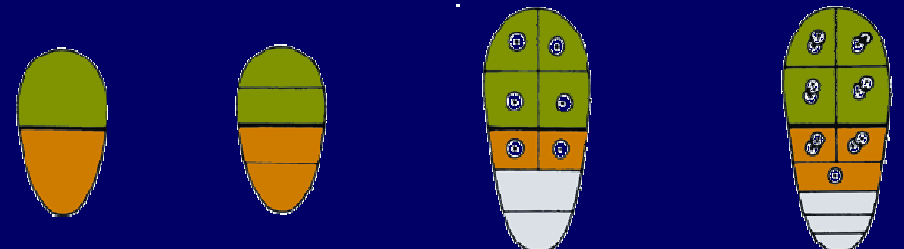
# Comparison of embryo development in Angiosperms



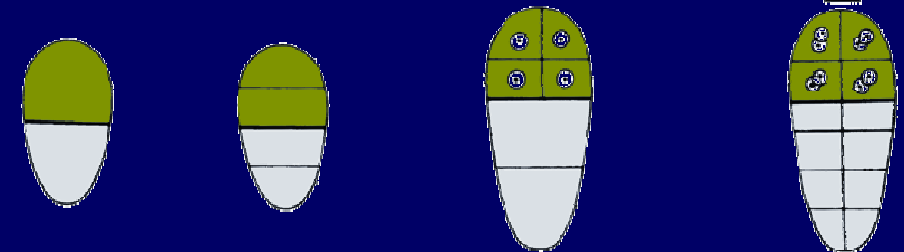
Caryophyllad



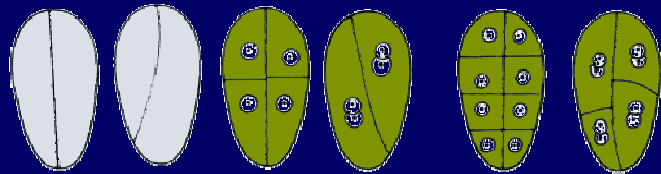
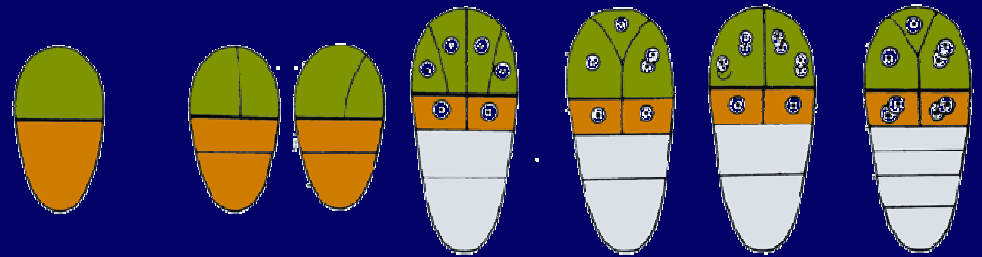
Chenopodiad



Solanad



Asterad



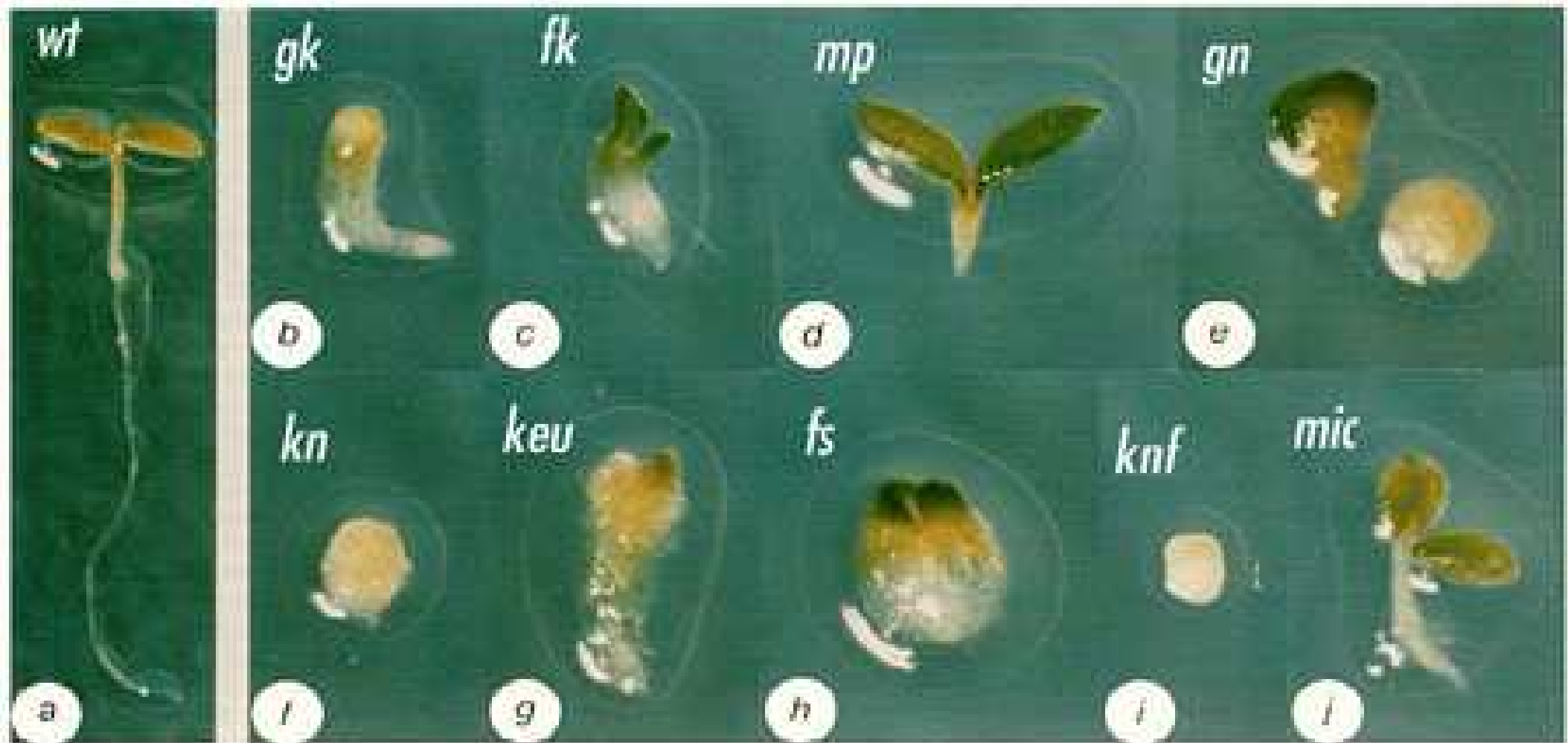
Piperad

Modified after  
Johri et al. 1992

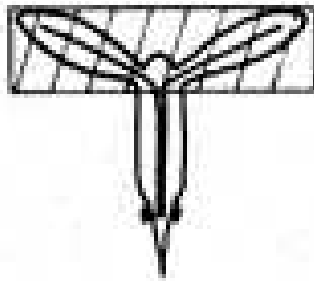
How can such a protected system be investigated experimentally?



# Mutant screen at seedling level



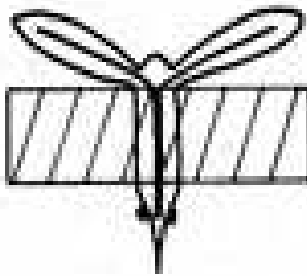
# Patterning mutant types



APICAL



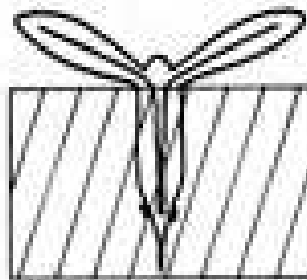
*(gurke)*



CENTRAL



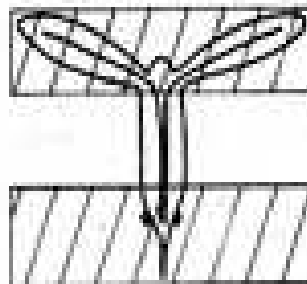
*(fackel)*



BASAL



*(monopteros)*



TERMINAL



*(gnom)*

Mutations in the ***BODENLOS*** (*bdl*) and ***MONOPTEROS*** (*mp*) genes lead to very similar deletions of basal pattern elements

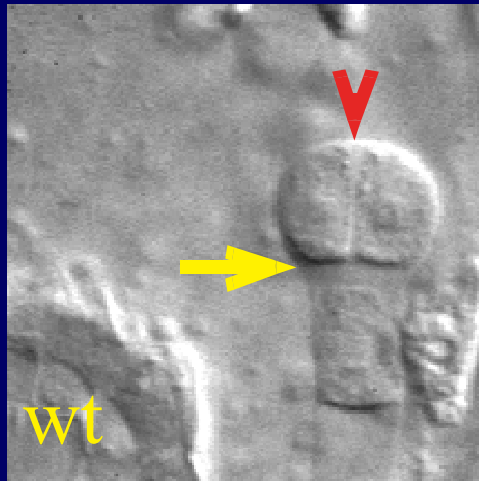
- *mp* seedling



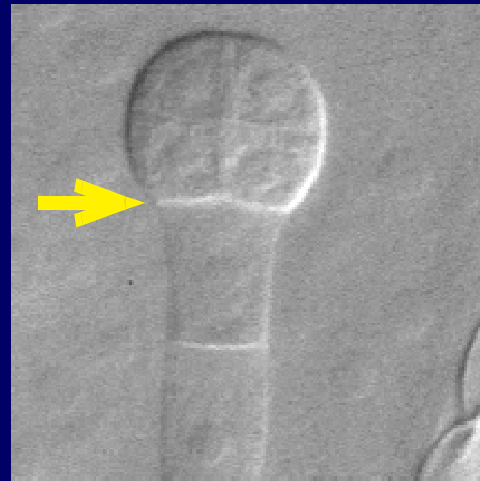
- *bdl* seedling



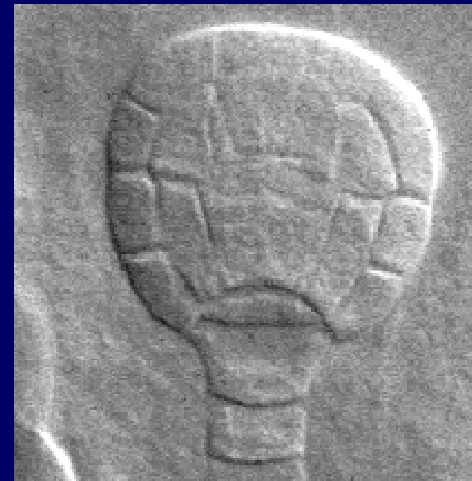
# The *bodenlos* (*bdl*) root meristem defect



Two-cell stage



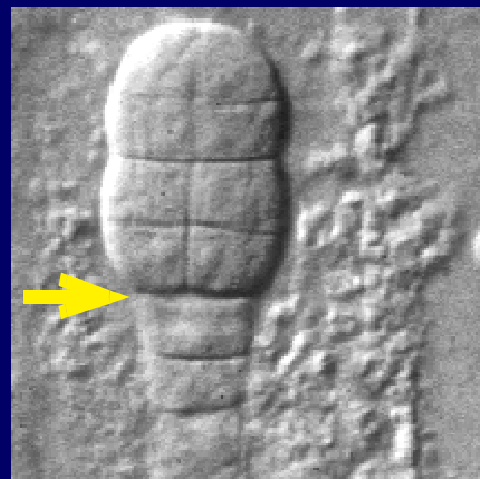
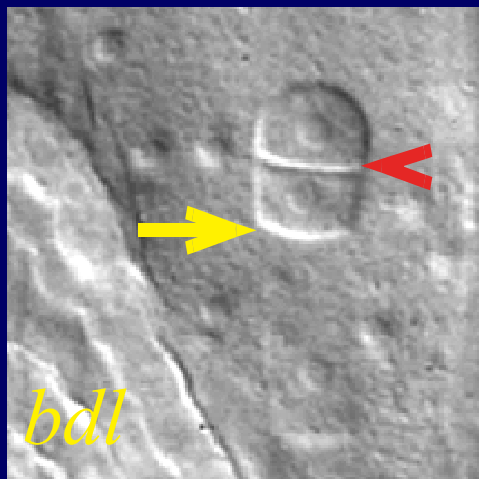
octant



globular

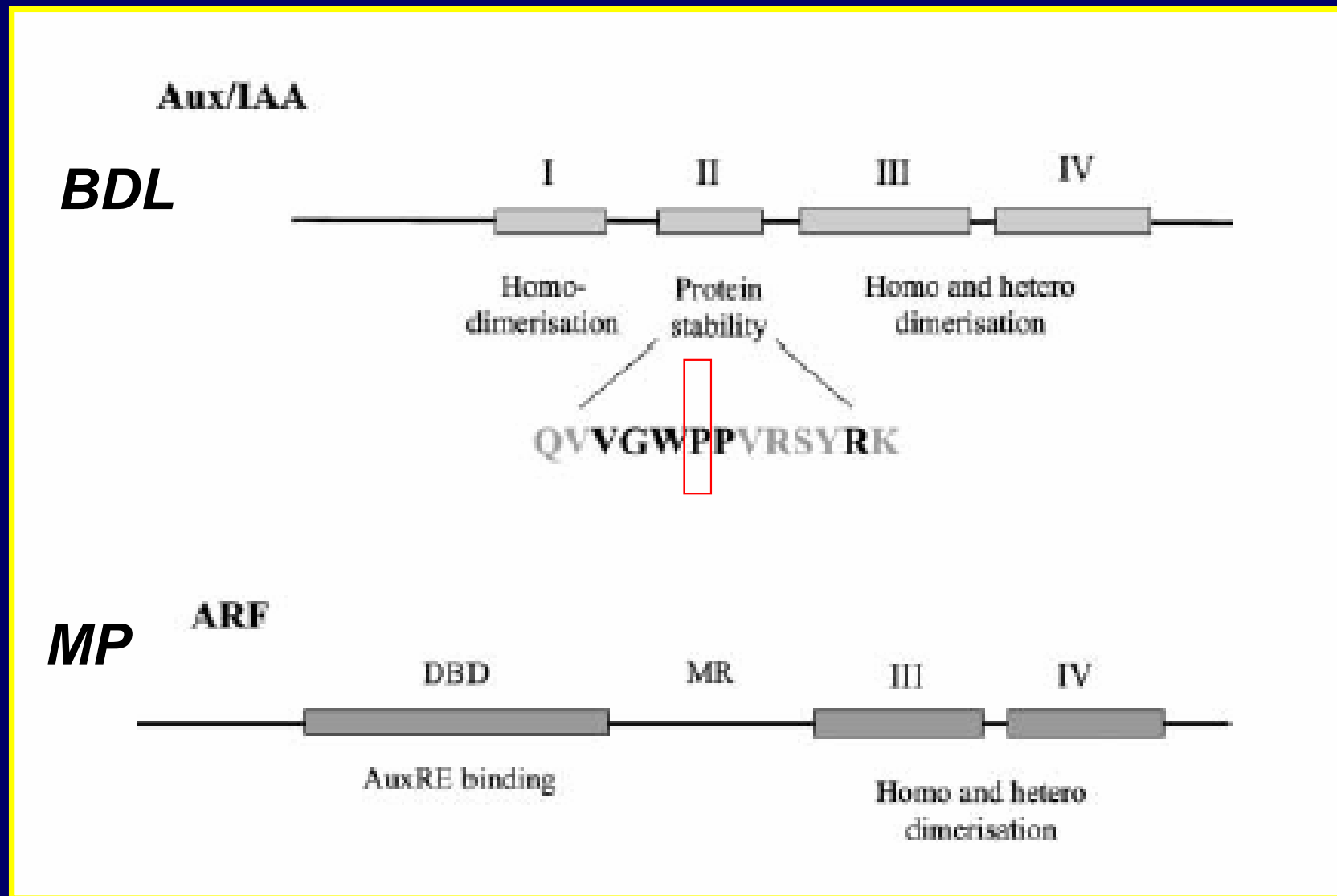


heart



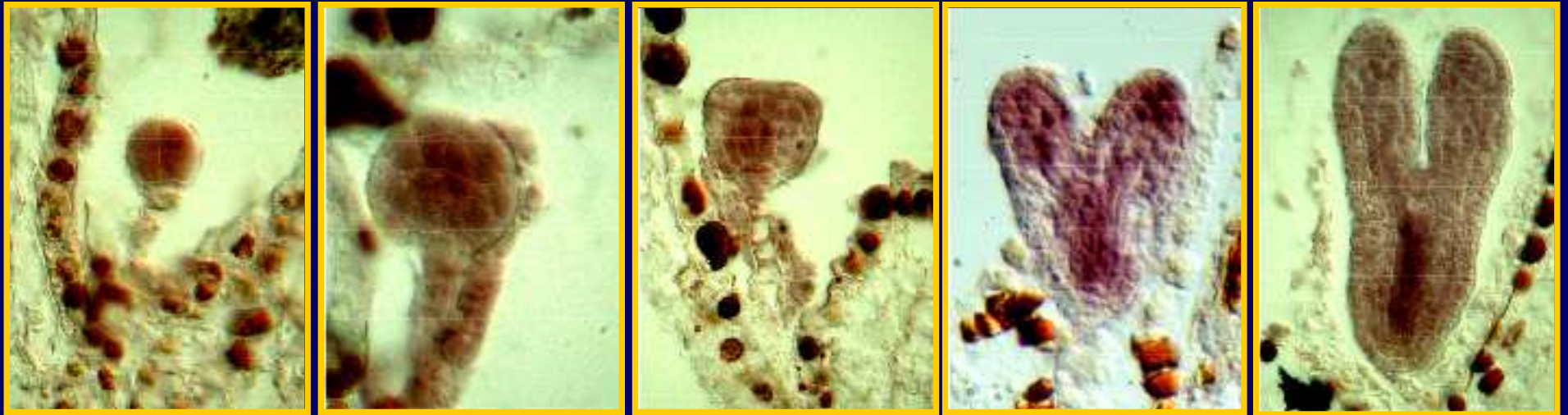


*MP* encodes for ARF5, an **activator** of auxin response, whereas *BDL* encodes for IAA12 the corresponding **repressor**

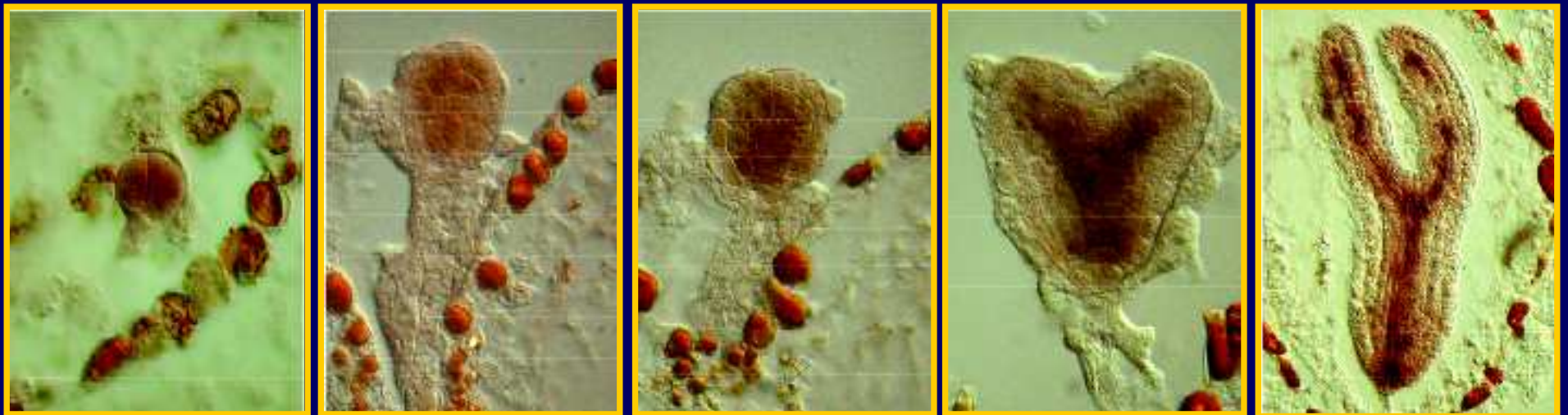


# Expression patterns of BDL and MP

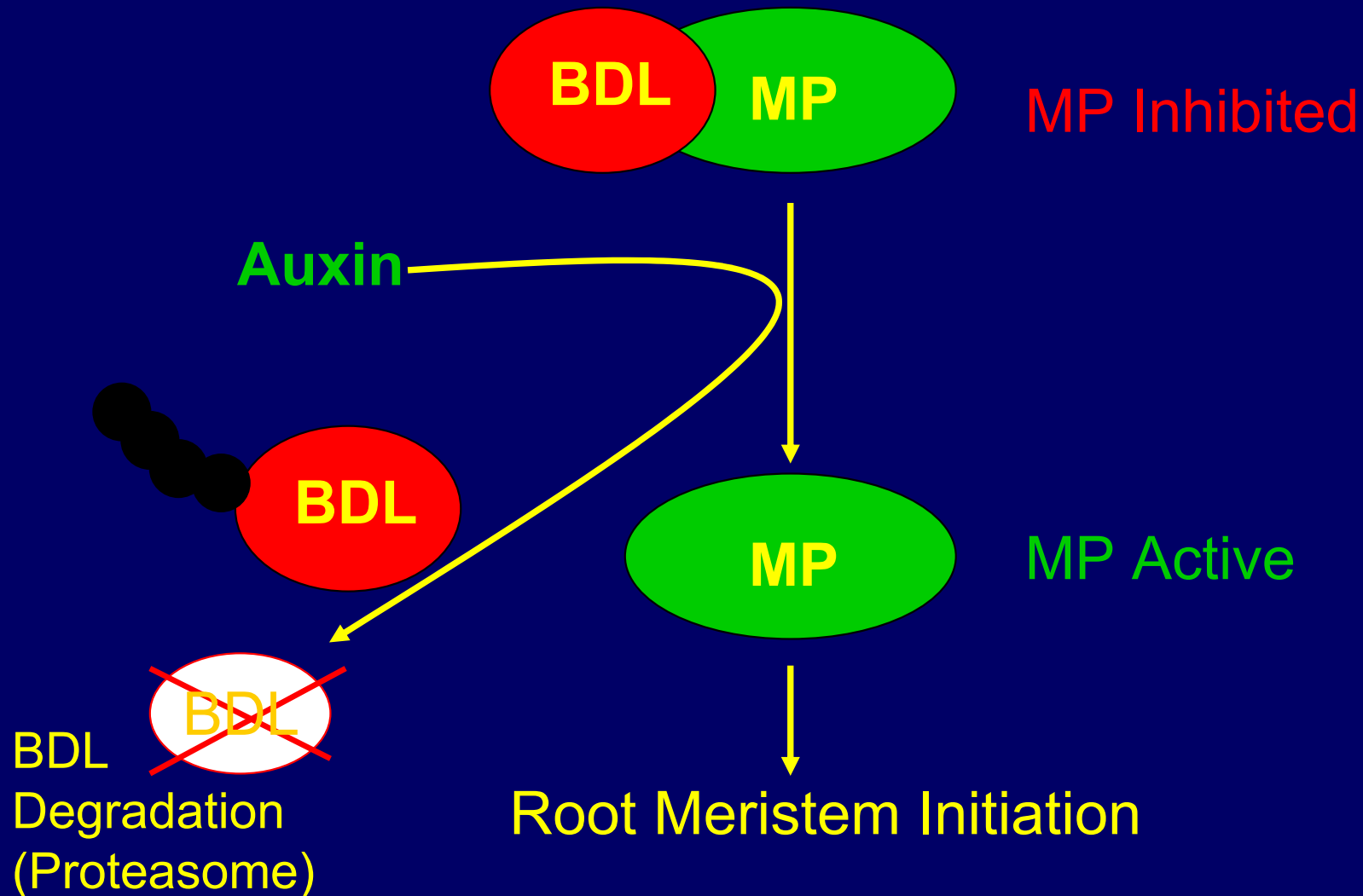
*bdl*



*mp*

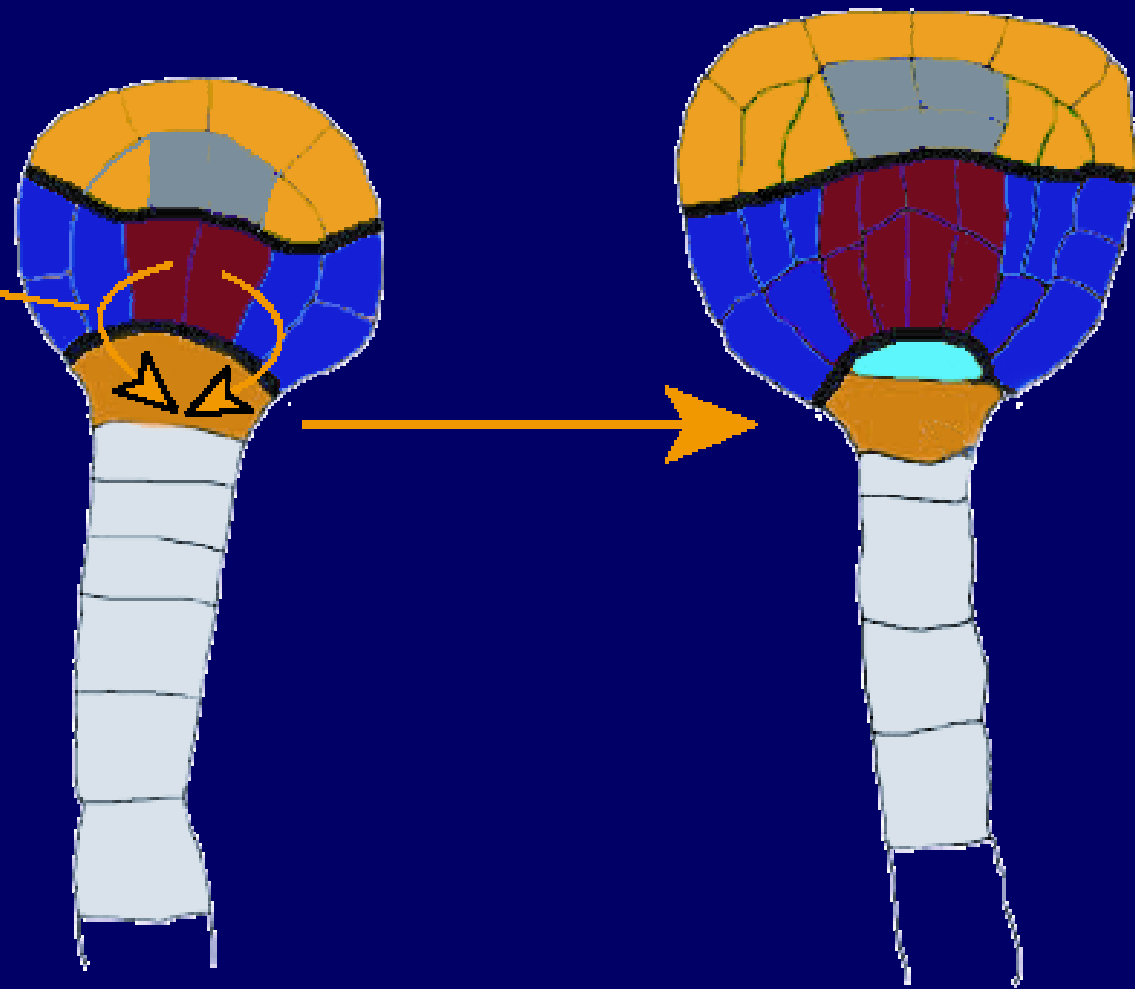


# Model of BDL/MP interaction

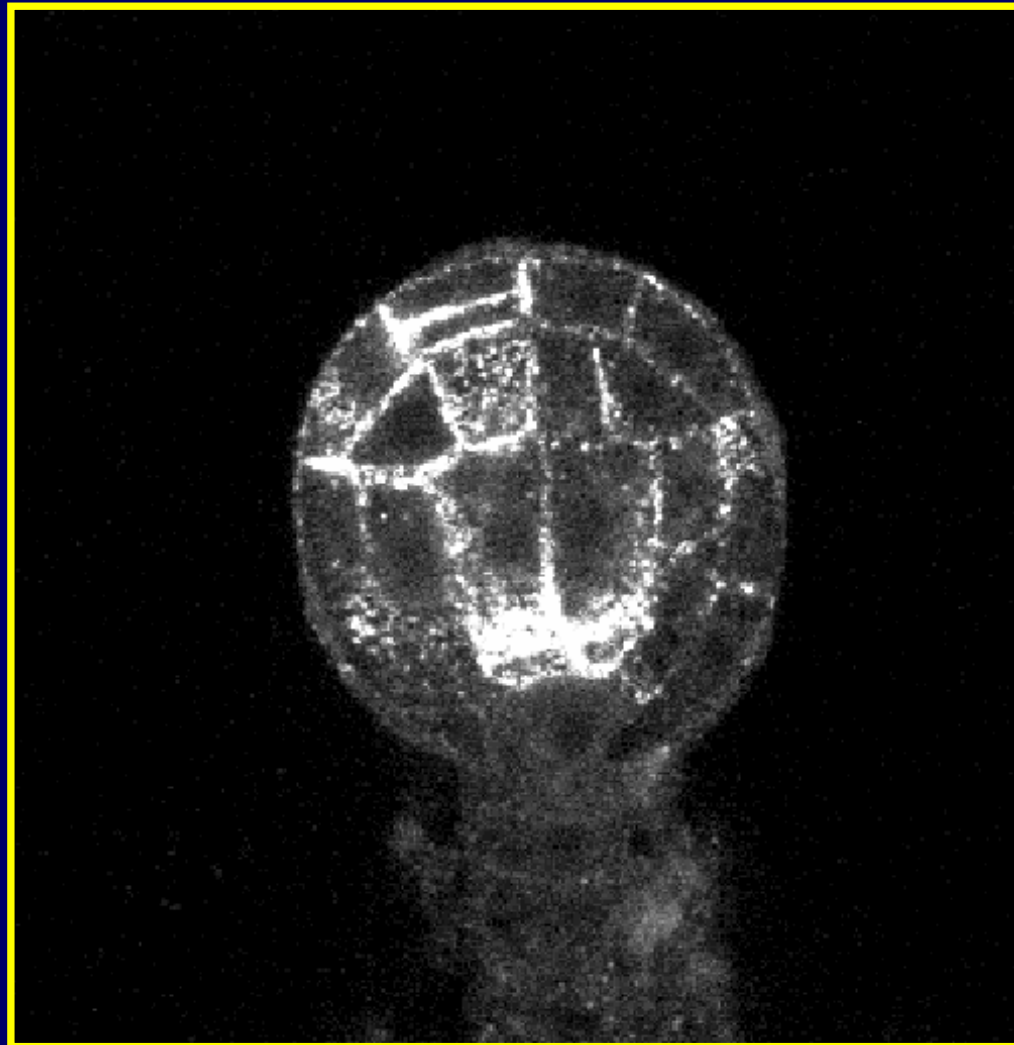


# BDL/MP act non-cell autonomously to induce hypophyseal cell fate

**Auxin or  
unknown MP/BDL  
dependent signal**



# PIN1 efflux carrier localisation suggests auxin flux towards the hypophysis



# Genetic Interference with Auxin Response and Transport Disrupts Embryo Patterning



*monopteros*

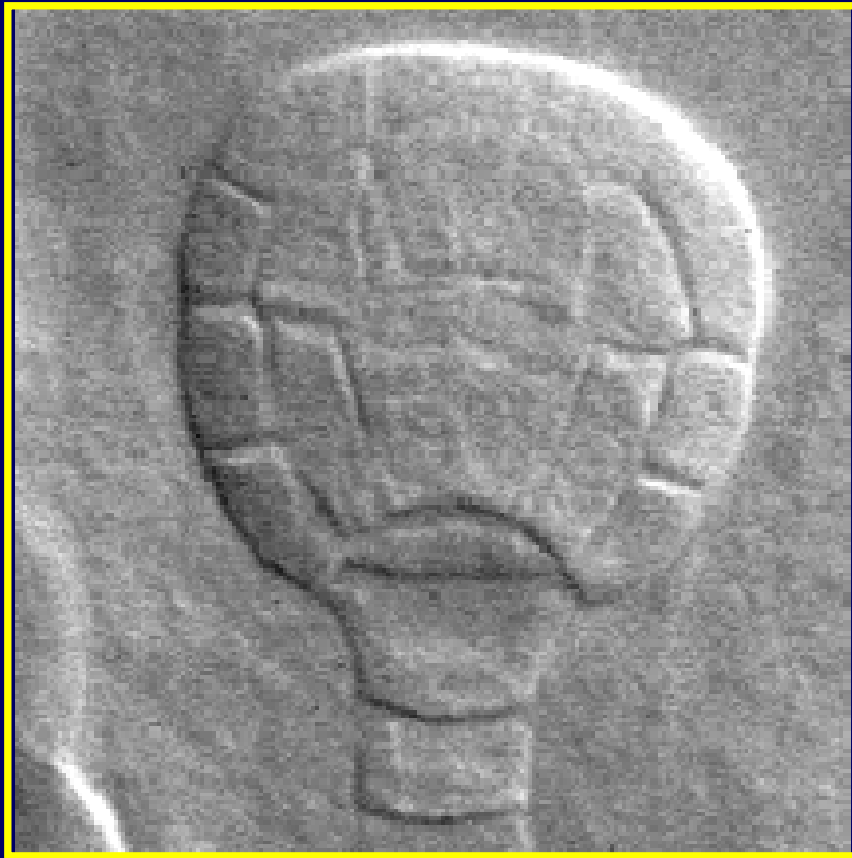


*bodenlos*

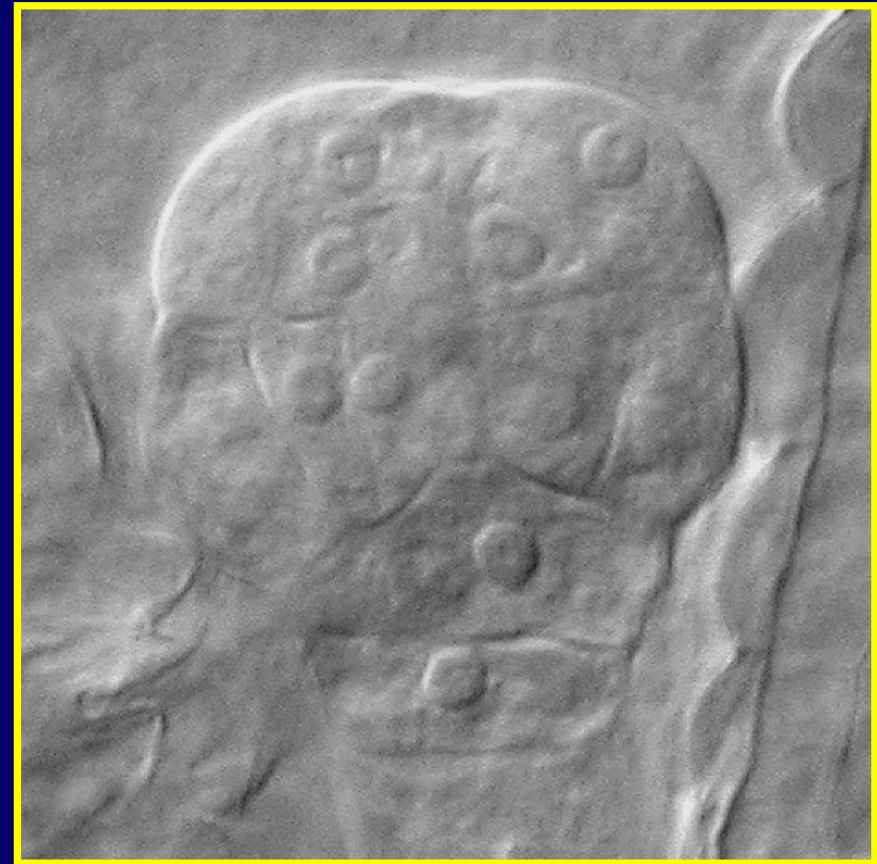


*gnom*

GNOM, a putative auxin transport mutant  
has similar defects in hypophyseal cell fate  
specification



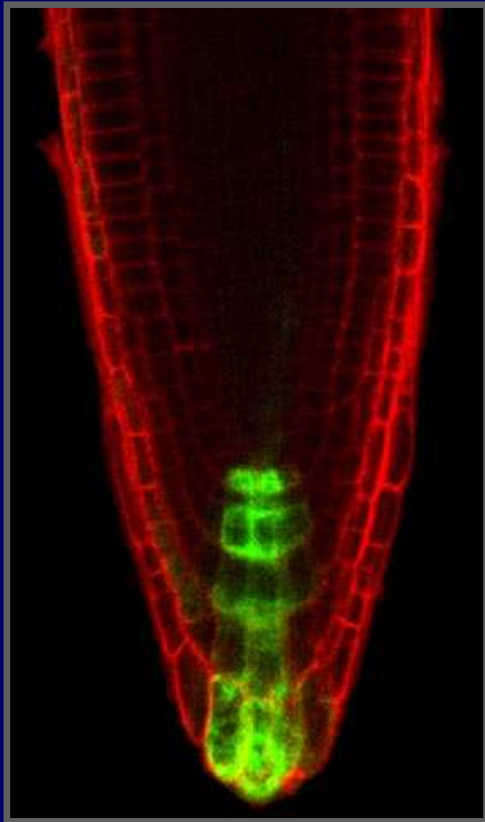
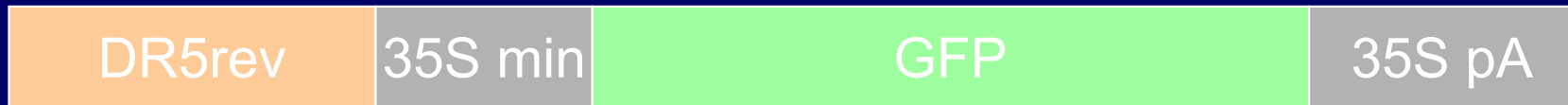
*wt*



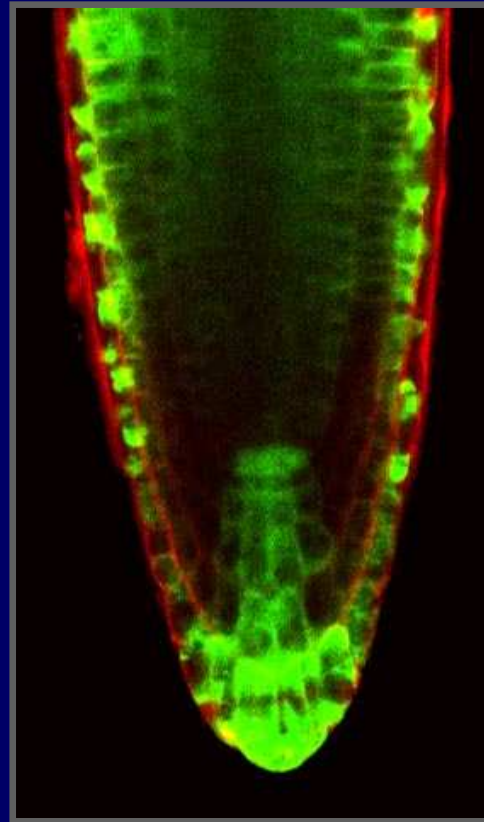
*gn*

# *DR5::GFP* Auxin Reporter

→  
5' CCTTT TGTCTC 3'  
9x inv.



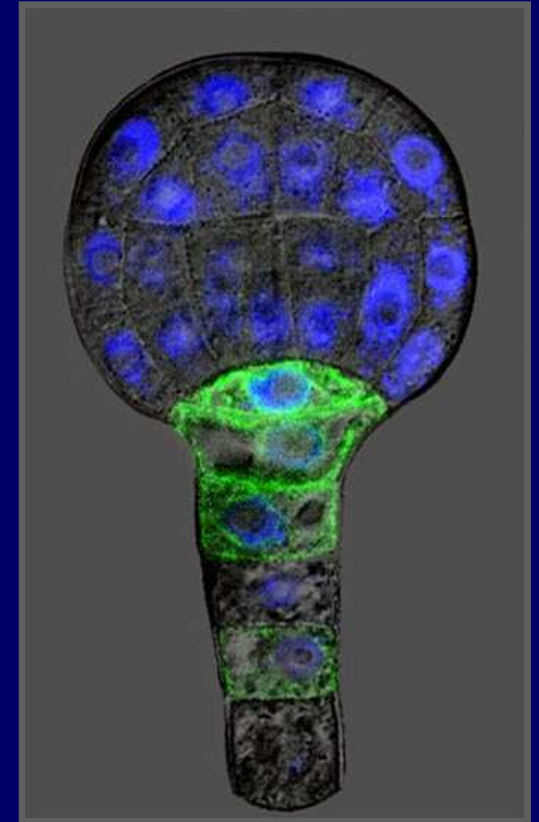
Root



Root + Auxin



anti-IAA AB



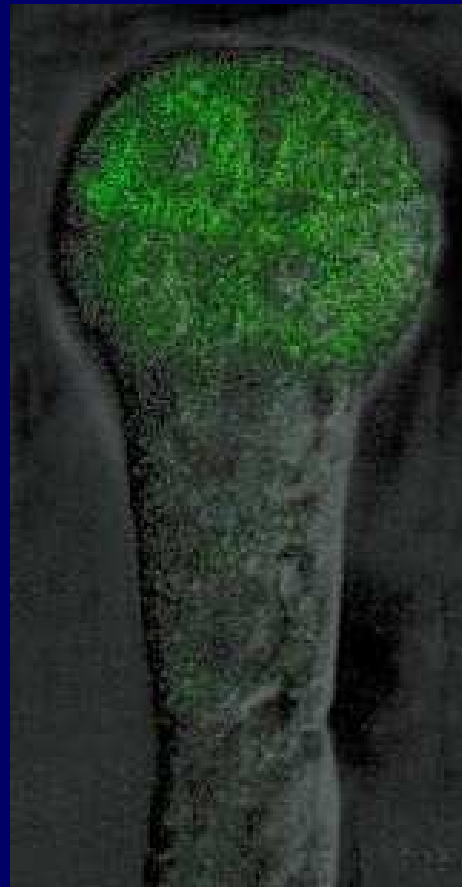
Embryos



# Auxin in Early Embryogenesis

*DR5::GFP*

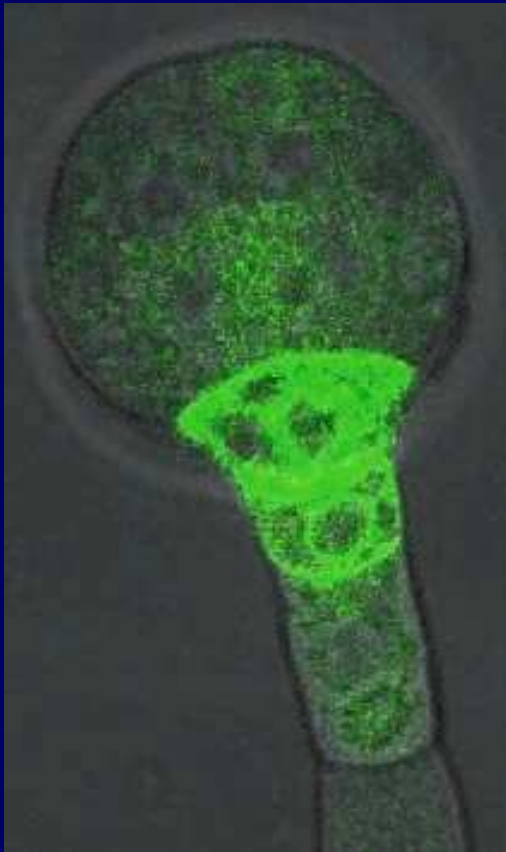
IAA  
localisation



# Auxin in Embryogenesis

*DR5::GFP*

IAA localisation



# *DR5::GFP* in Embryo Mutants

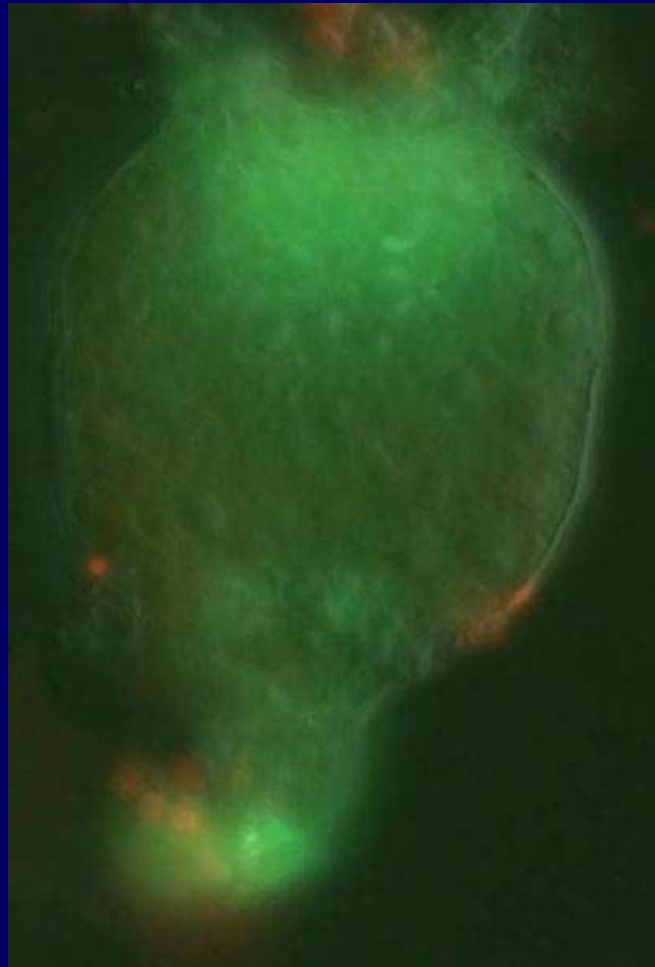
Auxin signaling

Auxin transport

BFA treatment



*monopteros*

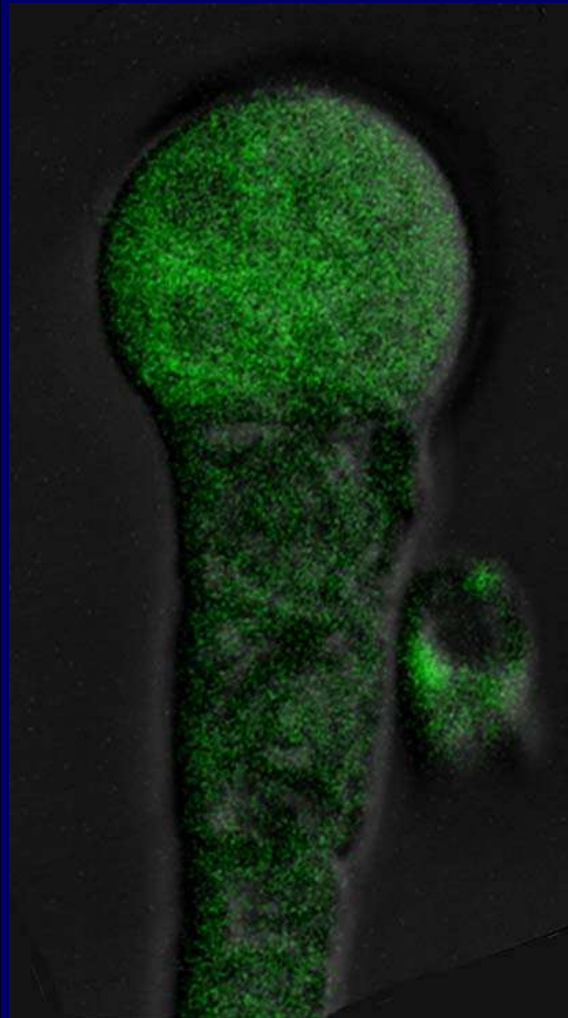


*gnom*



# *DR5::GFP* – *in vitro* Culturing

Preglobular embryos – short time treatments



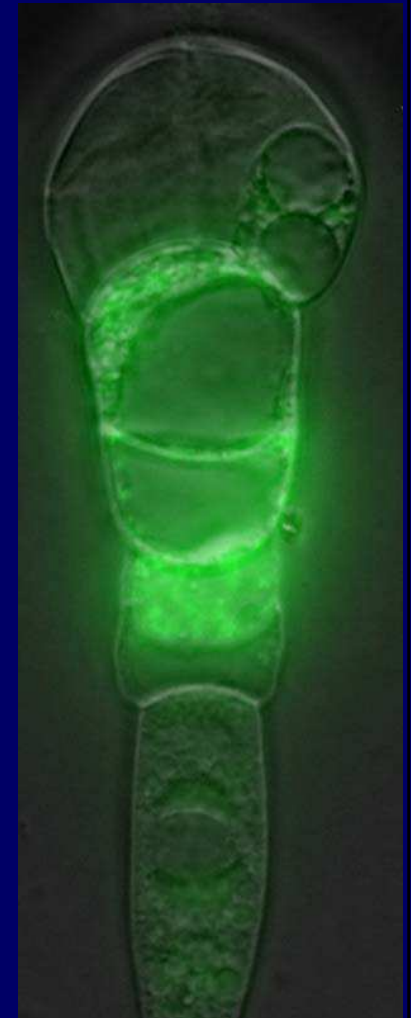
Control



0



NAA



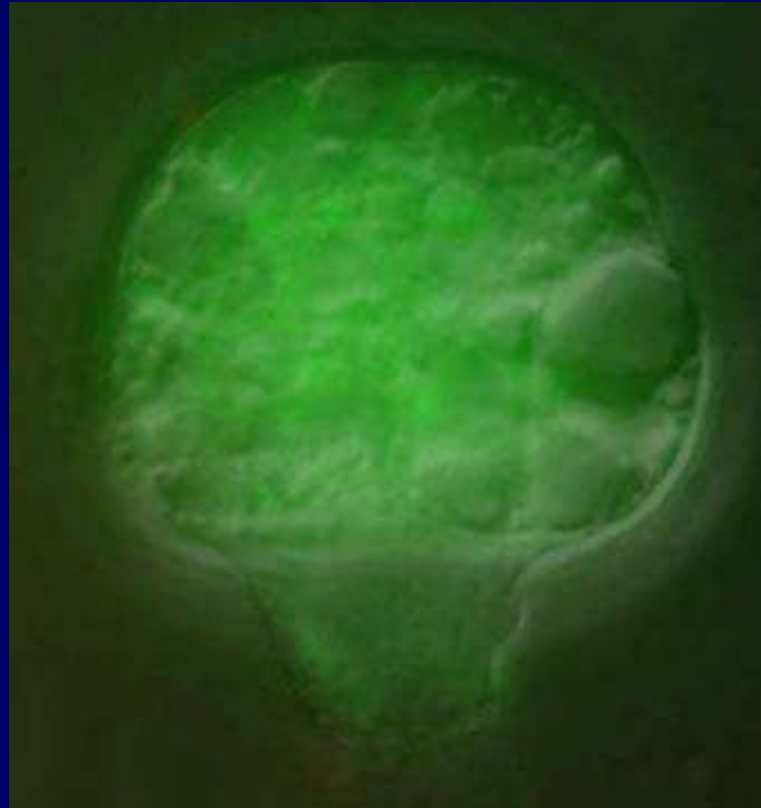
NPA

# *DR5::GFP* – *in vitro* Culturing

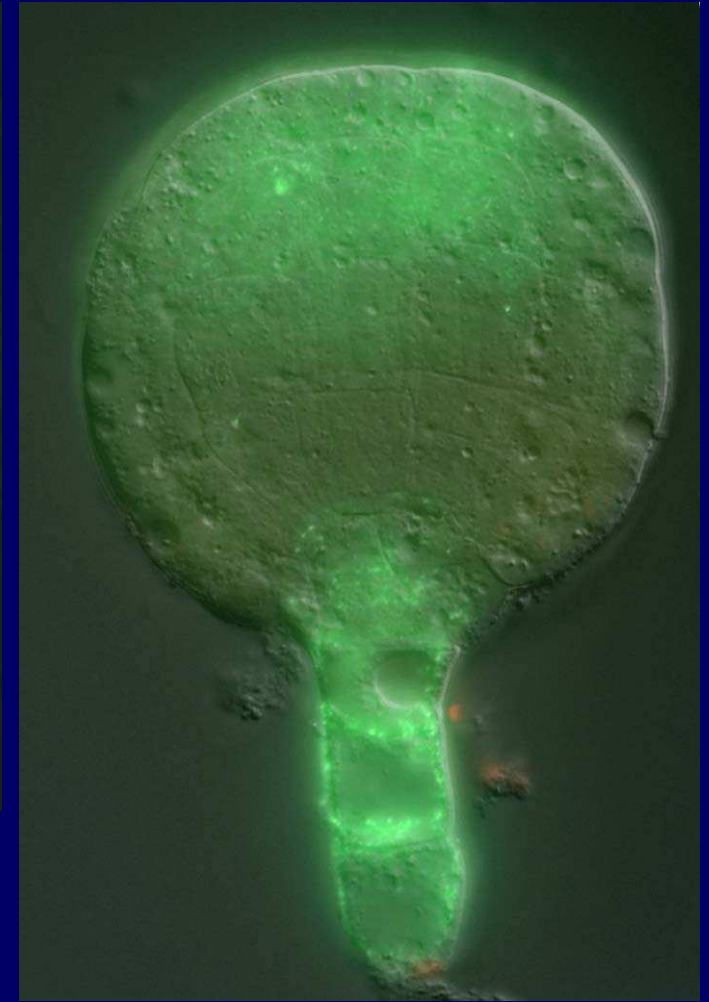
Globular embryos – short time treatments



NAA



2,4D



BFA

# *DR5::GFP* – *in vitro* Culturing

Long time treatments



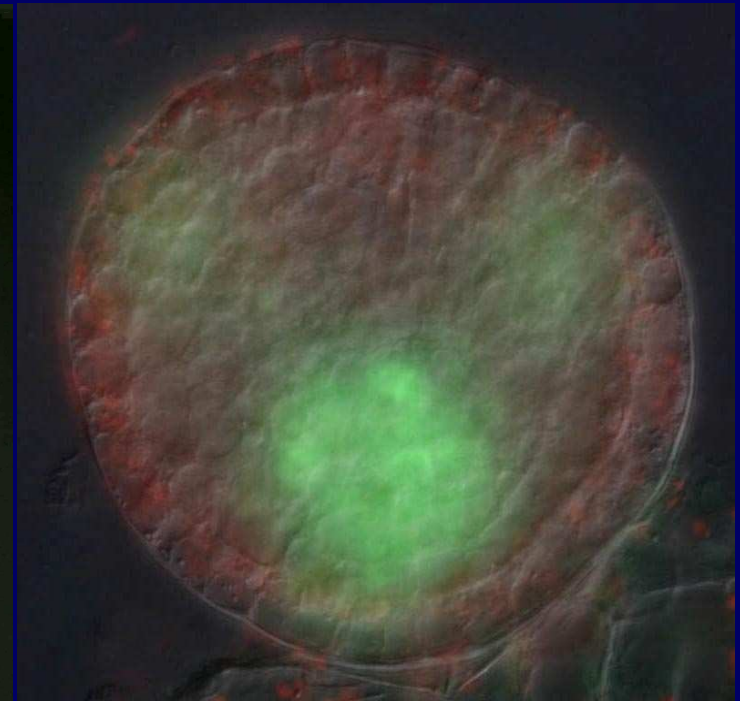
Control



NAA

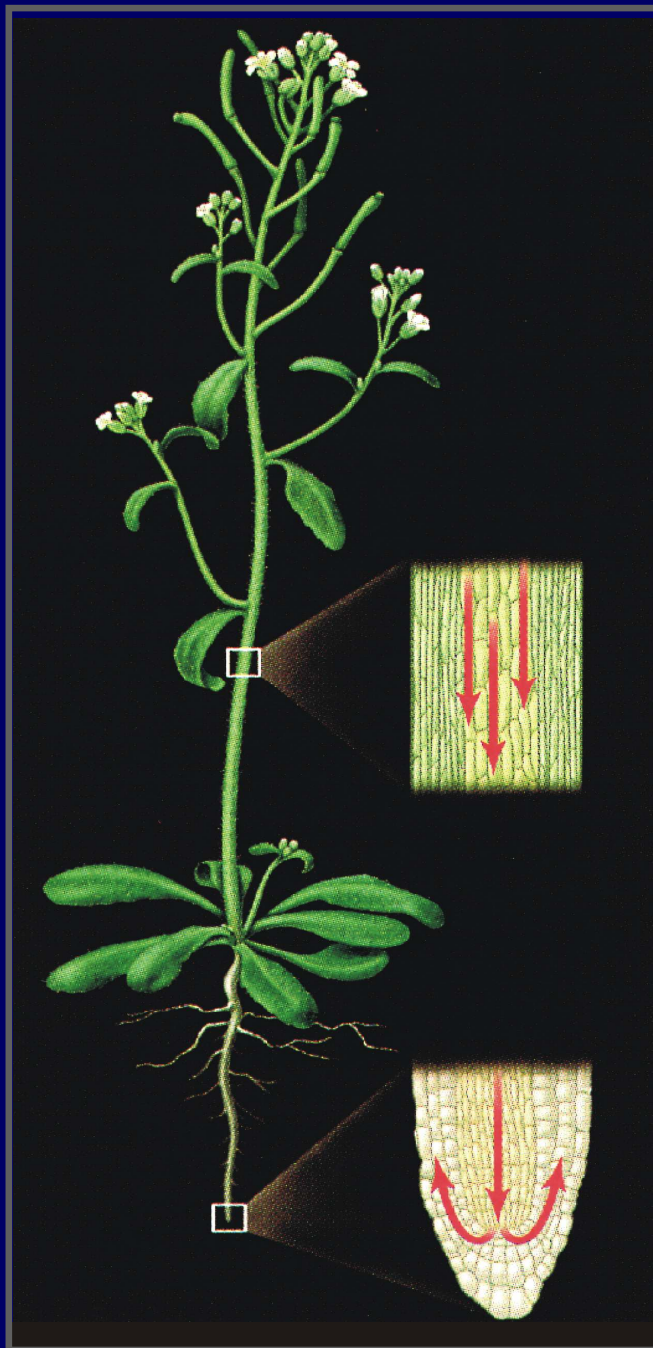


2,4D

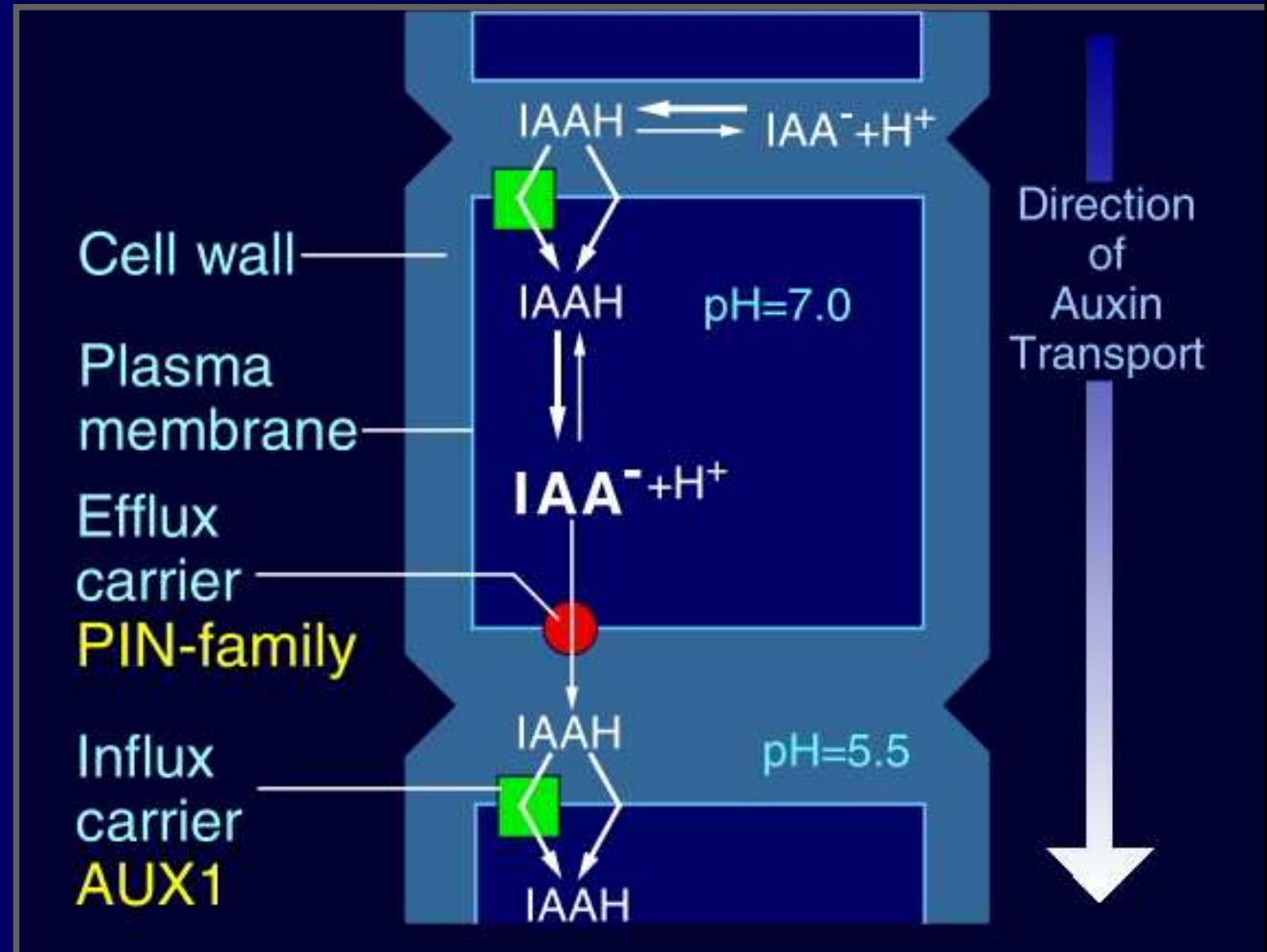


NPA or BFA

# Auxin Transport

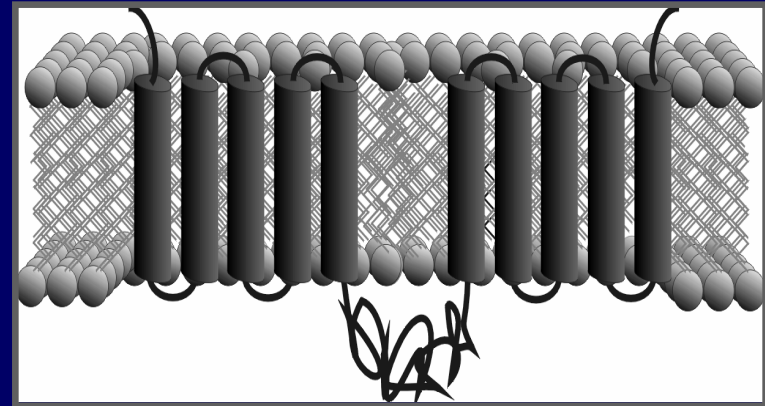
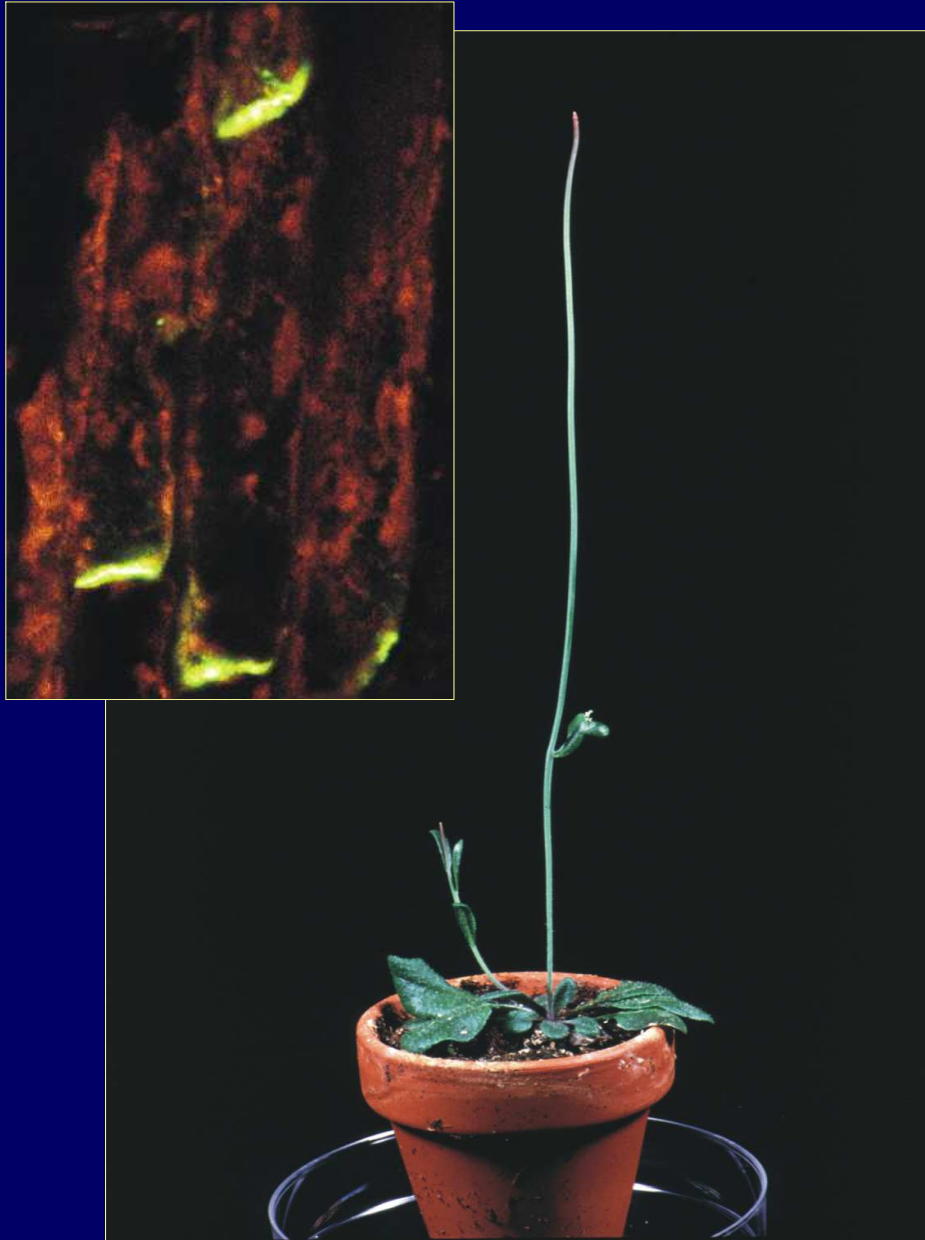


## Chemiosmotic hypothesis



# Molecular Genetics of Auxin Efflux

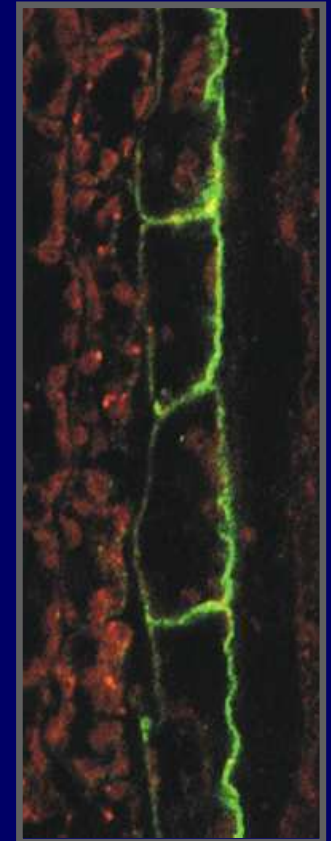
**PIN1**



**PIN2**



**PIN3**

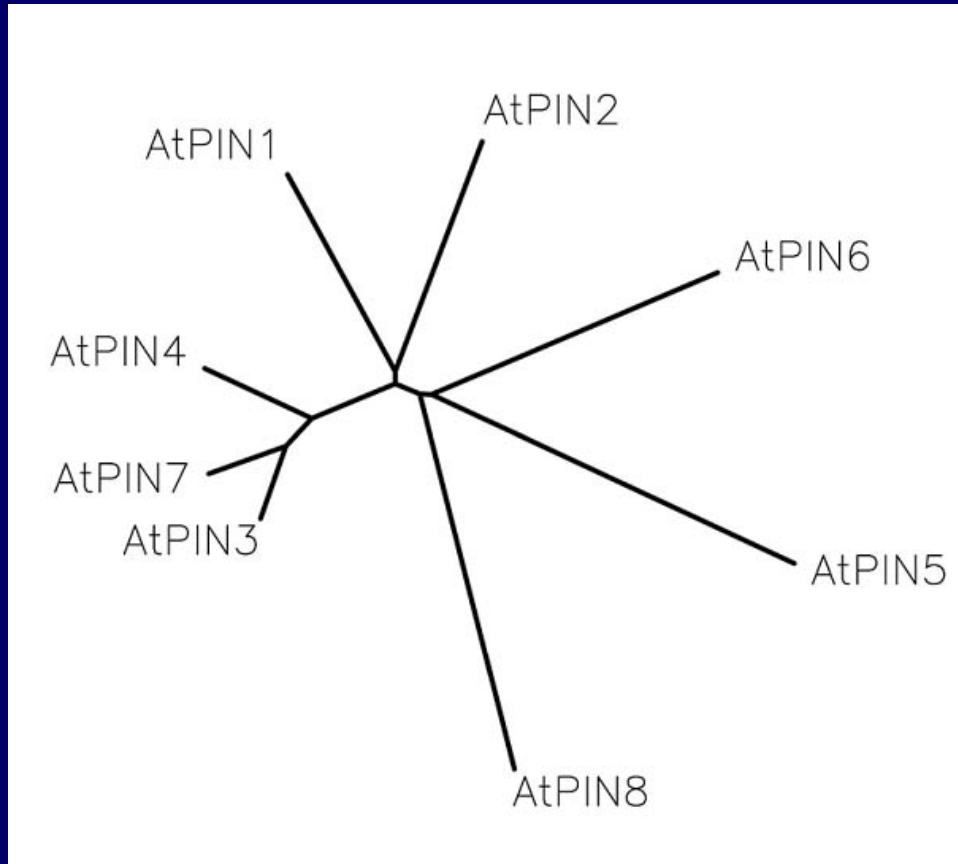




# Arabidopsis PIN Protein Family

Phylogenetic tree

Homology of PIN proteins



AtPIN1	1	METAADEHVMEMVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFAANNPYAMNRELAADSLOKVEVSELEFF...ECLLSRNSG EDWTELFLSGLTLPNTLV
AtPIN2	1	MITGKDMEDVLAAMVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...GQAFSRSSG LEWMTLFLSGLTLPNTLV
AtPIN3	1	MISWHLETVLAFAVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...WAFNFRSSG LEWMTLFLSGLTLPNTLV
AtPIN4	1	MITWHLETVLAFAVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...WALFRKNSG LEWMTLFLSGLTLPNTLV
AtPIN5	1	MKCGVYKILAMVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...WAFYNSKSYG WSPFSLFCHTNGELV
AtPIN6	1	MITGNEFYVMCAAPLRFAMFVAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...WAFNFRKASG EDLHLETFLSGLTLPNTLV
AtPIN7	1	MITWHLETVLAFAVPLVAMLLAYGSVKWRIETPDQCSGINRFVAVFAVPLLSFHFISSNDPYAMNRELAADSLOKVEVSELEFF...WAFNFRSSG LEWMTLFLSGLTLPNTLV
AtPIN8	1	MESHLIHHVSGTWPLVGMTEGFLPARKLHSHRQAGINRQVAKRSTELLESQIHSNNFRKMSKLESLITLQKFLVVVAVMLRFHPPTGGRGOKGVWRCGLSIVLNTLII
AtPIN1	116	MGIPLEKMGVNFSGDLVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...DNGSIVSLHVDSDIMSLDGRQPEFTEAEATEDEGKGLHVVVRNRSAS...SDIYSRRSQELS...ATF
AtPIN2	116	MGIPLEKMGVNFSGDLVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...ETAGSITSRVSDVSLDNGREPECDABIGDDGKGLHVVVRNRSASMSISFPNKSNGHLSMSLTFP
AtPIN3	116	MGIPLEKMGVNFSGDLVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...ETAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...RSEFC...EPN...MFP
AtPIN4	116	MGIPLEKMGVNFSGDLVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...ETAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...REIM...MEP
AtPIN5	116	VGVPLAKAMYQQAVDQVQSSVPAVWVWVLLLVLELFRK...EAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...RSEFC...EPN...MFP
AtPIN6	116	KGELKQAMGGYQVIMVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...EAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...RSEFC...EPN...MFP
AtPIN7	116	MGIPLEKMGVNFSGDLVQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...EAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...RSEFC...EPN...MFP
AtPIN8	121	LMELHSHKYGDEAASILEQIVVLOCIWYIEMLEFPEYRGAKLLISEQFF...EAGSIVSSEKVESDVLSDGHEPEETDABIGDDGKGLHVVVRNRSAS...RSEFC...EPN...MFP
AtPIN1	229	RPSNLTNAEISYQSSRNPTFRGSSFNHT...DEFS...MNASG...EG...NSNFGF...GE...AFGCKGPTFRSNYEDGGPAKPTAATAGAGRPHQSGGSGGGG
AtPIN2	235	RASNLGVEIYSVQSSREPTFRASSENCT...DEFA...MNASKAPSPRHGYNSYGG...AGAGGGDVSLSQSSKVEPTFRSNEDEE...VMKTAKKASRGGSRMSGELYNNNSVP...
AtPIN3	224	RPSNLTGAEIYSIST...FPRGNSFNHS...DEYN...HMGFP...GG...KLSNFGF...ADMYSVQSSRPTFRSNEDEE...VMKTAKKASRGGSRMSGELYNNNSVP...
AtPIN4	221	RPSNLTGAEIYSIST...FPRGNSFNHS...DEFS...HMGFP...GG...KLSNFGF...ADMYSVQSSRPTFRSNEDEE...VMKTAKKASRGGSRMSGELYNNNSVP...
AtPIN5	158	...SFSNNISDVPQVDNIN...EGKRETVVVGKESF...LE...
AtPIN6	228	RASNLGAEIYSIST...FPRGNSFNHS...DEFS...HMGFP...GG...KLSNFGF...ADMYSVQSSRPTFRSNEDEE...VMKTAKKASRGGSRMSGELYNNNSVP...
AtPIN7	227	RPSNLTGAEIYSIST...FPRGNSFNHS...DEFS...HMGFP...GG...KLSNFGF...ADMYSVQSSRPTFRSNEDEE...VMKTAKKASRGGSRMSGELYNNNSVP...
AtPIN8	169	...SALEREQNDQ...REANI...EDEKKEDEEVEIVRTRSVGTMK...
AtPIN1	325	GAHFEADNMGSPNPTGGGGCAANAVV...VGRKQDQNGRDRHFWVWSASAPVSEVFE...GGGHHADYSTMTNDHQEDVWSGGNS...DNQYVER
AtPIN2	342	EVEFPNMGFG...STSGASGVCKESGGGGGGGGVGGQGN...KEMNFWWSASAPVSE...ANAKAMTRKGSFDVSDPKVSLFPH...NATRAMQNLNMSFPR
AtPIN3	305	AGSYFAPNPEE...STSTANKSVNKNPDRVNTNQQTLPDTCSSNSHDARELHMFVWSNGSPVSDRAHLVFGAPDQDGGRDQ...GAREHMLVEDQSHNGTRAVNHPASGDFGE
AtPIN4	303	AGSYFAPNPEE...STGAGVSTKP...NIPKE...NQQLQEKDSNA...SHDAKELHMFVWSNGSPVSDVFE...GAGDHWATQEQE...KSNRGGDDIGG...
AtPIN5	192	...
AtPIN6	313	LDVNNQGT...VMK...SAAGR...ITROSSPKMWSQQRHARENGSWEKESIFSDALKRPAQPTAAGG
AtPIN7	308	PGSYFAPNPEE...STGKKGSMAPKE...NHHV...GKSNNDARELHMFVWSNGSPVSDRAHLVQDNDA...EQVGKEDQGGAREHMLSDHTQGEN...RGMNGDYG...
AtPIN8	210	...
AtPIN1	423	EFSFGNKDDSKVL...ATDGGNNISNKT...QAKVMPPTVMTRLLIMVWRKLRNPNYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN2	443	KG...HVMDDQGNNS...GKSPYMGKSGSDVEDGGGPRKQMPFASVMTRELLIMVWRKLRNPNYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN3	424	QQFSPKGEKAEAPKDAEMSNKLA...PMSWALQSKTGLGGAASQKRNPPASVMTRELLIMVWRKLRNPNYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN4	407	...LDSGEGEREIK...ATALNKMGSSEALEAAGDGGGNGG...THMPEVMTRLLIMVWRKLRNPNYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN5	192	...VMSLVWKLATNPCKYSCILGIAWAFINENHLELGLLEGLSLSKAGTSTAMFNHIF
AtPIN6	381	ASMEGAAGKDT...TFVVAIG...KQEMBSIVMRLITVCRKLSRNPNTYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN7	411	...ESESIVKVPVPLHRLKRCNS...LMPKRALESQEVVVKV...GASVSMELL...EHWKRLRNPNTYSYSLFPTISISIFKWNIEEMALAKESILSDAGLGAMFSLGF
AtPIN8	210	...MLKAWRLIINPNTAELEGIAWAFINENHLELGLLEGLSLSKAGTSTAMFNHIF
AtPIN1	526	MALNREIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN2	551	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN3	544	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN4	520	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN5	253	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN6	474	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN7	523	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--
AtPIN8	271	MALQPKIACGRNREAFAMAVREVEPAMVLAASYVQLRLELHAIQALPGQVVPVFAKEYNVHPPISLAVIFGMLALP...LLELYLGLL--

# PIN1 in Early Embryogenesis

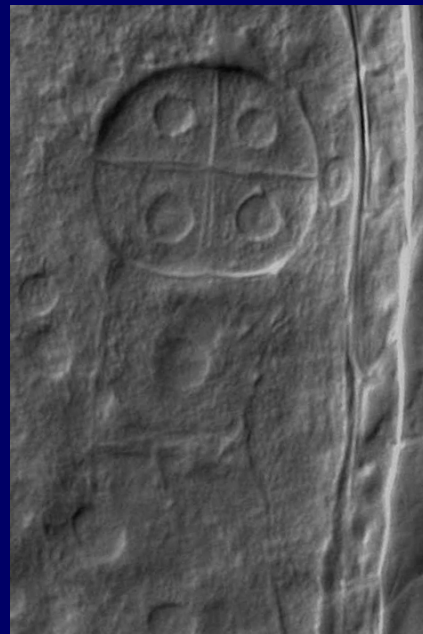
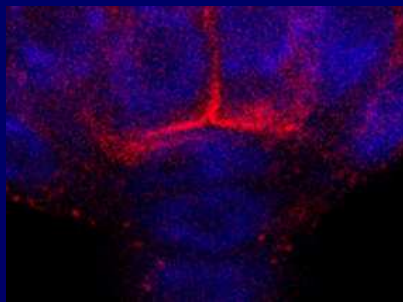
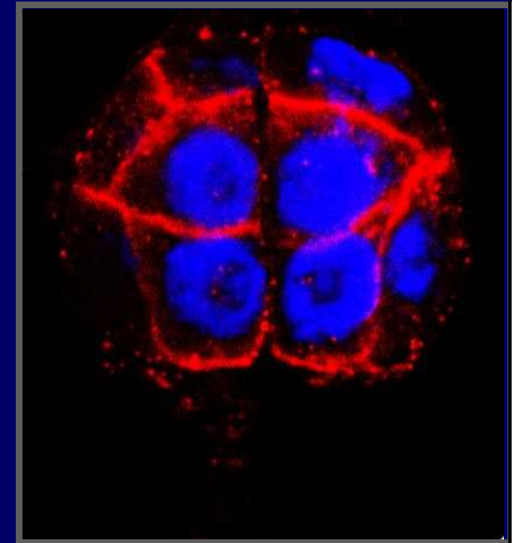
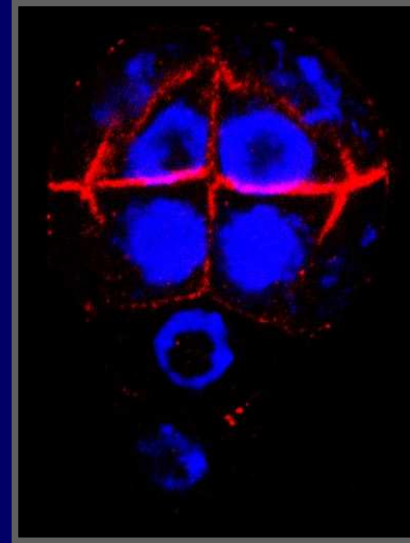
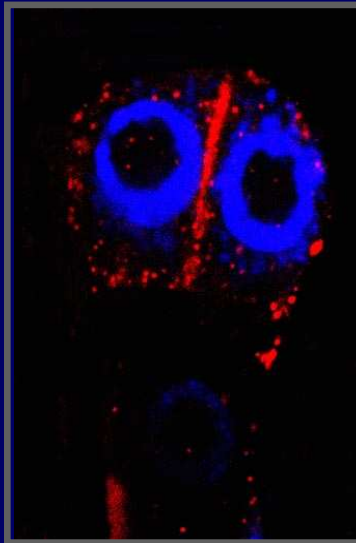
*GUS*



mRNA



Protein



*Enk*  
6,1%

*pin1*  
30,4%

# PIN7 in Embryogenesis

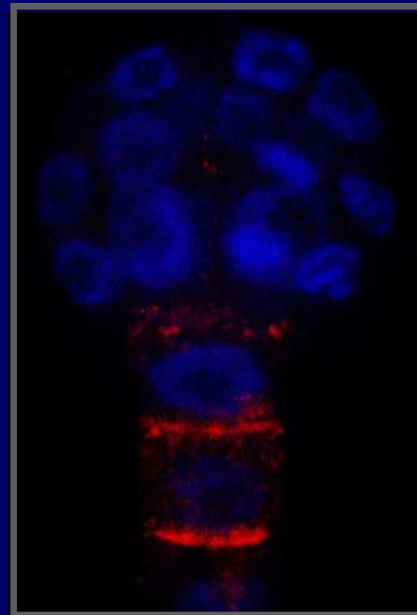
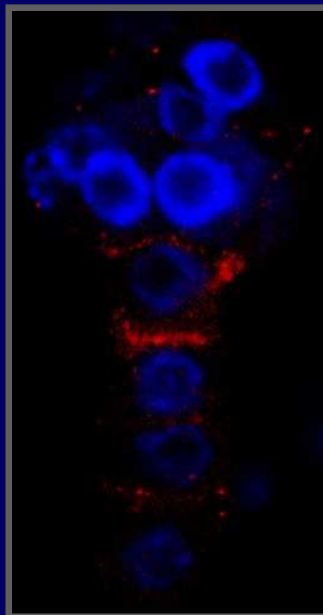
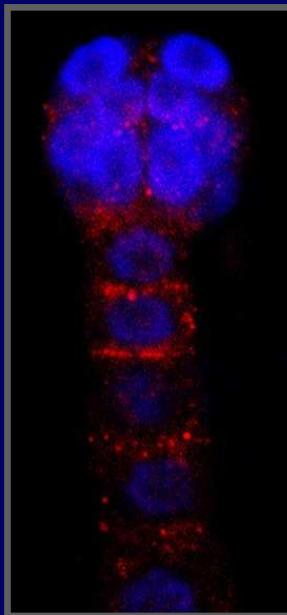
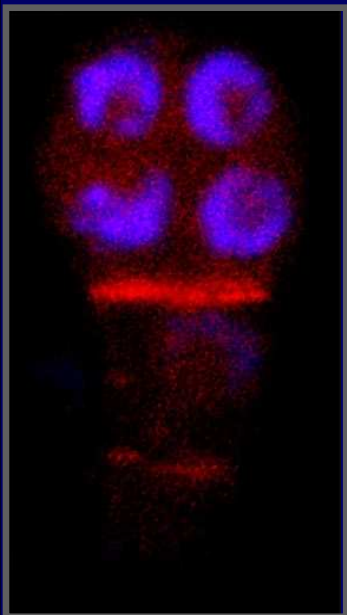
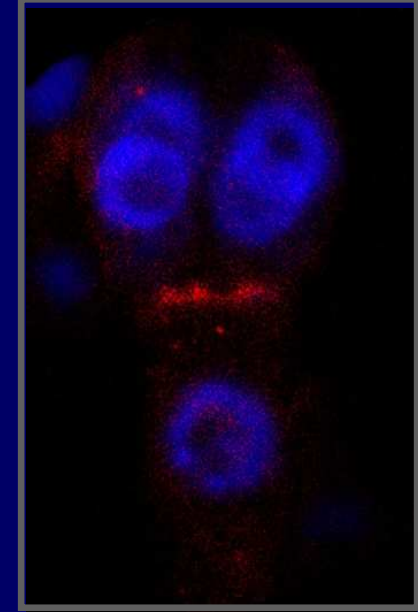
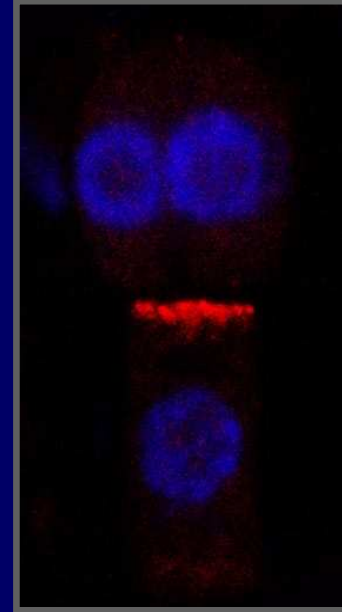
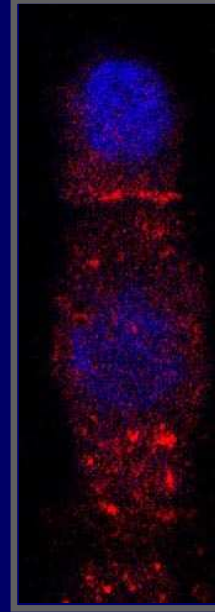
GUS



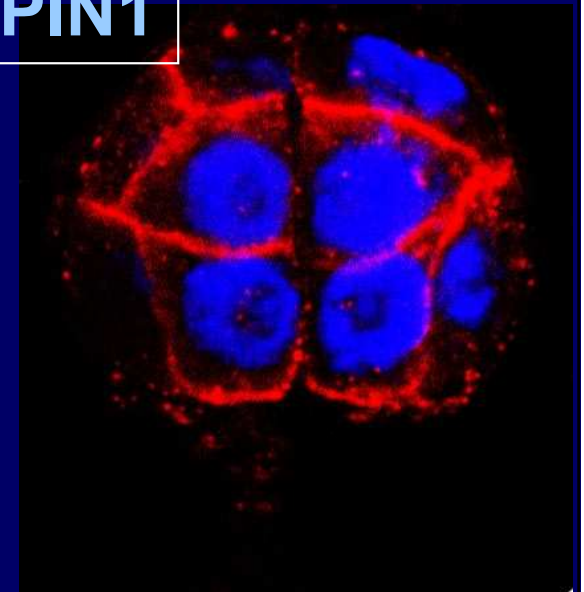
mRNA



Protein

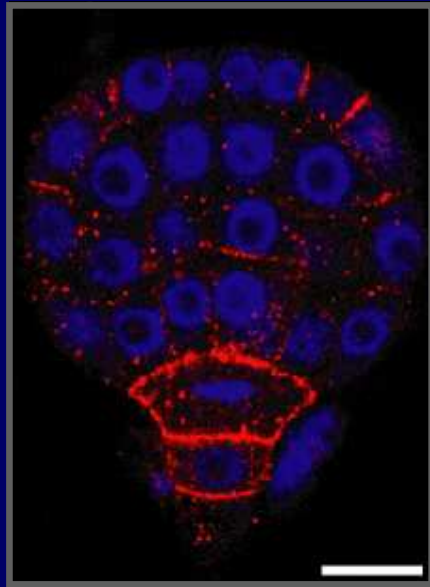


PIN1



# PIN4 in Embryogenesis

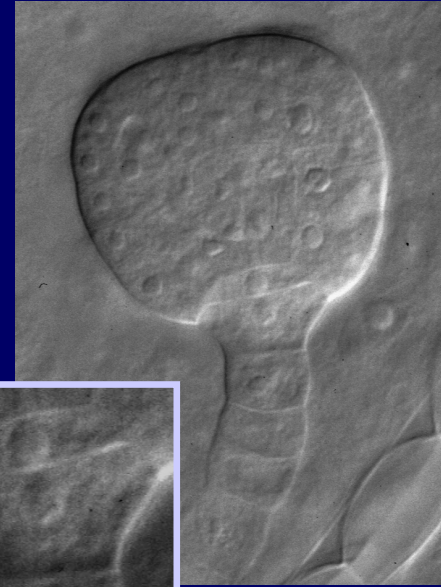
PIN4 protein



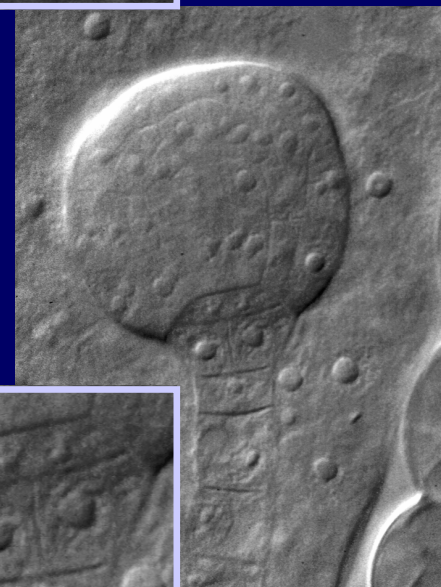
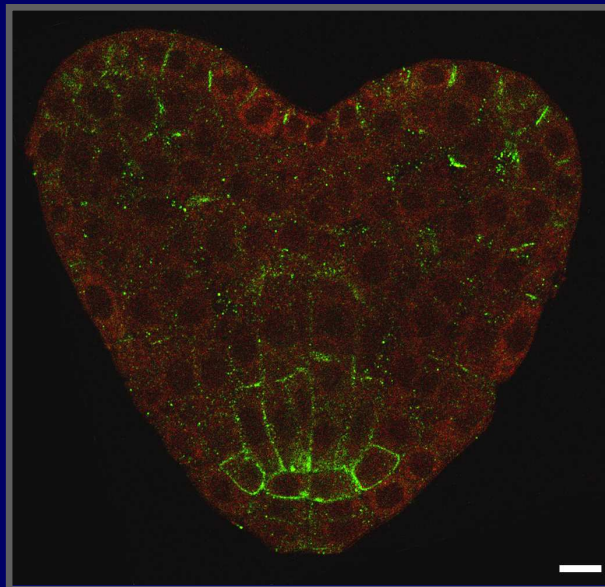
DR5



basal defects

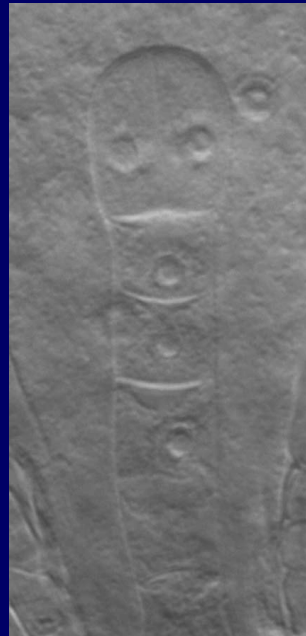
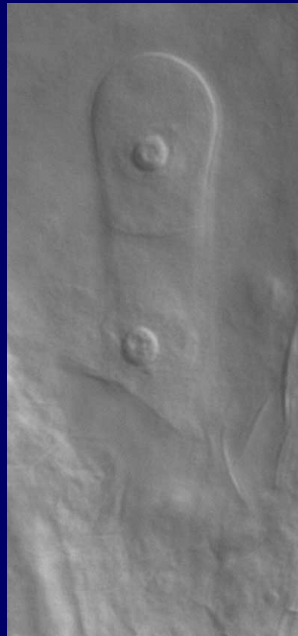


QC marker

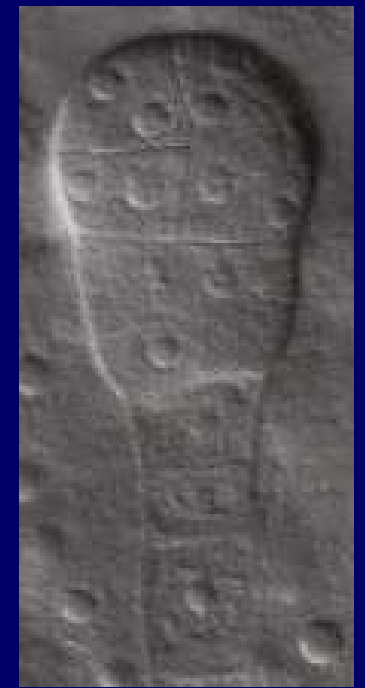
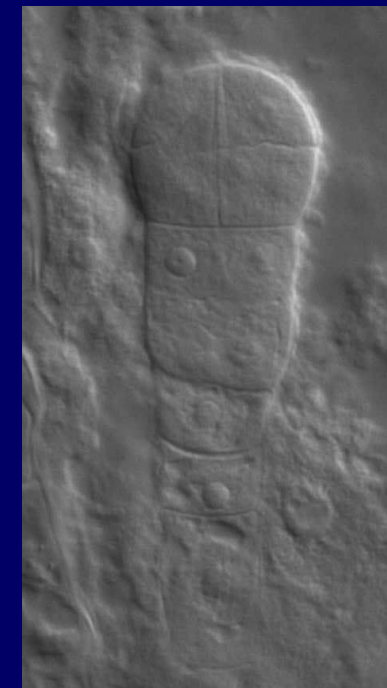
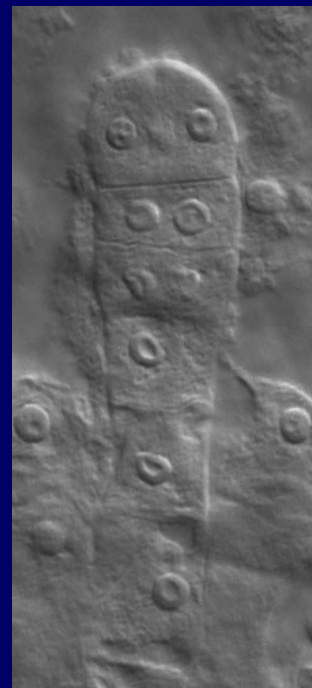
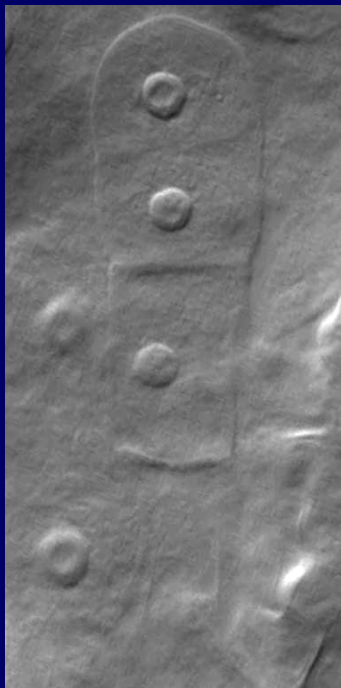


# Embryo Phenotype of *pin7* Mutants

**Col-0**

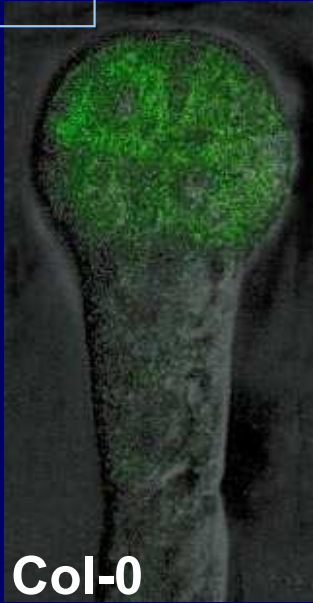


***pin7***

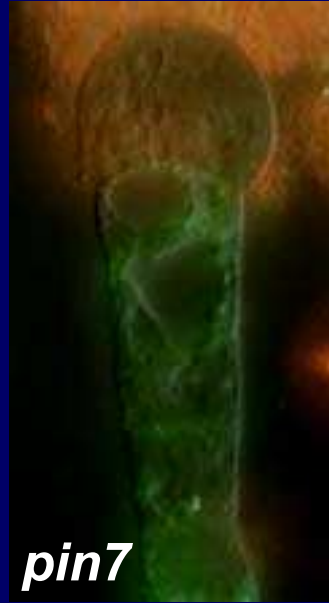


# Analysis of Markers in *pin7*

DR5



Col-0



*pin7*

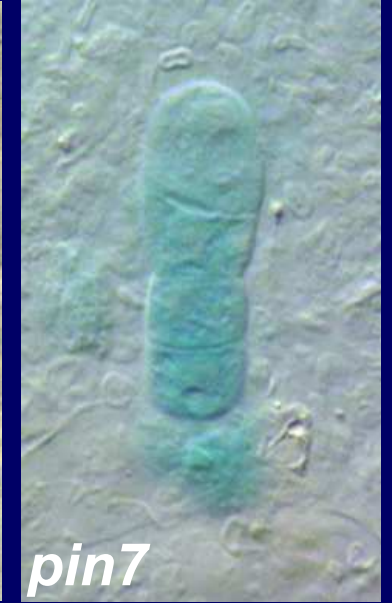


NPA

*PIN7::GUS*



Col-0



*pin7*

*PIN1::GUS*

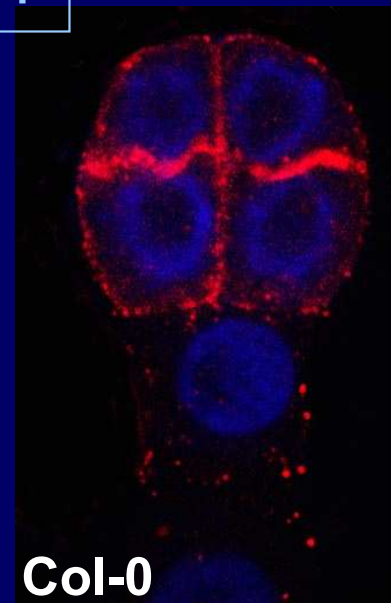


Col-0



*pin7*

PIN1

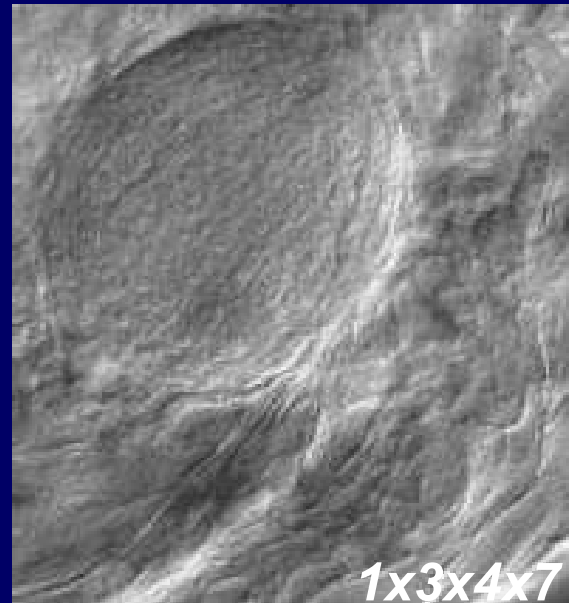
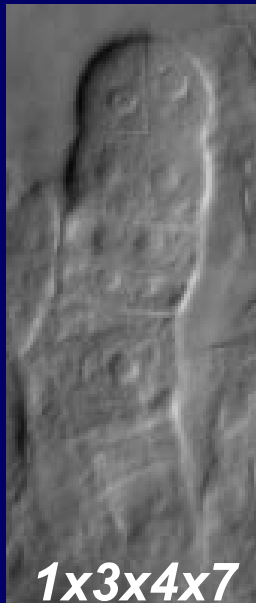


Col-0



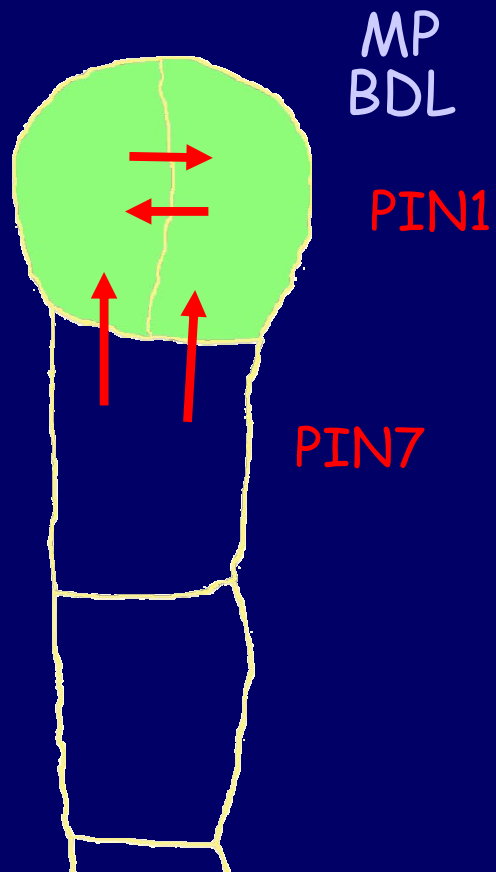
*pin7*

# Phenotypes of *pin* Multiple Mutants



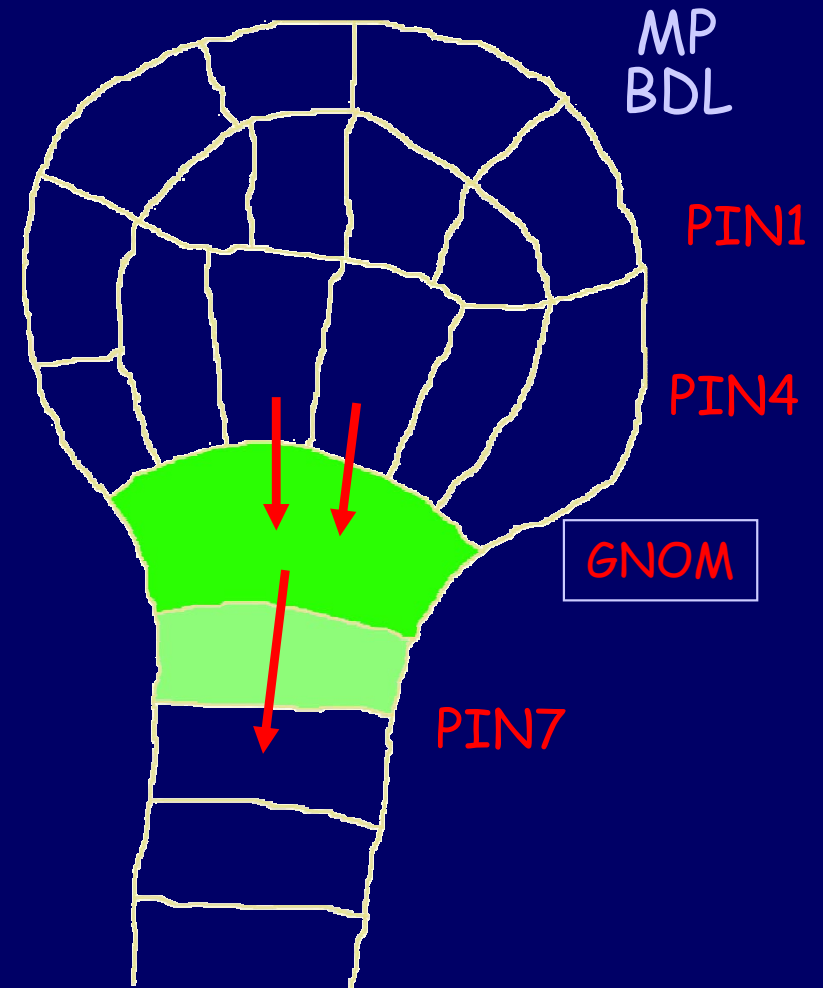
# Auxin and Embryogenesis

Apical pole specification



Two-Cell

Root pole specification



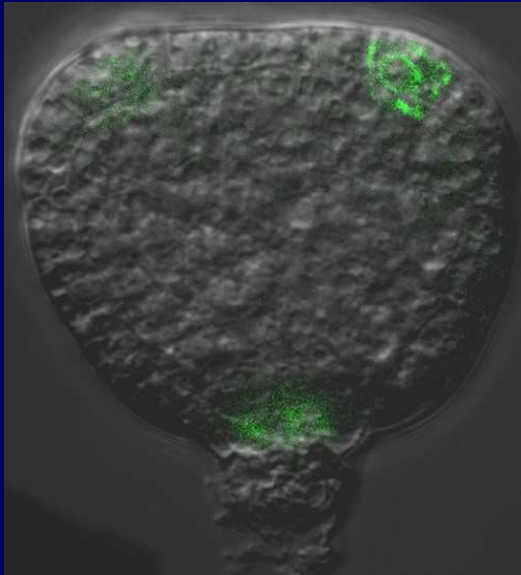
Globular



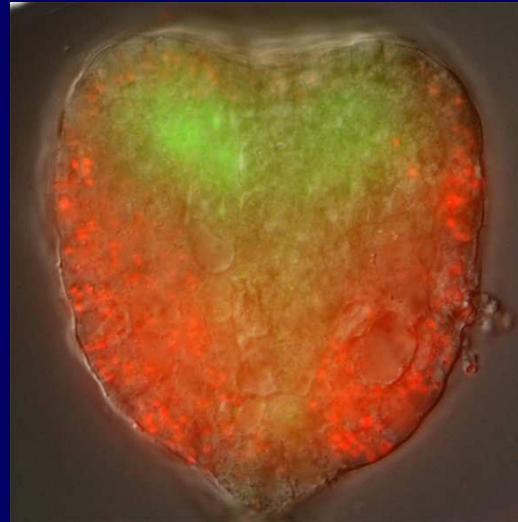
# Organogenesis

# Auxin in Cotyledon Formation

**DR5**



**BFA**



*pins*



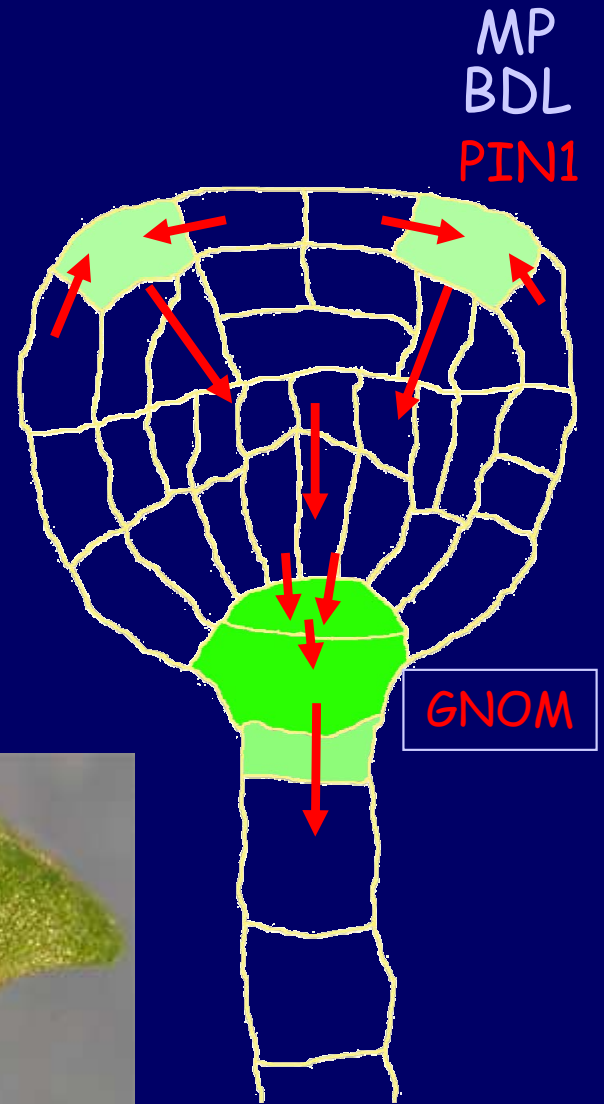
*gnom*



**IAA**



*pin1*

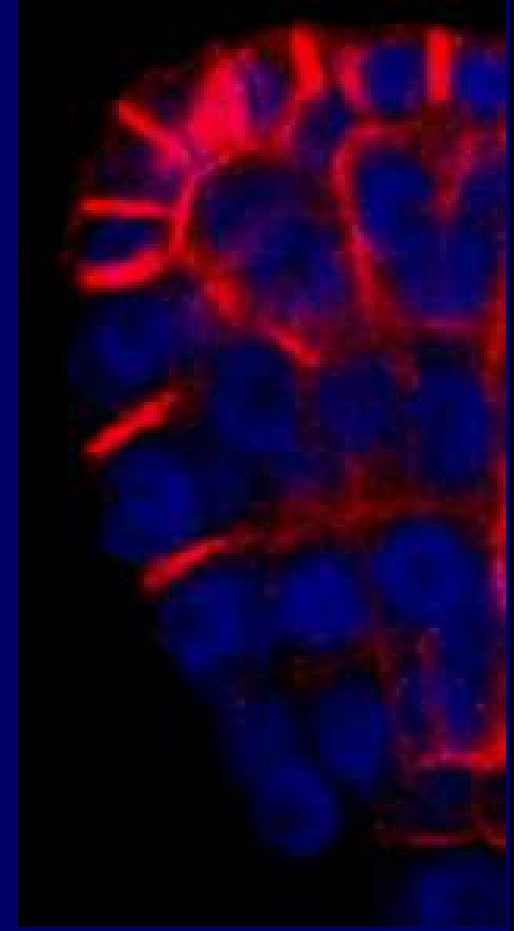
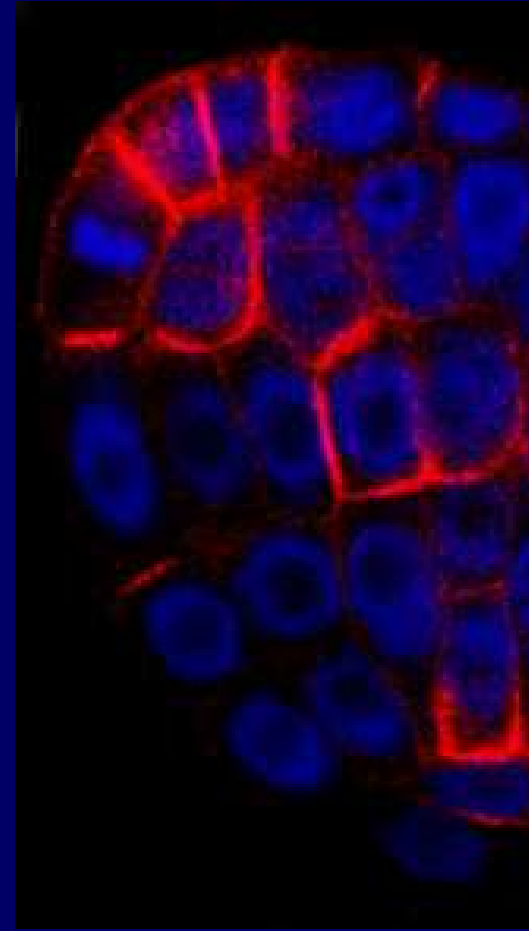
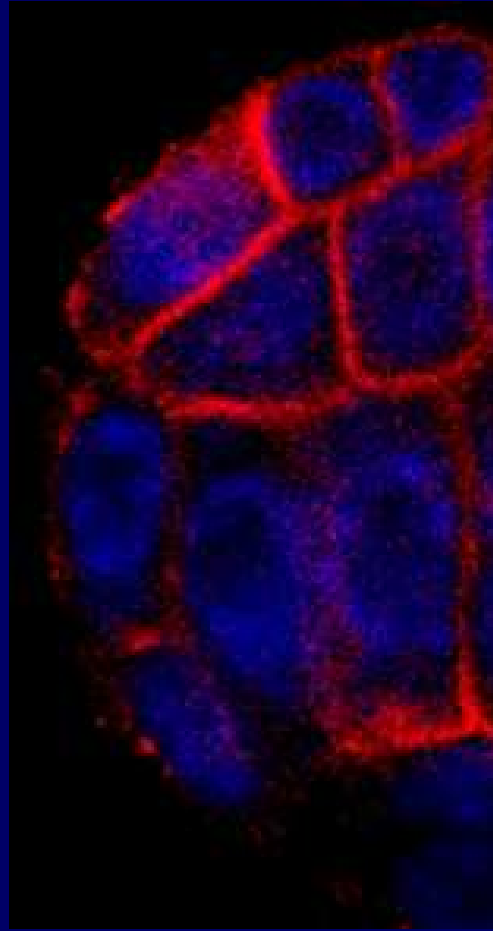
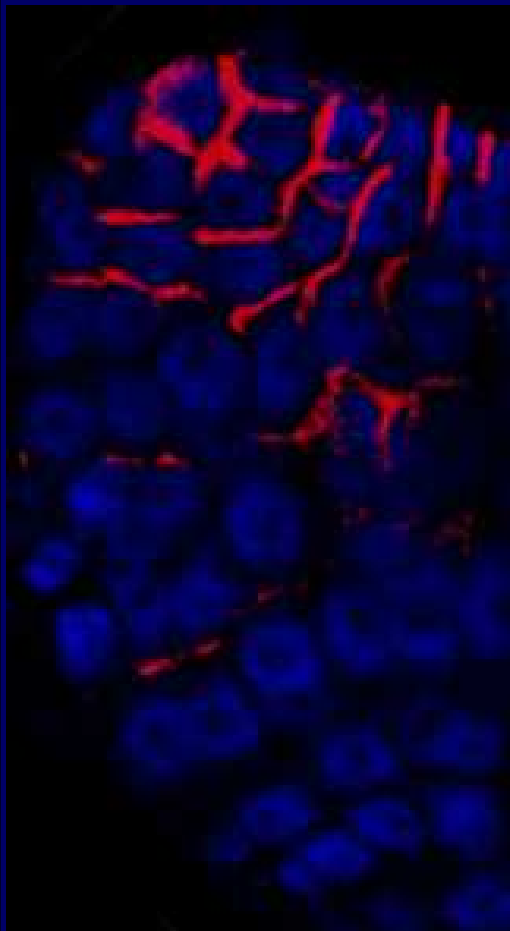


# PIN1 Polarity in Cotyledon Formation

Outer layer

Inner layers

BFA treatment



Heart

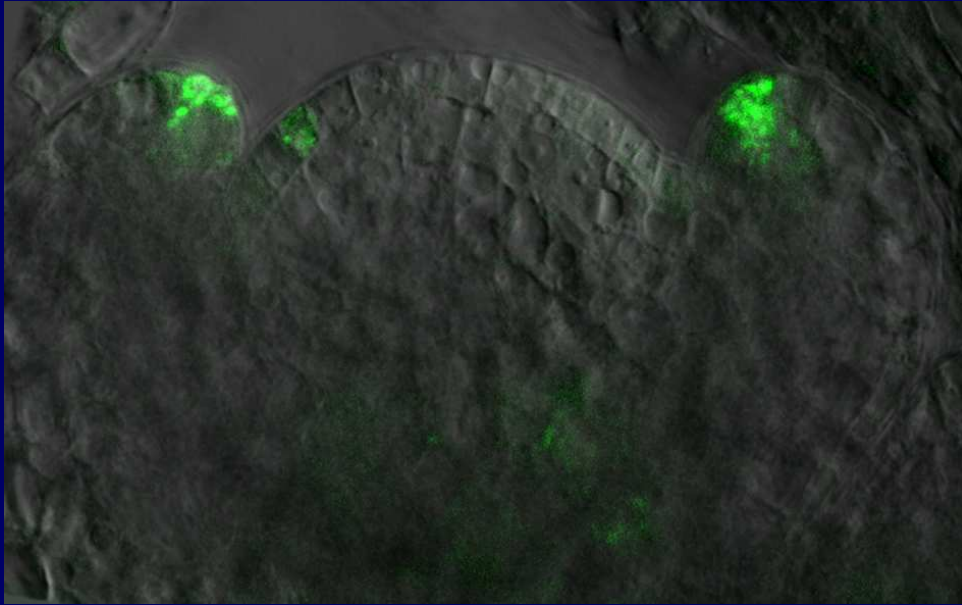
Globular

Heart

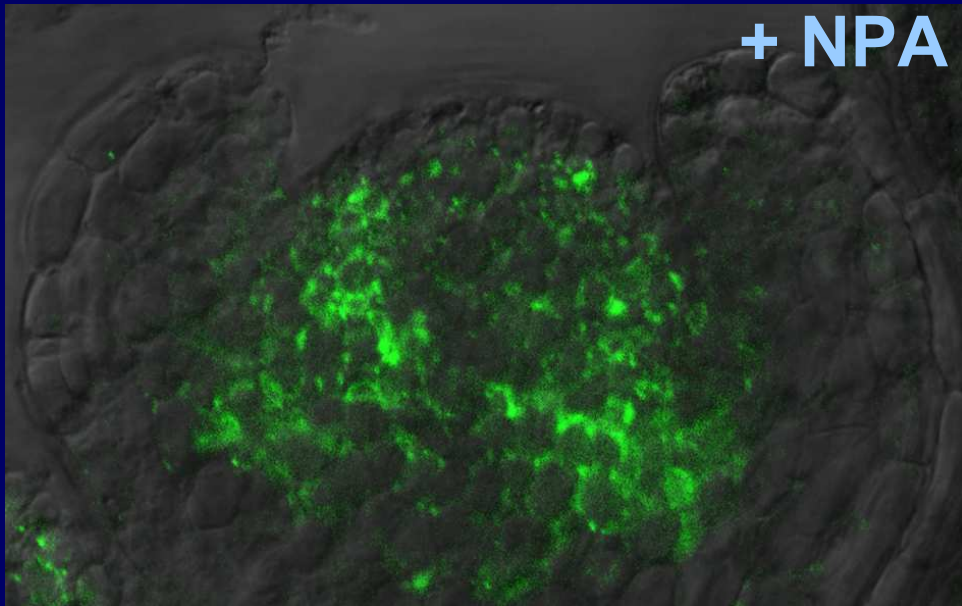
Heart

# Auxin in Flower and Leaf Formation

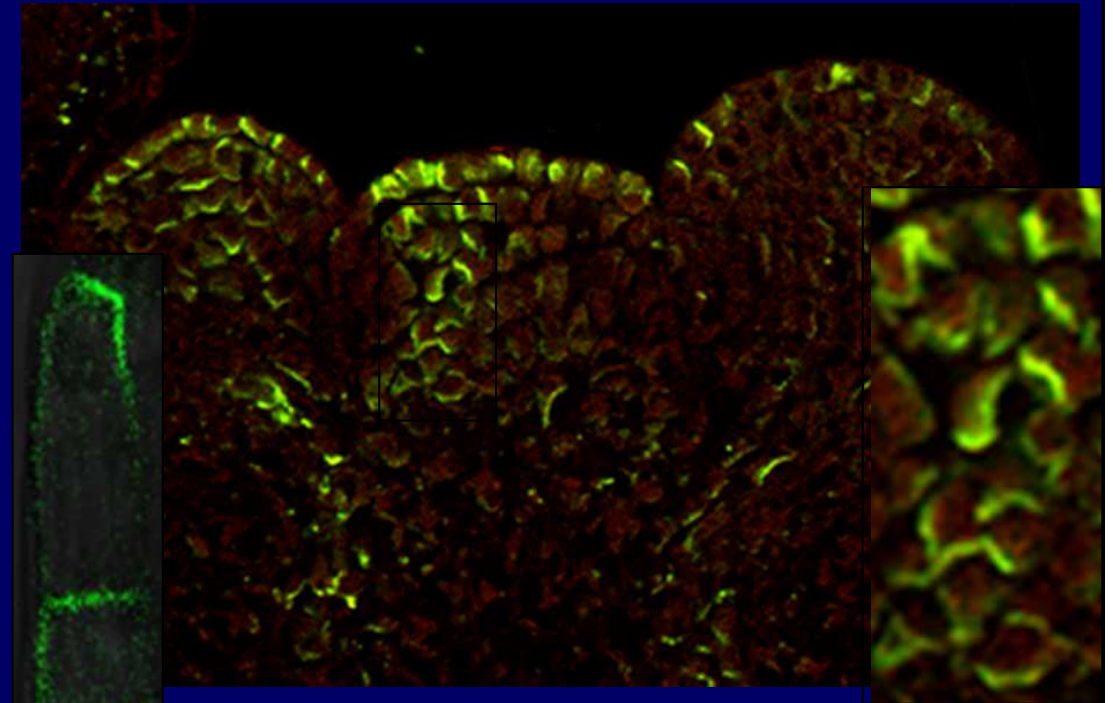
*DR5rev::GFP*



+ NPA



PIN1 localisation



+ NPA

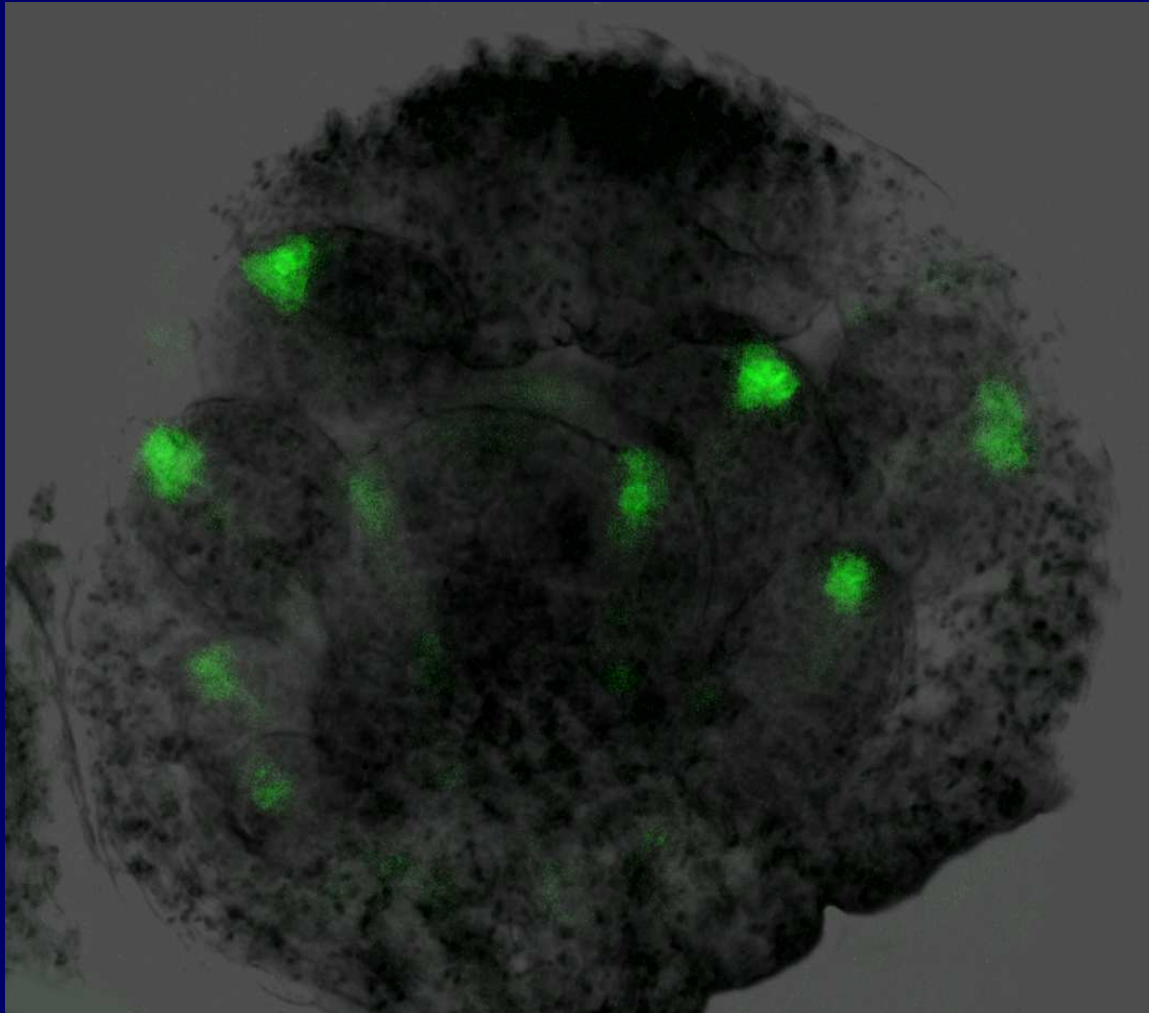


*pin1*

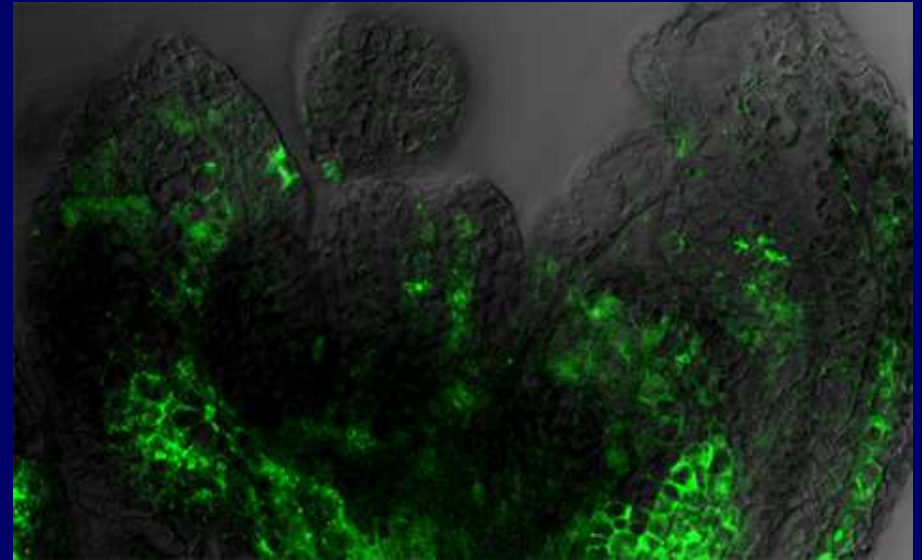


# DR5 in Floral Organ Formation

*DR5rev::GFP*



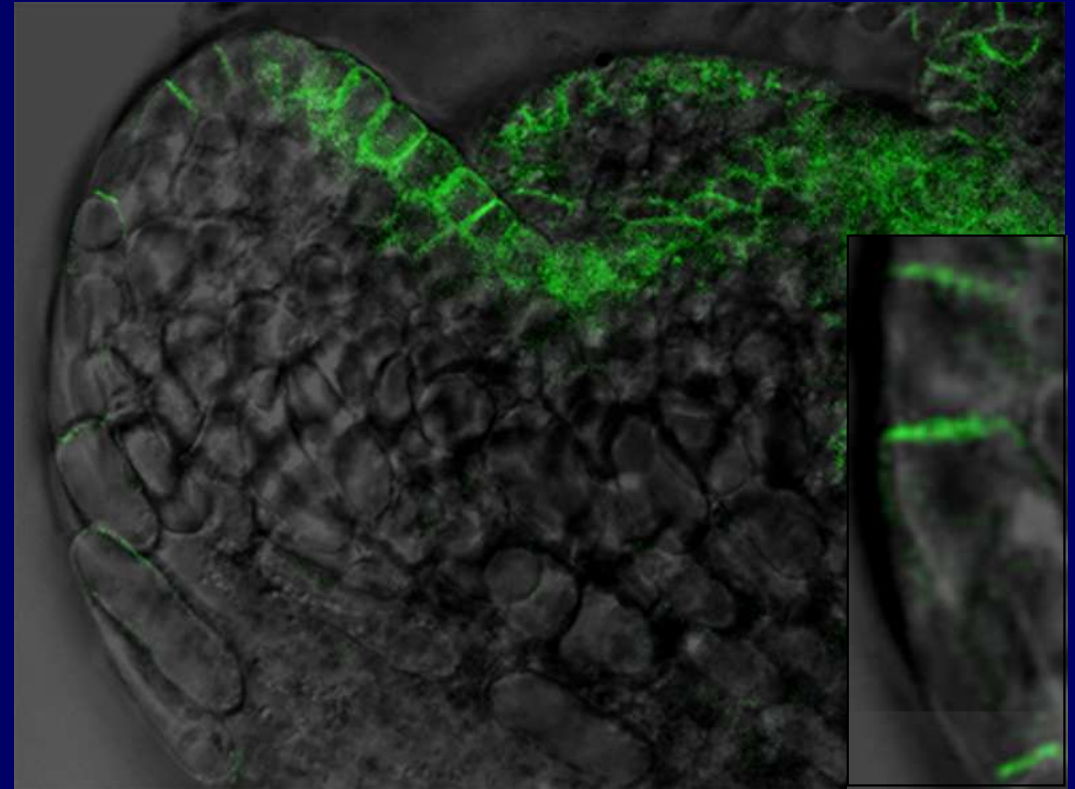
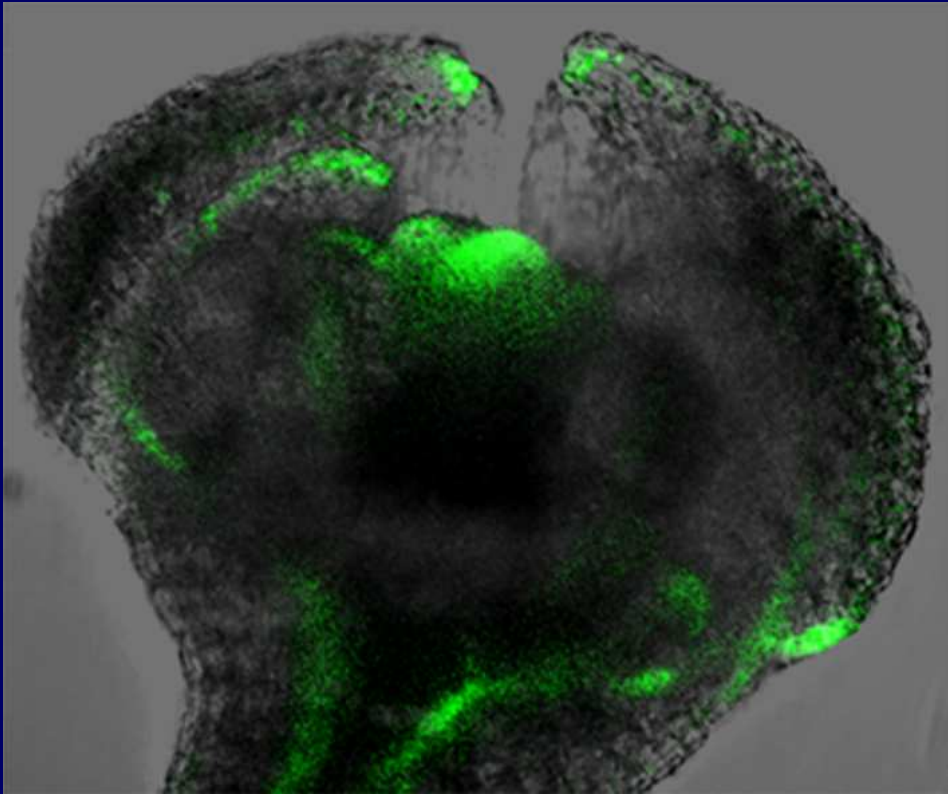
+ NPA



*pin* mutants

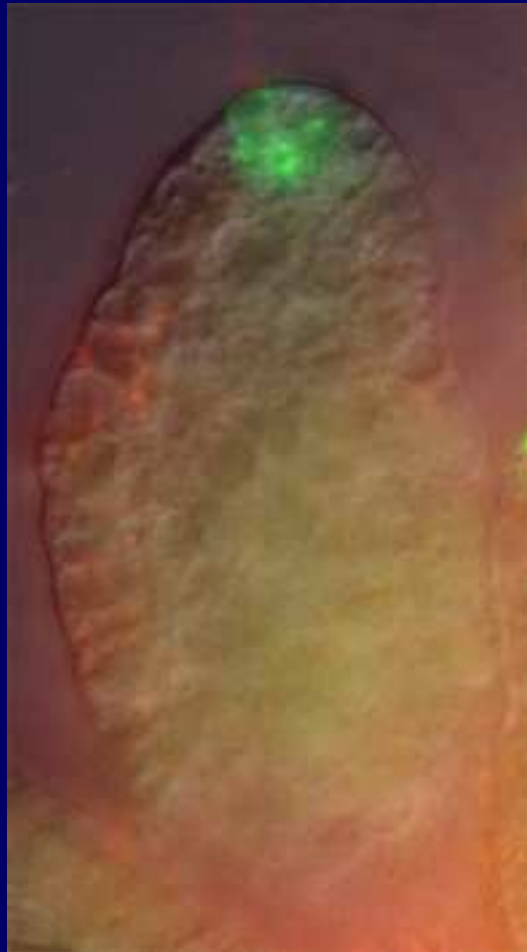


# PIN1 in Floral Organ Formation



# DR5 in Ovule Formation

Ovule  
primordium



Ovule with  
Integuments  
primordia

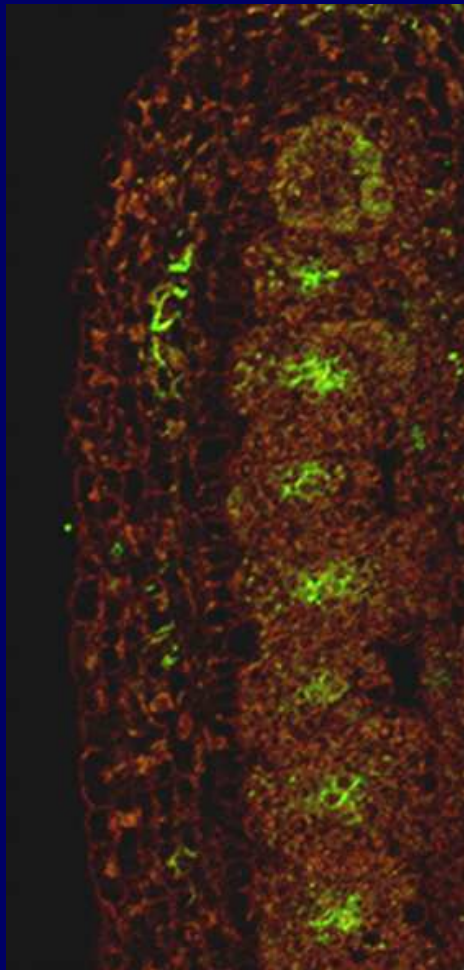


Ovule defects  
in *pin1*

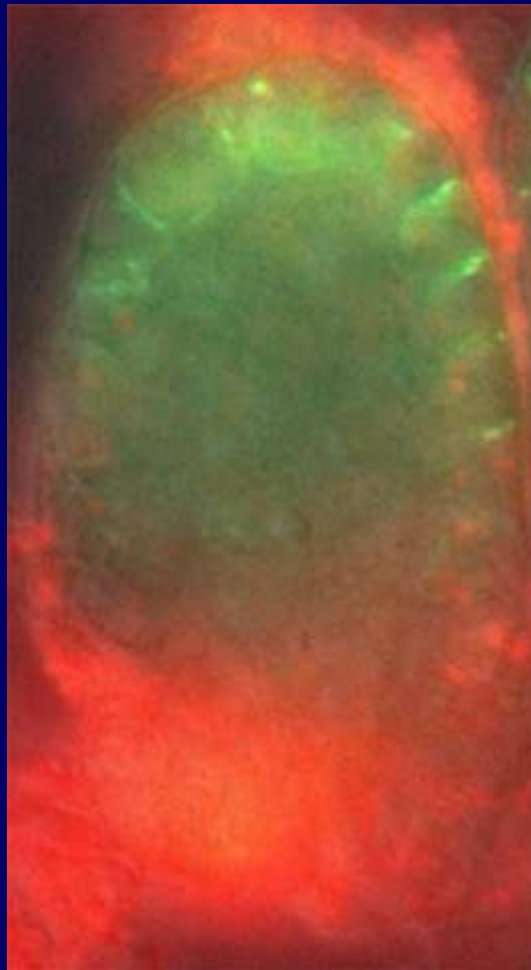


# PIN1 in Ovule Formation

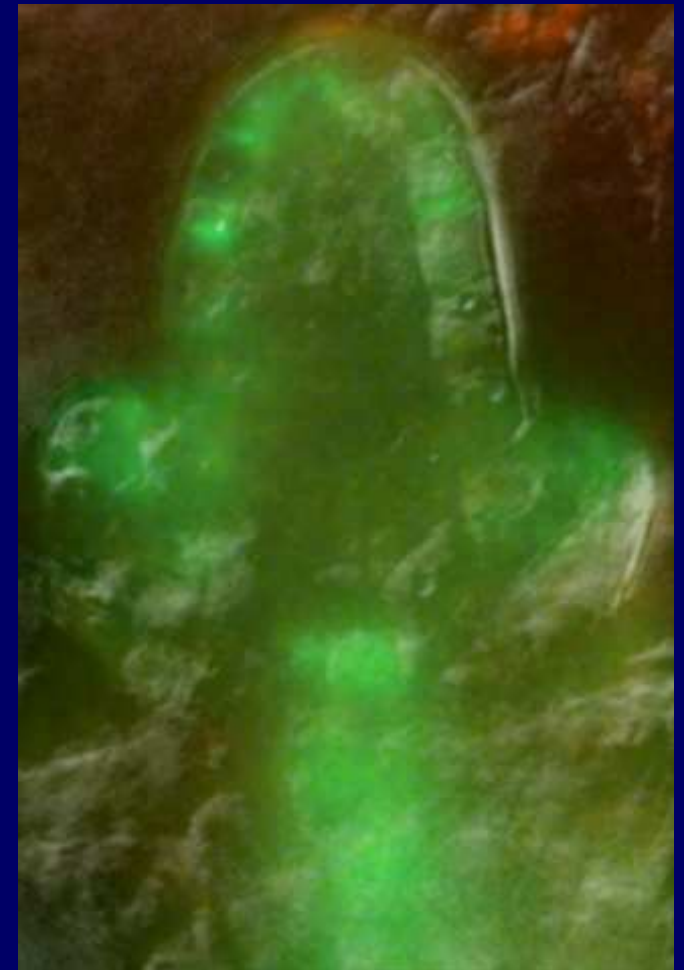
**Gynoecium  
with ovule primordia**



**Ovule  
primordium**

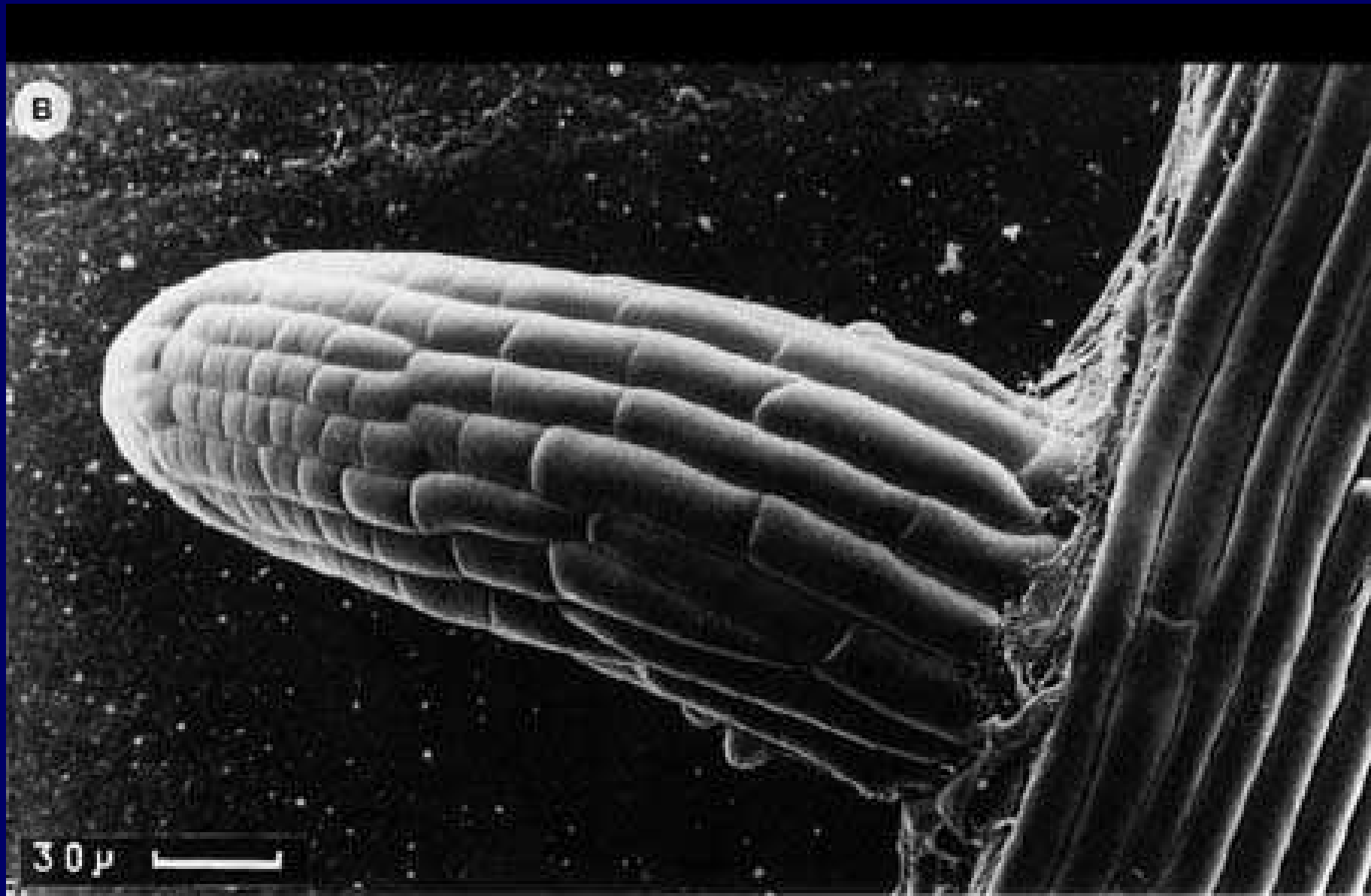


**Ovule with  
Integuments  
primordia**



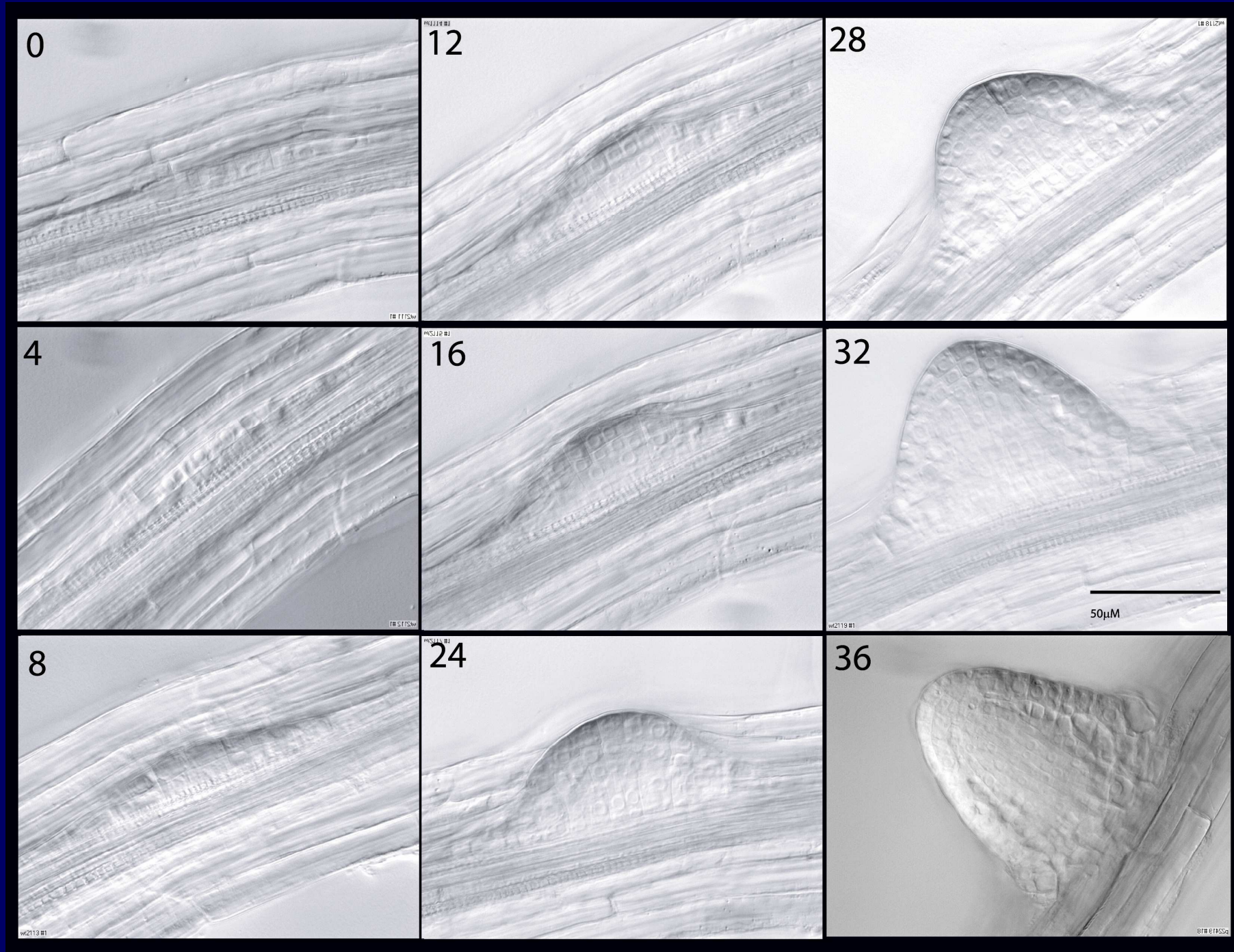


# Lateral Root Development

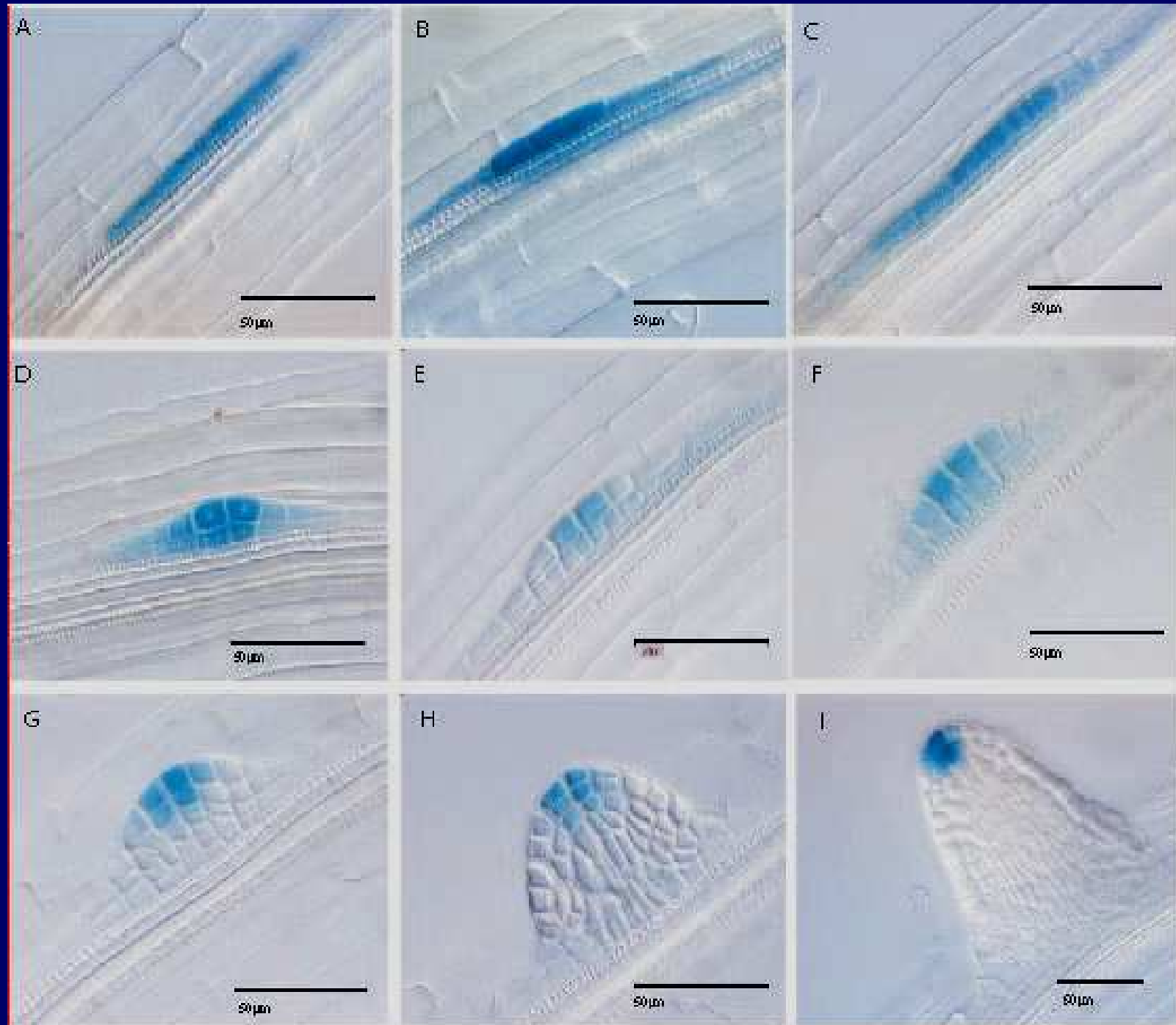


*Arabidopsis lateral root*

# Lateral Root Development in Time



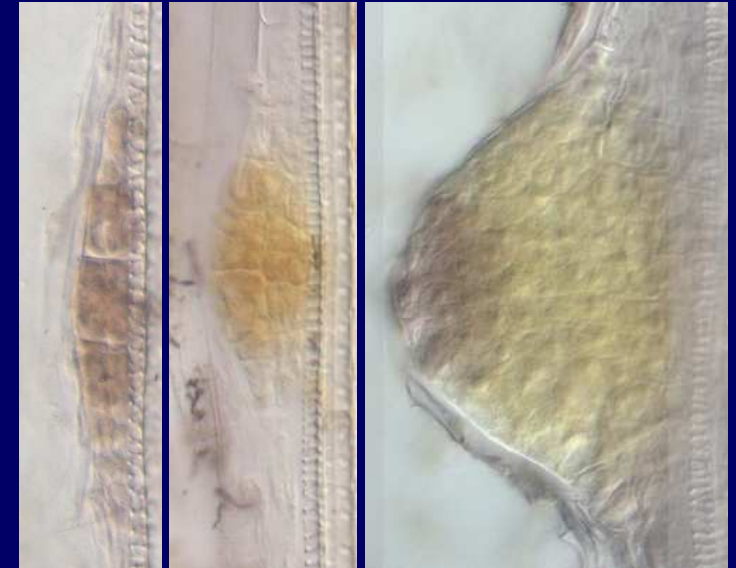
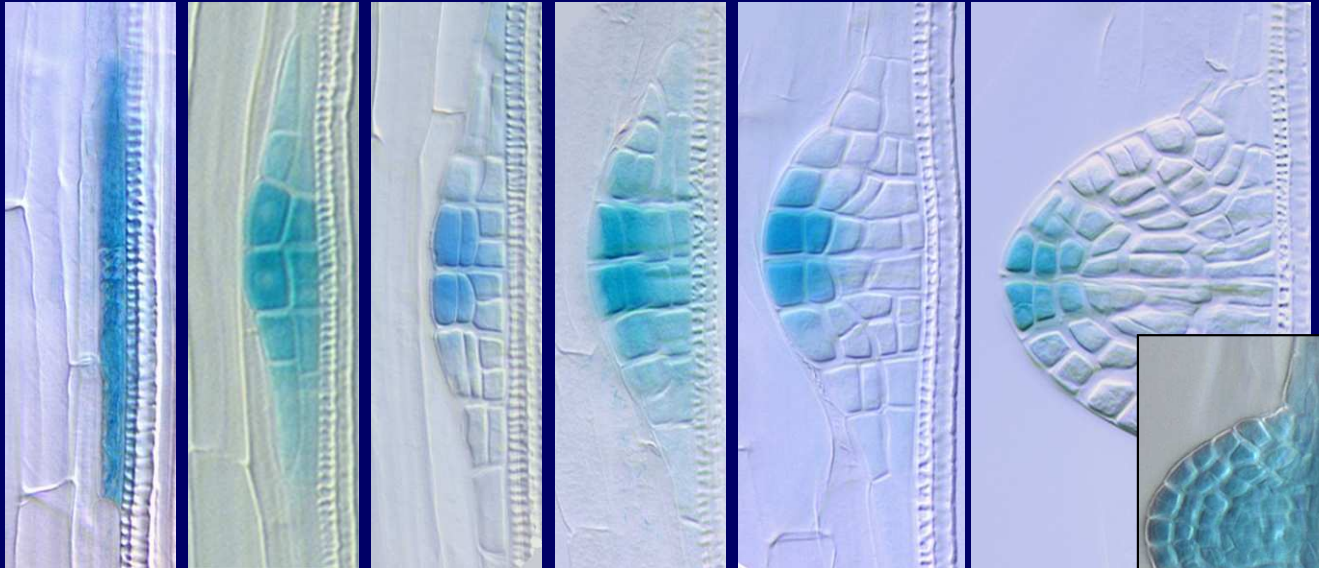
# Auxin in Lateral Root Development



# DR5 in Lateral Root Formation

*DR5rev::GUS*

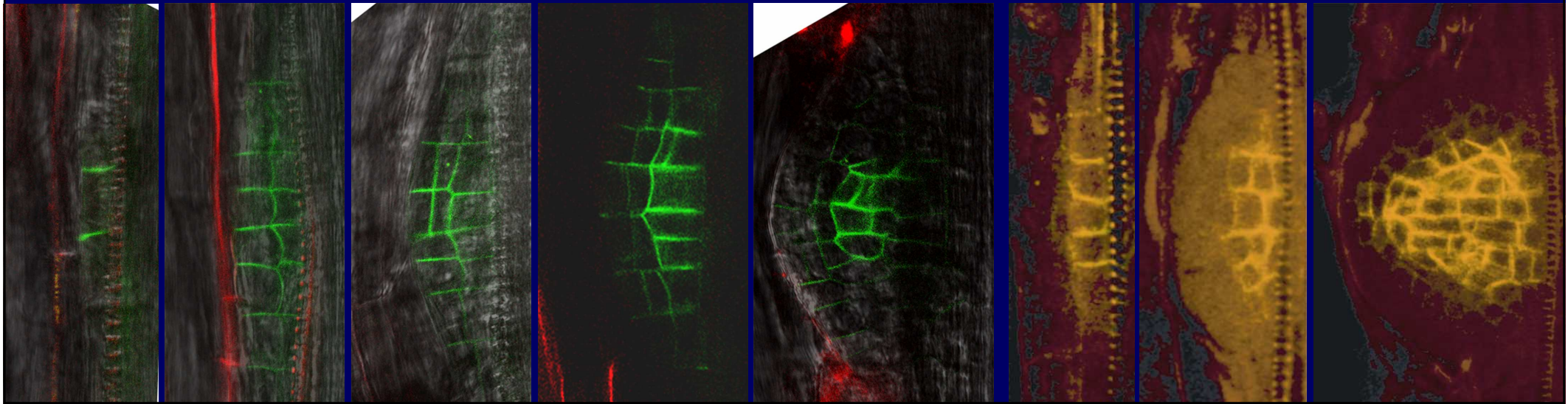
IAA



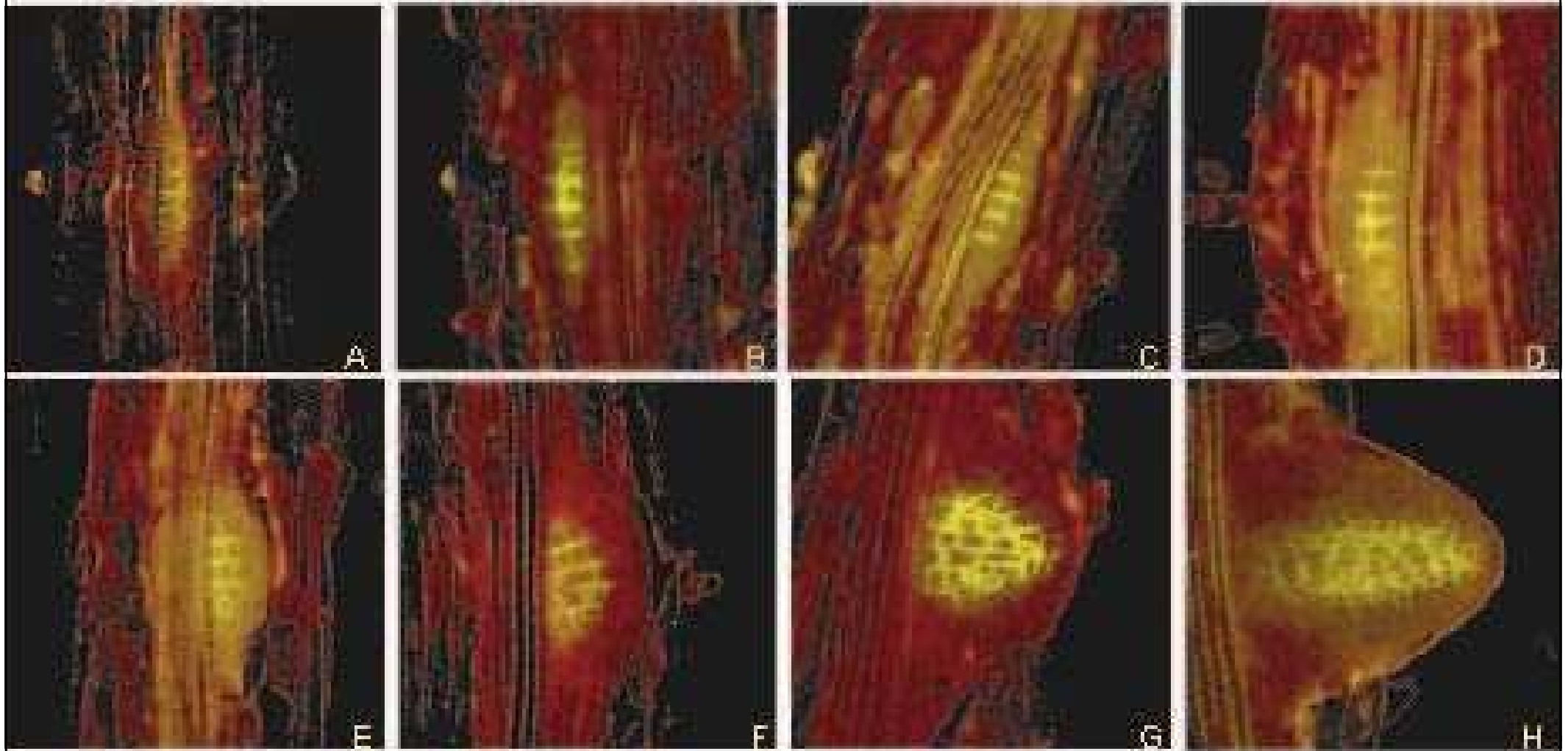
PIN1:GFP

+ NPA

PIN1



# PIN1 in Lateral Root Development



# Relocation > Gradients > Primordia

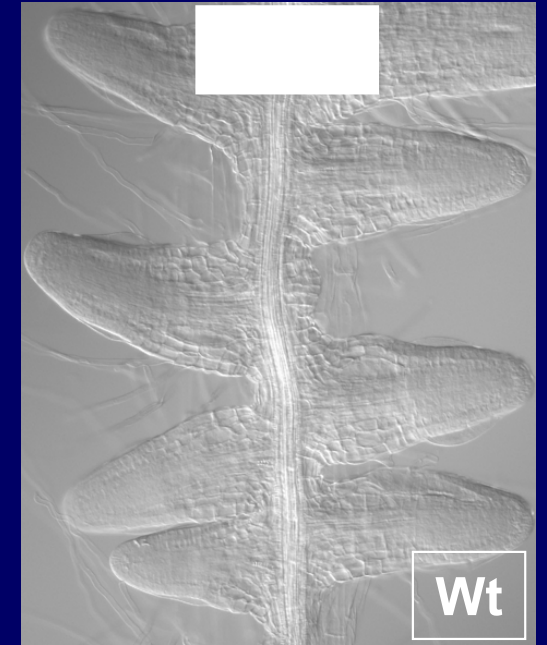
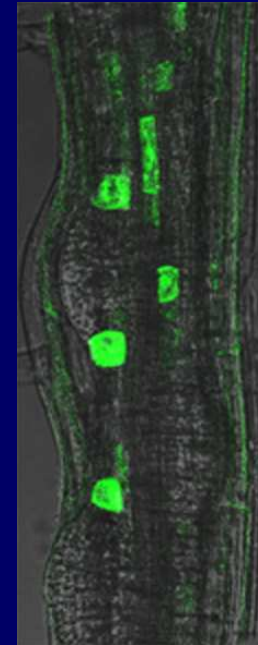
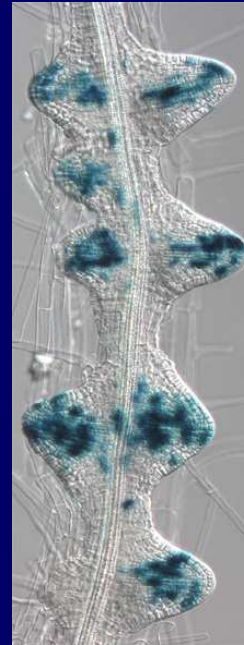
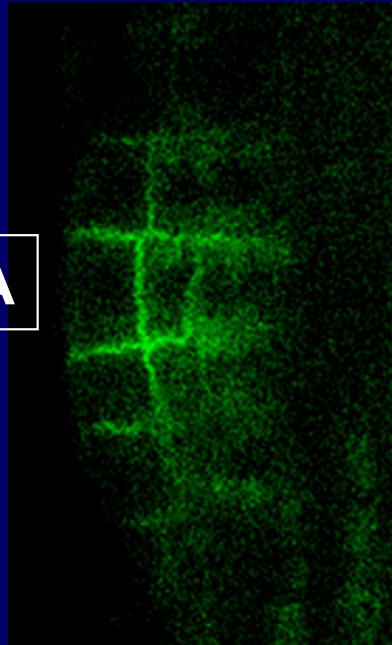
PIN1

DR5

CycB margins

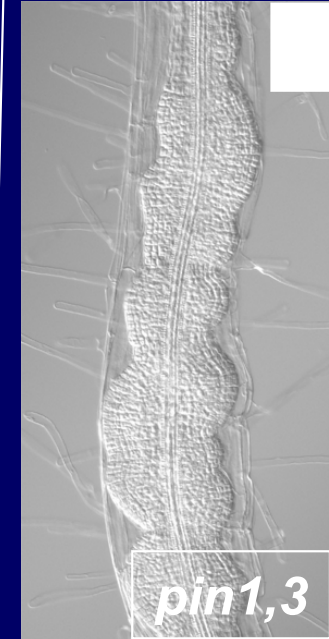
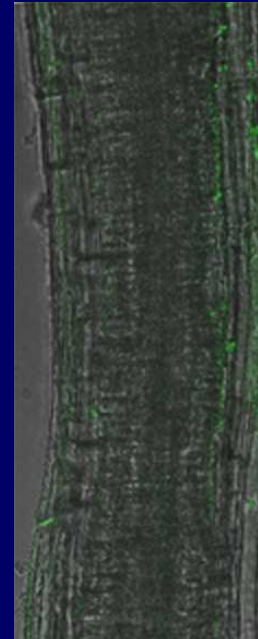
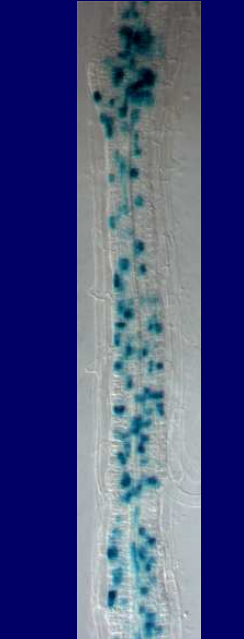
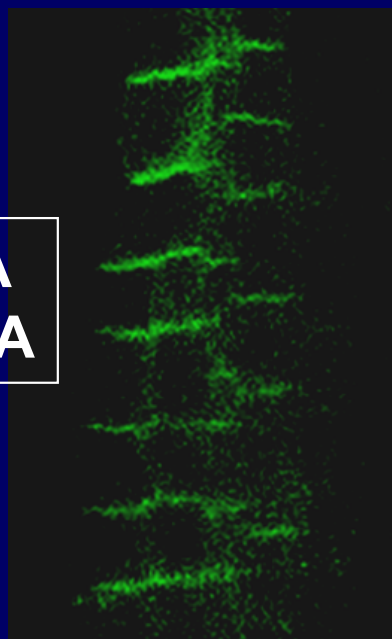
primordia

+ IAA

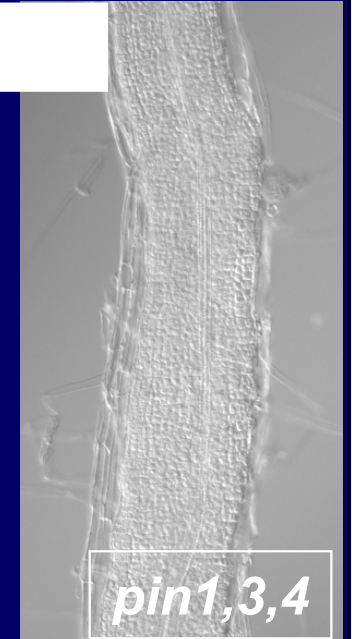


Wt

+ IAA  
+ NPA



pin1,3



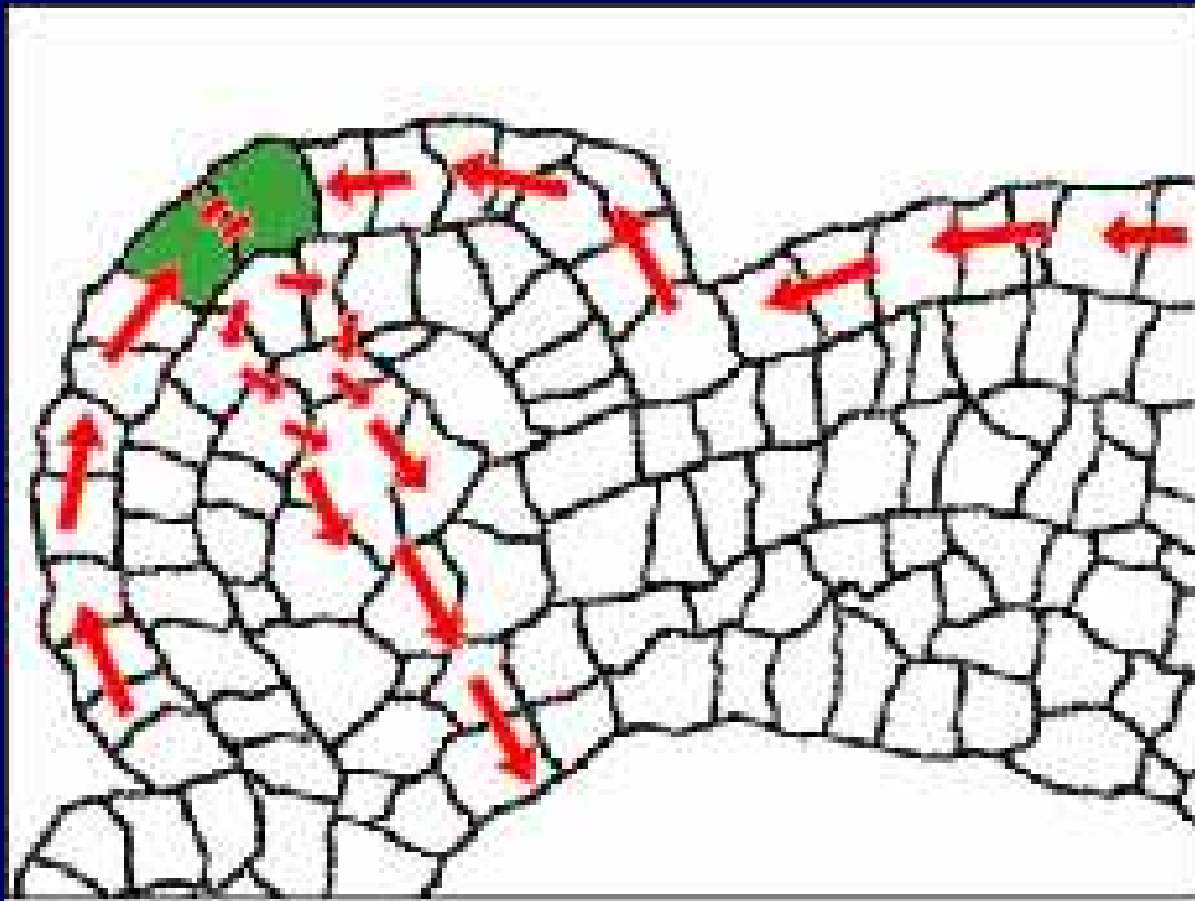
pin1,3,4

# Lateral Root Development

- Organogenic process involving re-entry into cell cycle and coordinated cell divisions and differentiation.
- Initiation (in pericycle) and development phases can be distinguished.
- Both phases require both long and short distance signaling probably by auxin and cytokinin.
- The lateral root meristem development is mediated by auxin gradient.

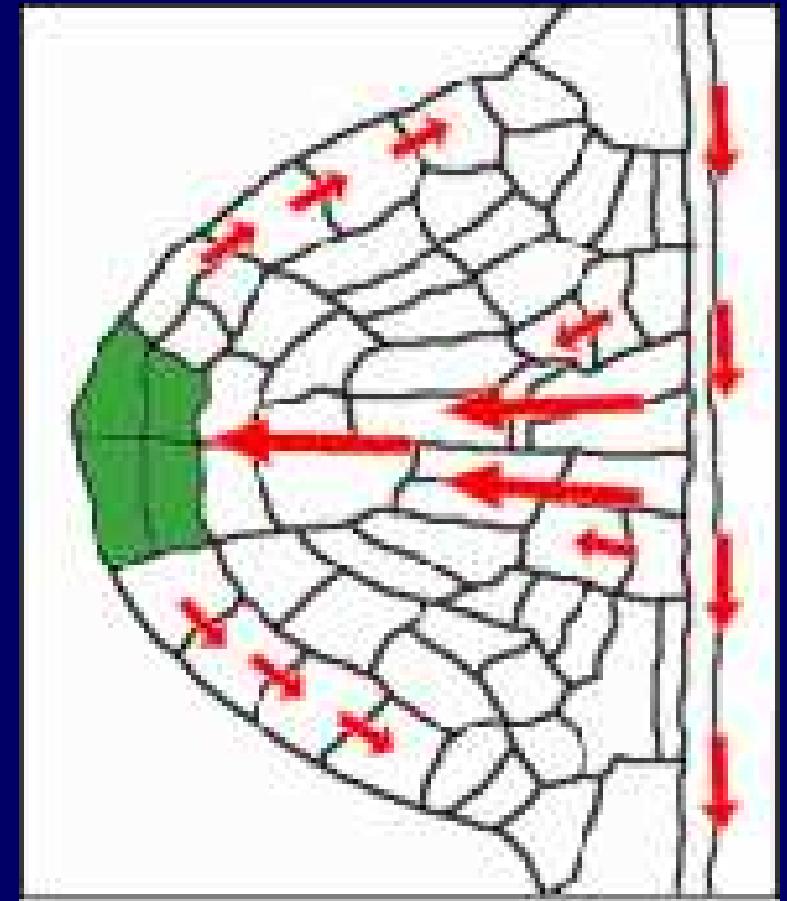
# Common module for organ formation

## Aerial organogenesis



Cotyledons, leaves, flowers,  
floral organs, ovules, integuments

## Underground organogenesis

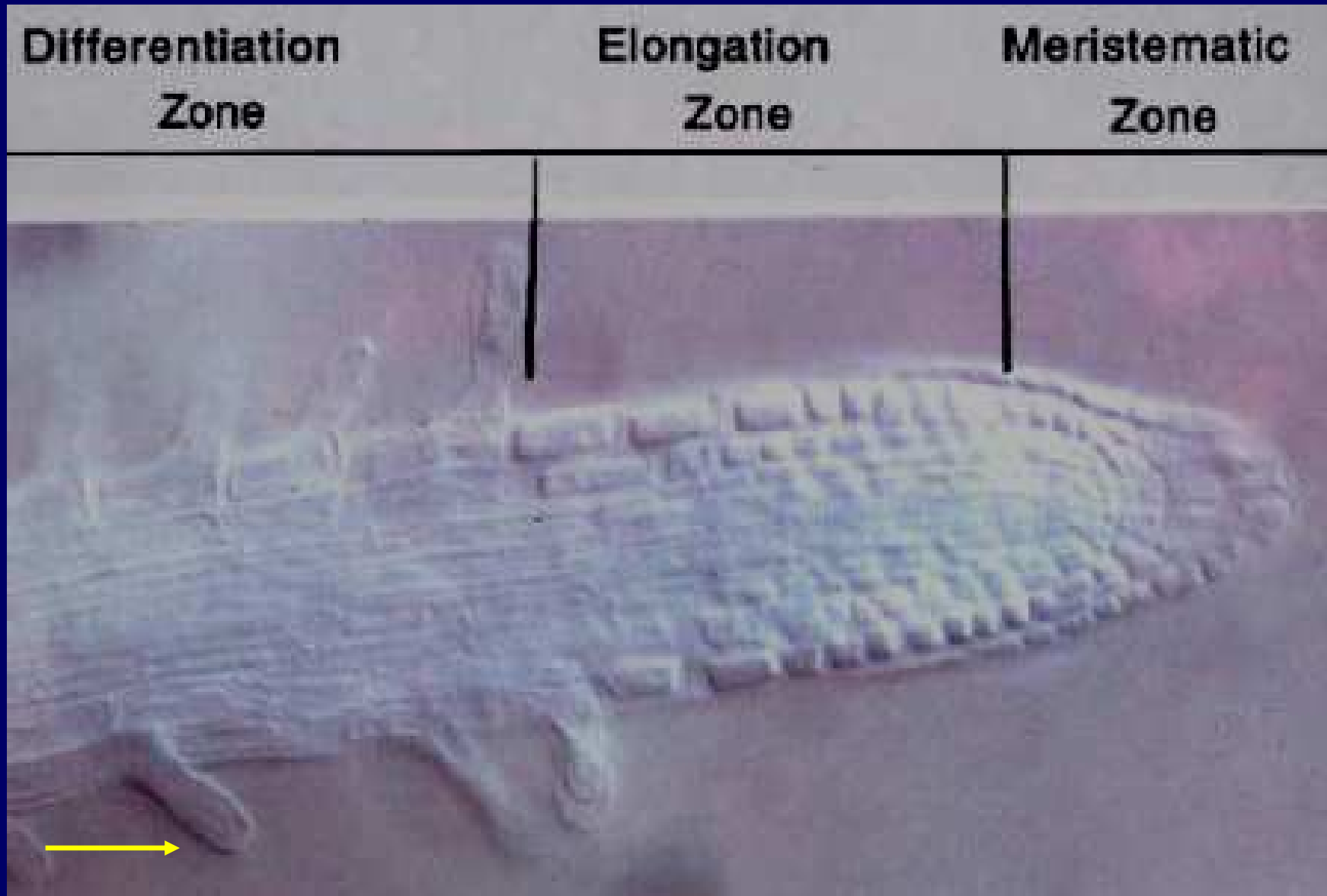


Lateral roots



# Root meristem

# Parts of the Primary Root



Differentiation  
Zone

Elongation  
Zone

Meristematic  
Zone

Root hair



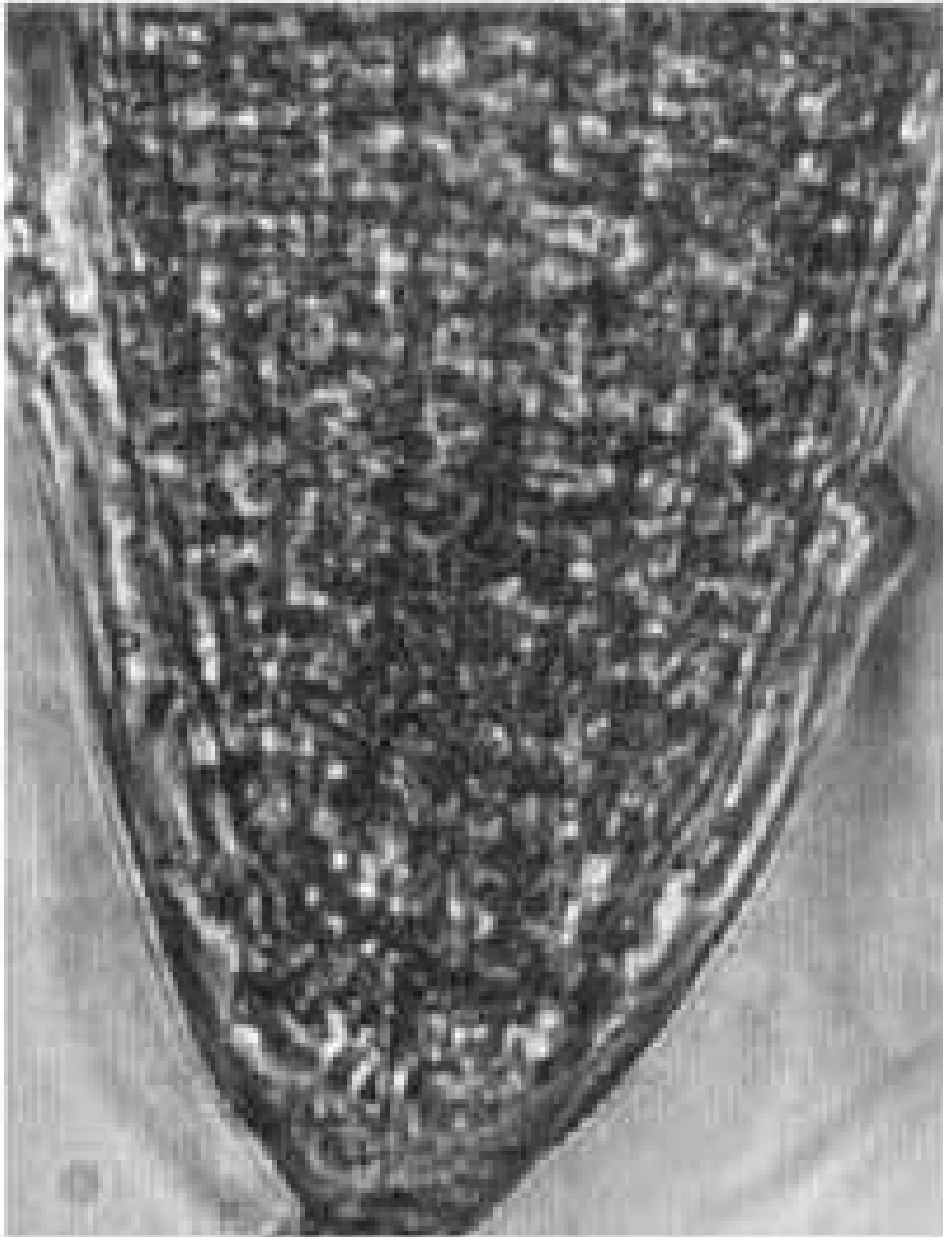
Differentiation

Elongation

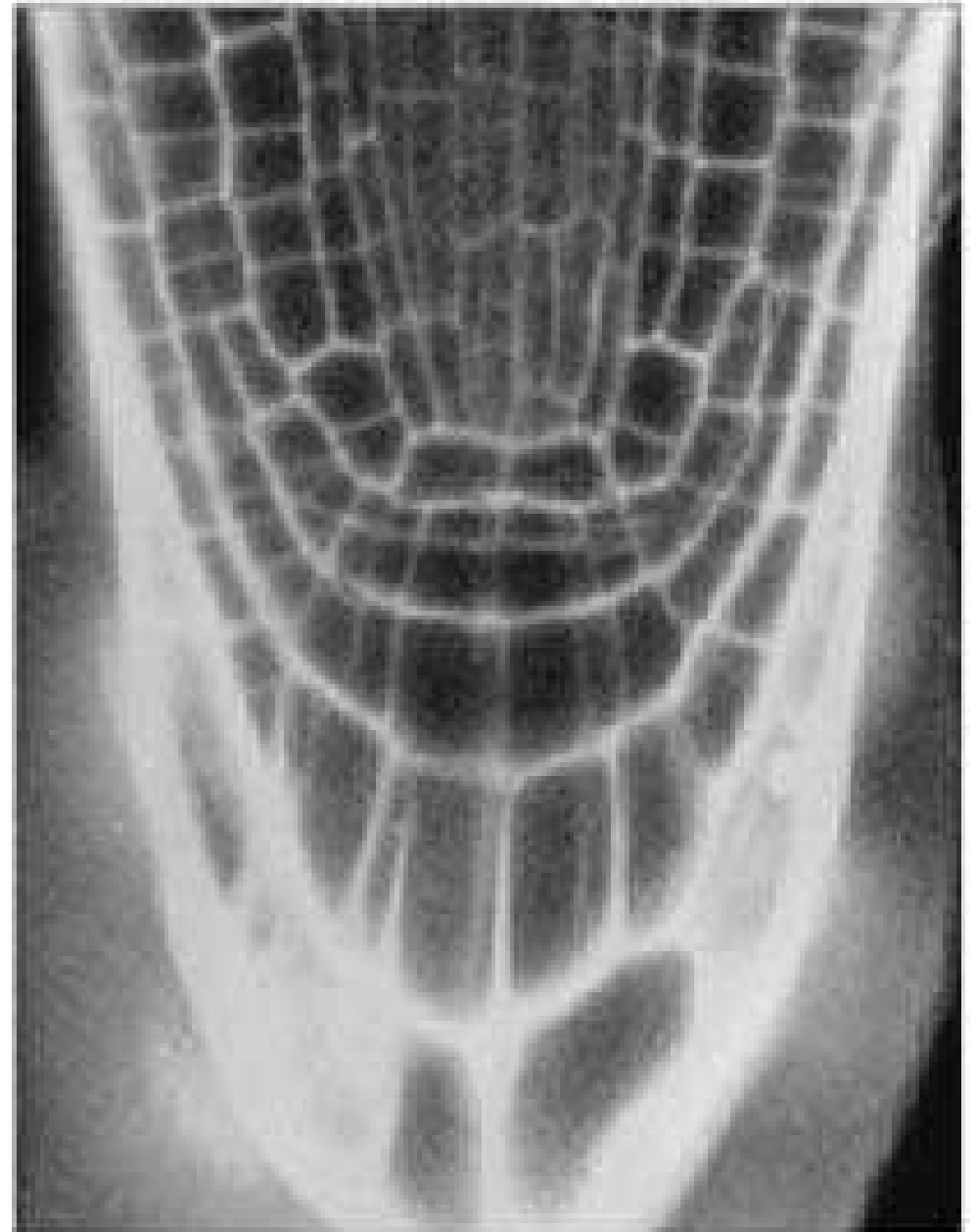
Division

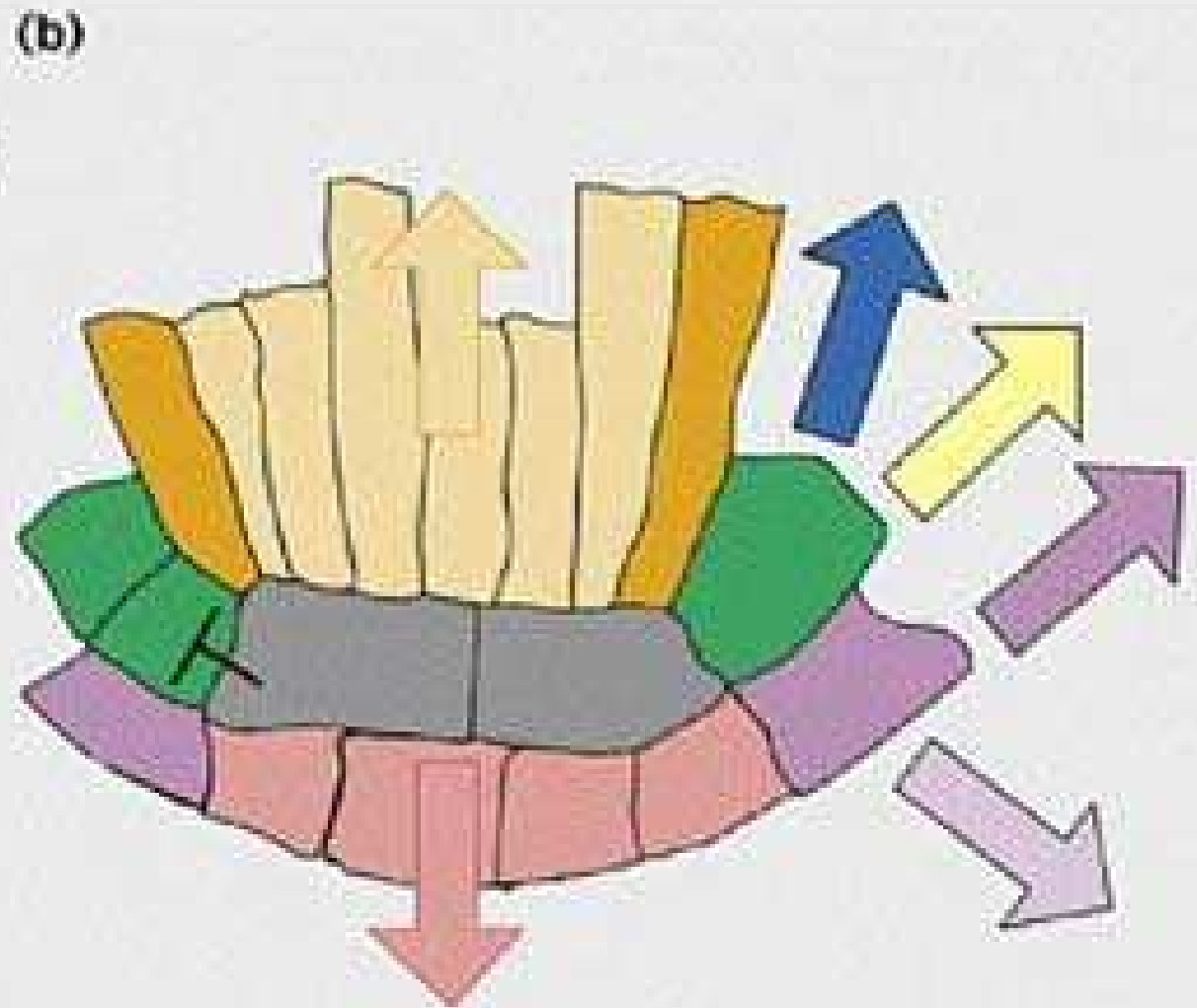
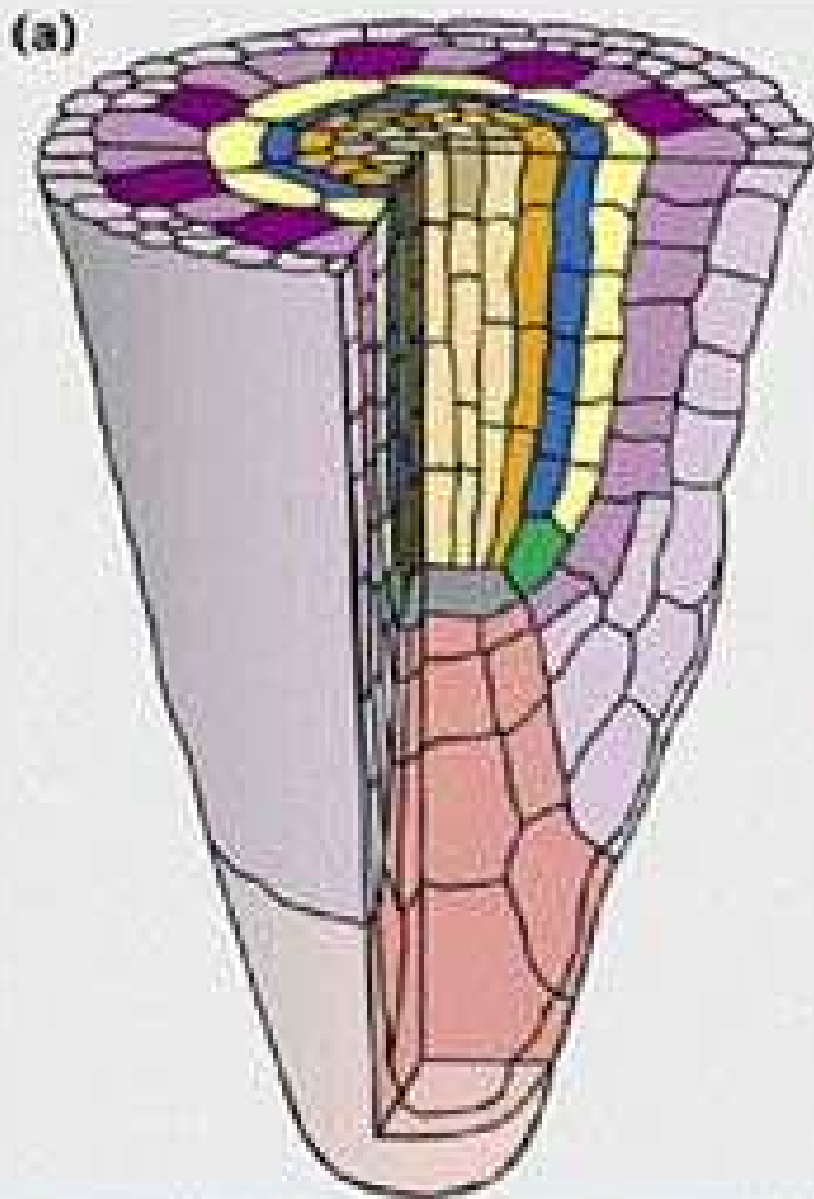
# Root Meristem

Light microscopy



Confocal microscopy





Current Biology

Xylem and phloem

Cortex initial

NH and RH epidermis

Quiescent center

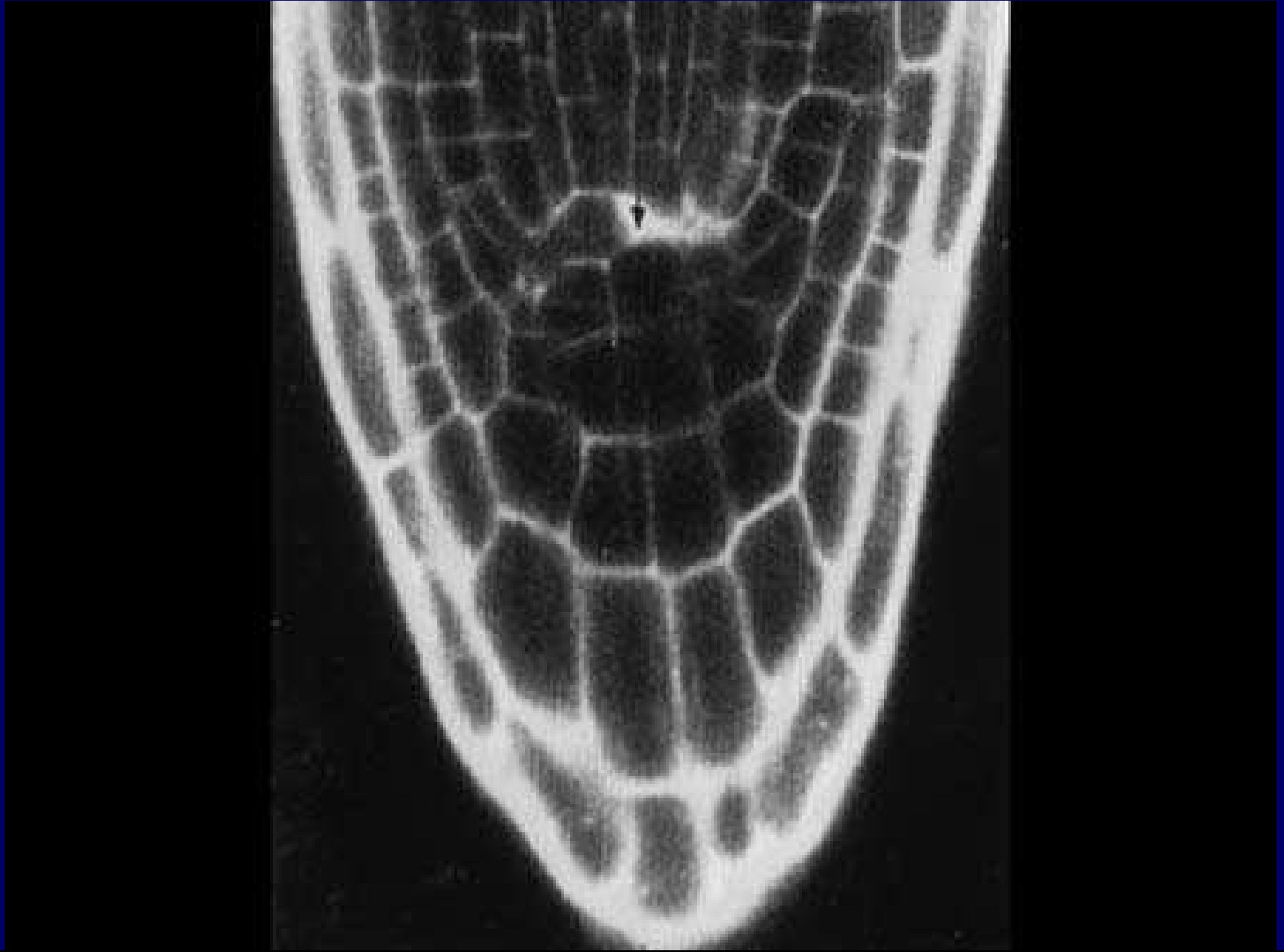
Pericycle

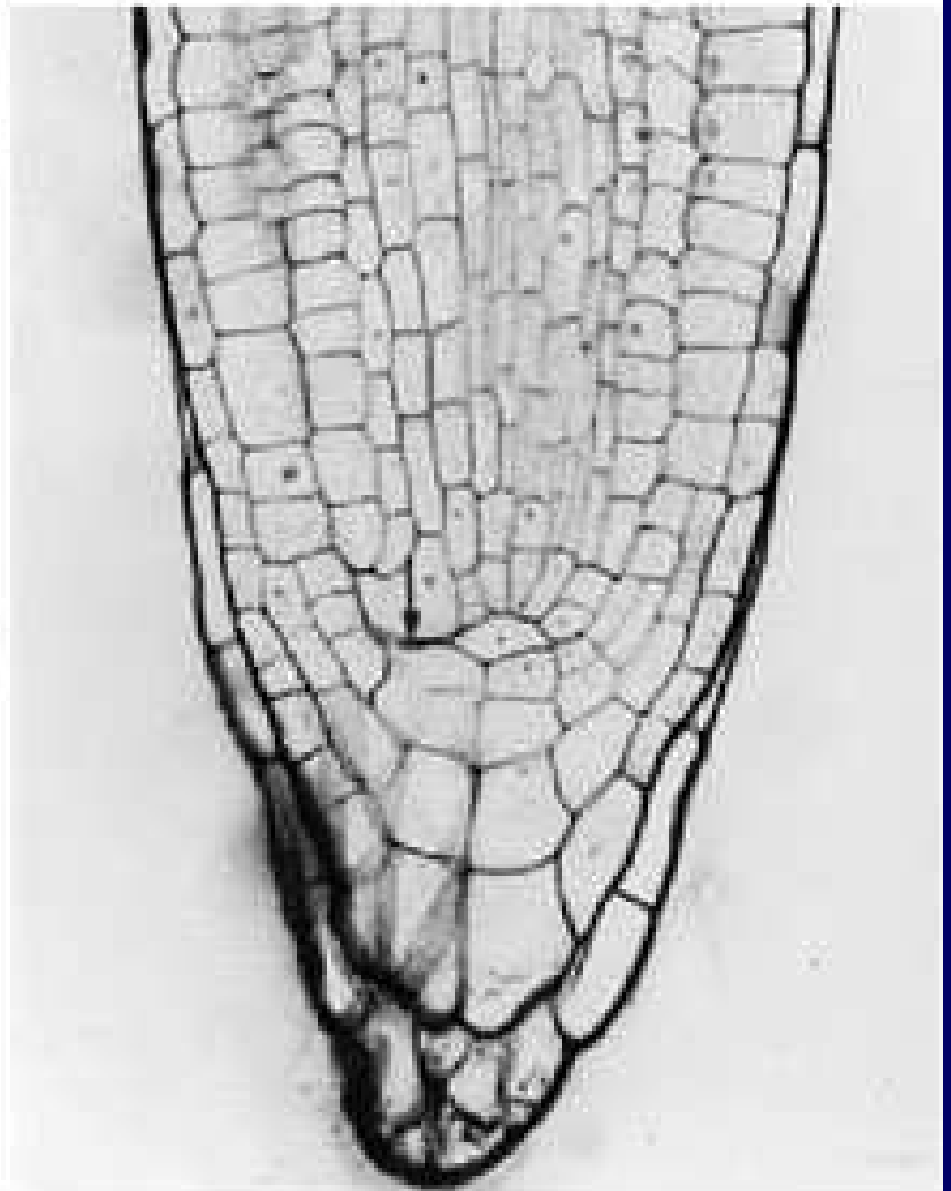
Cortex; endodermis

Lateral root cap

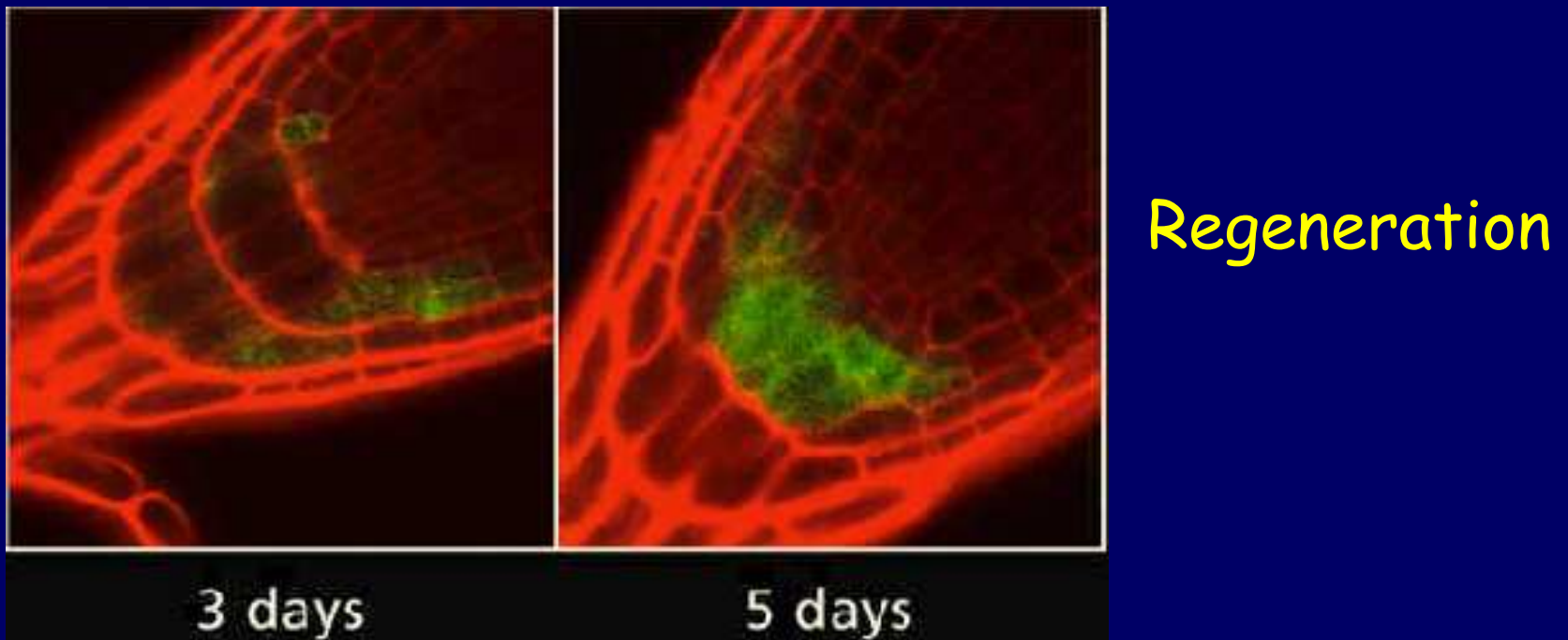
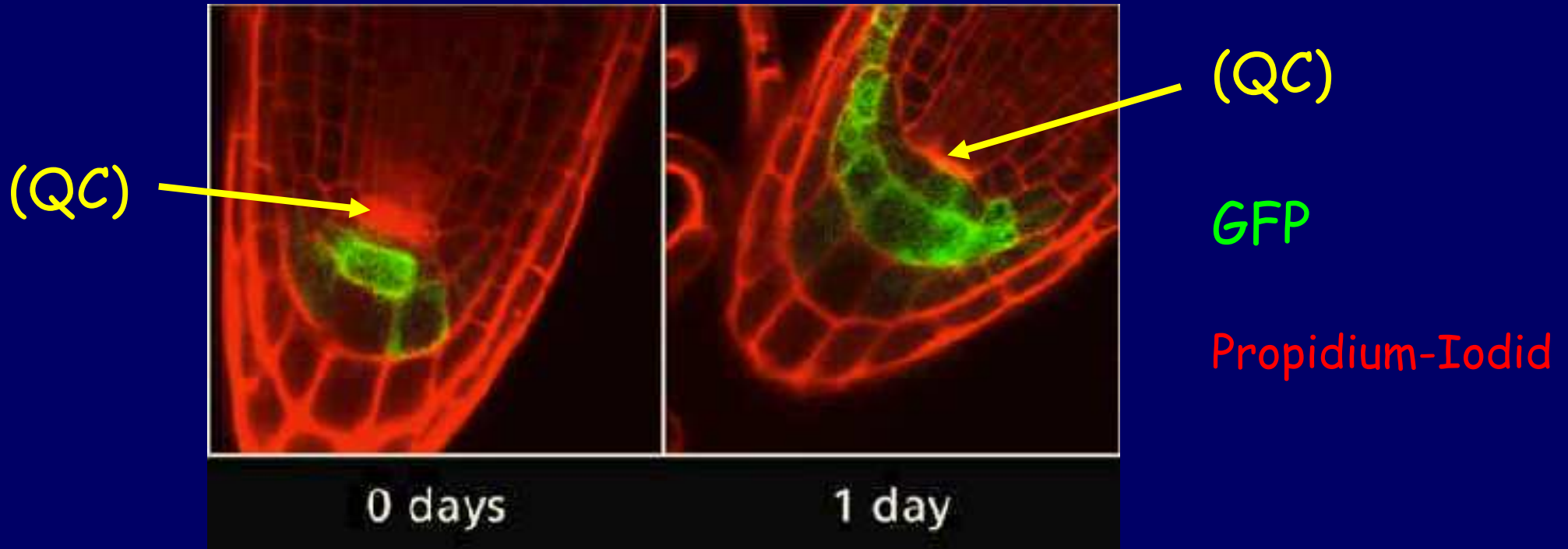
Columella root cap

# Laser Ablation of Single QC Cell



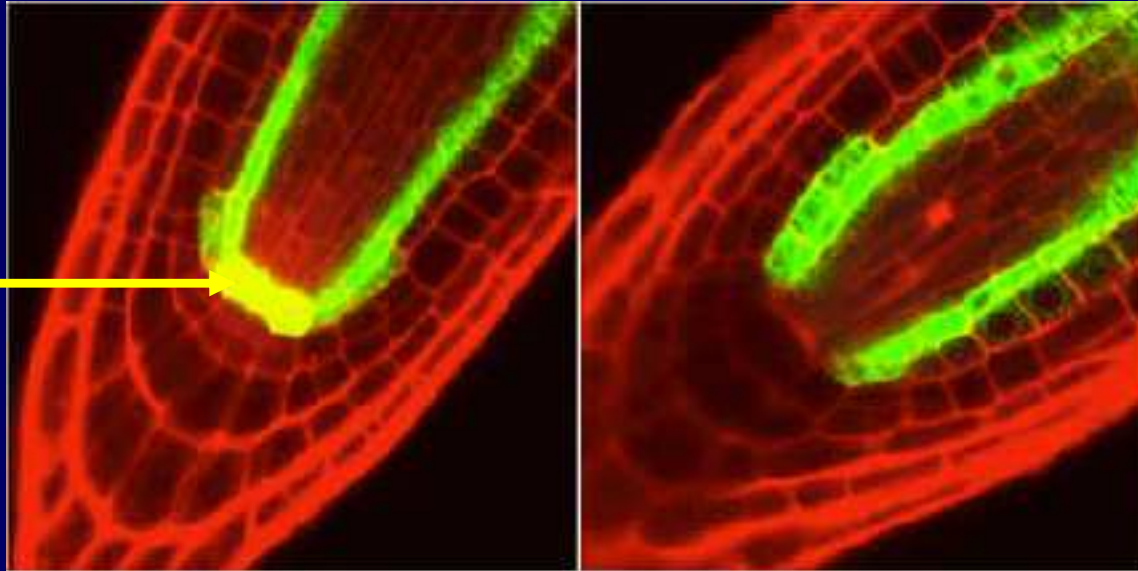


# Laser Ablation of Quiescent Centre



# Regeneration of Quiescent Centre

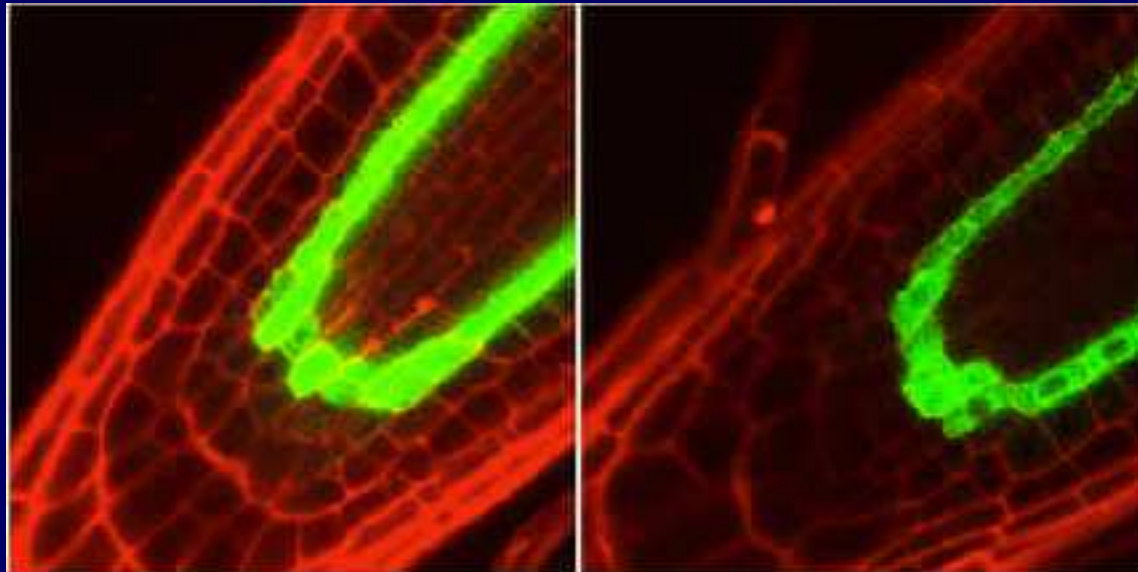
QC



0 days

1 day

SCR::GFP  
(Endodermis + QC)

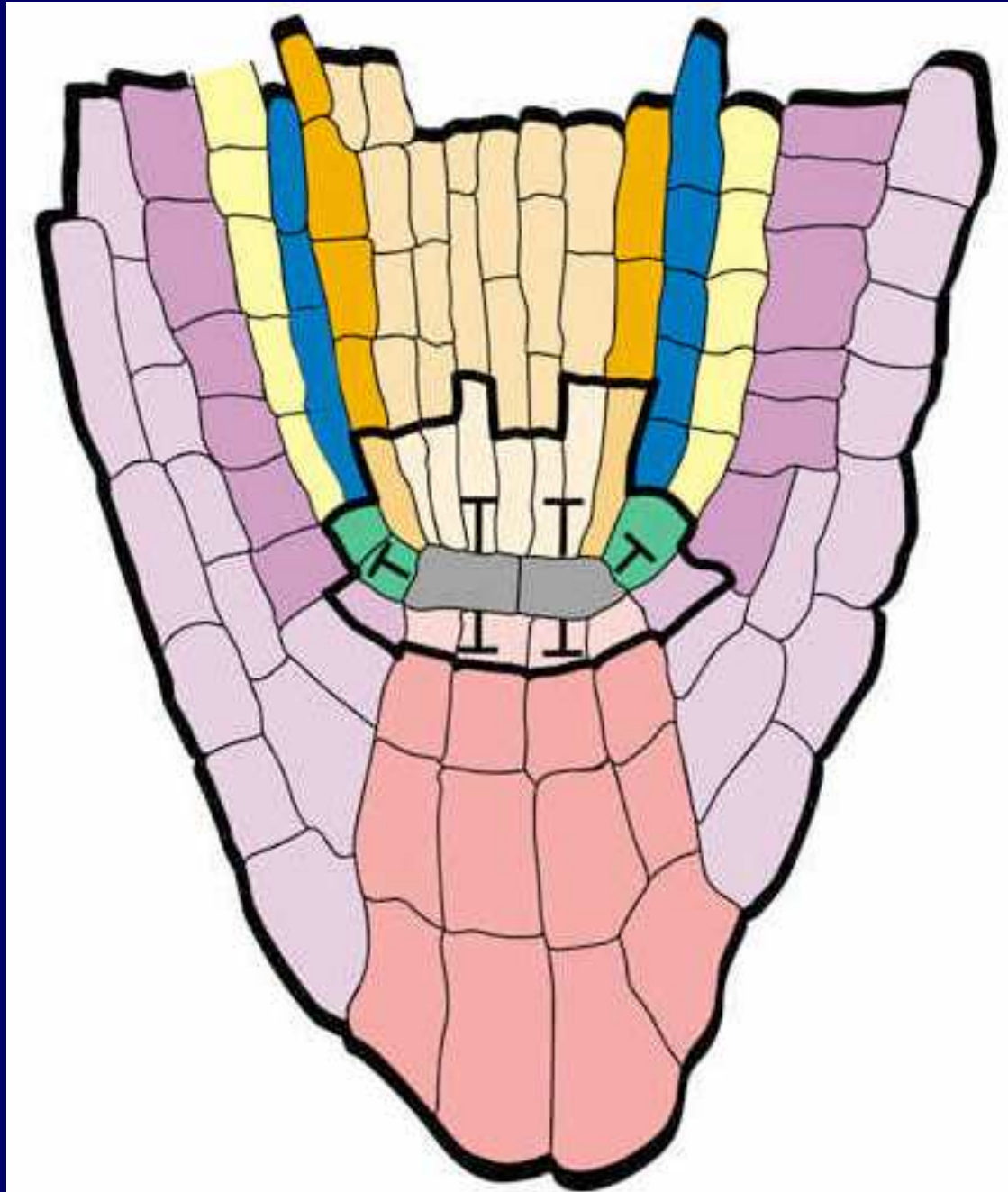


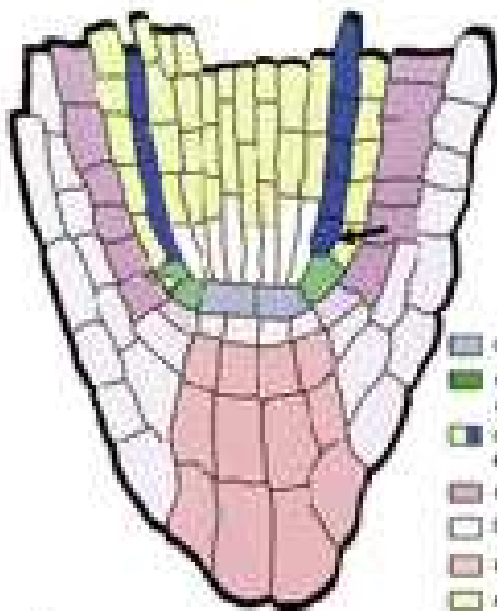
3 days

5 days



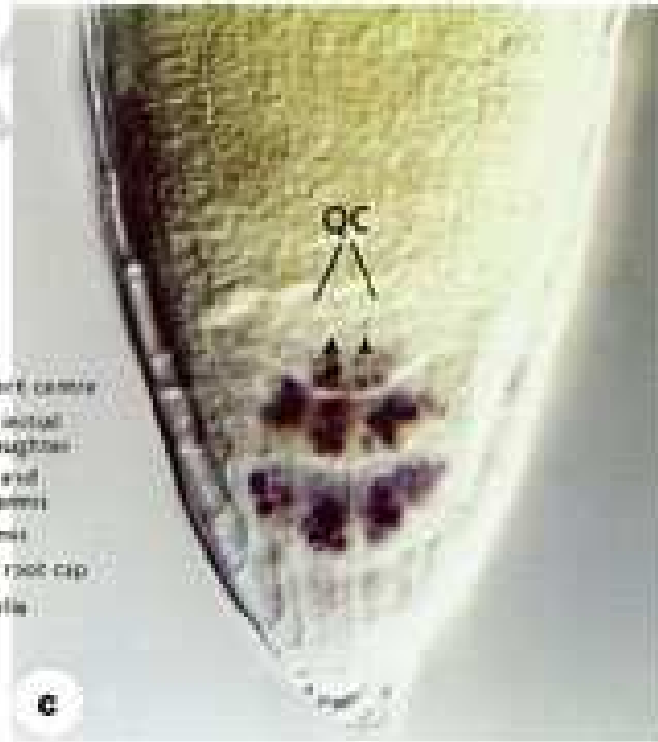
# Model for Role of QC in Keeping Stem Cells





- quiescent centre
- cortex initial and daughter
- cortex and endodermis
- epidermis
- lateral root cap
- columella
- stele

a



c



d

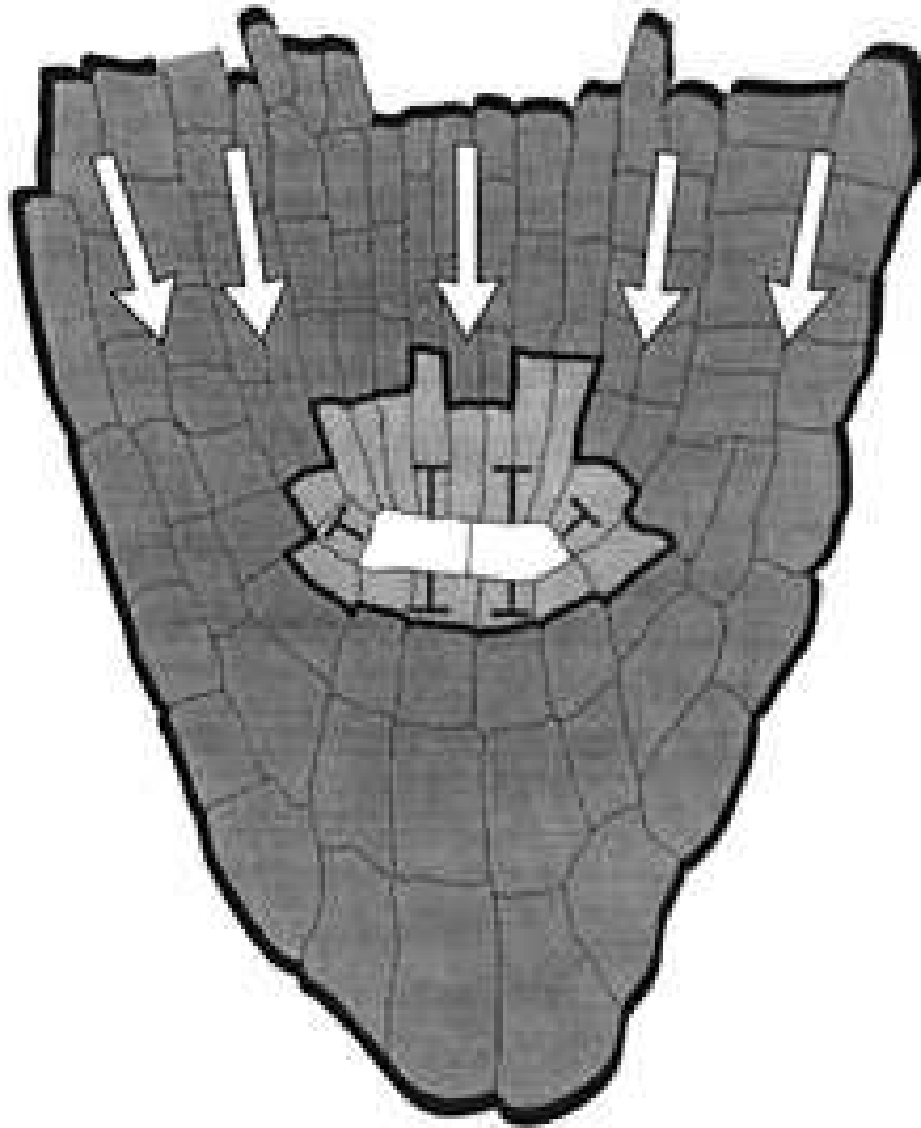


Fig. 4. A simple model representing two different regulatory signals within the root meristem. The quiescent centre inhibits differentiation of surrounding initials, whereas positional cues direct differentiation into different cell types

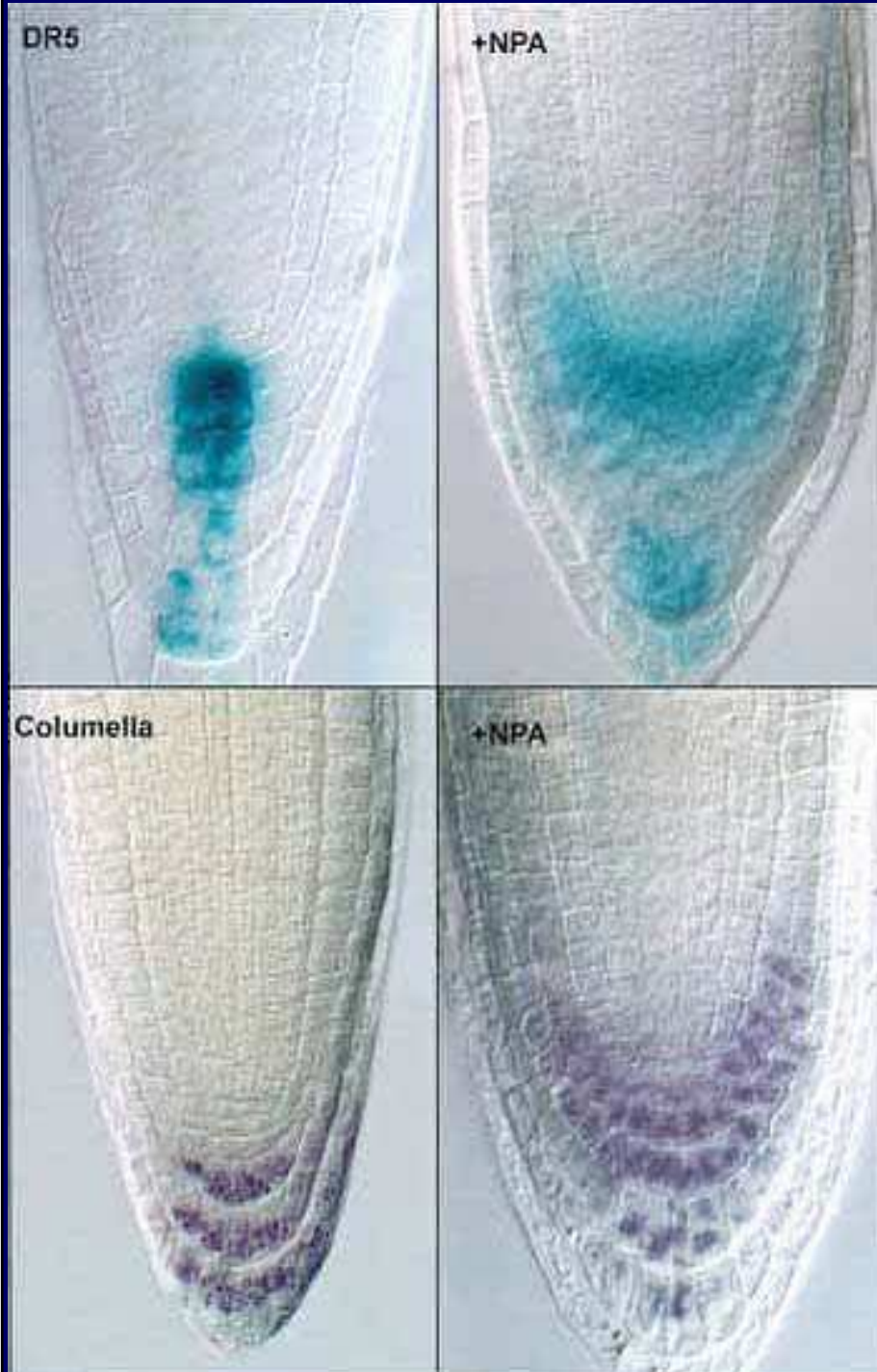
# Indirect Visualisation of Auxin

## *DR5::GUS* (Auxin) Response Reporter

→  
5' CCTTT TGTCTC 3'  
9x inv.



## Auxin and Root patterning



## Auxin related mutants affecting root pattern

Auxin resistant - *axr1*, *axr6*

AUX/IAA - bodenlos (*bdl*)

Auxin response factors  
- monopteros (*mp*)

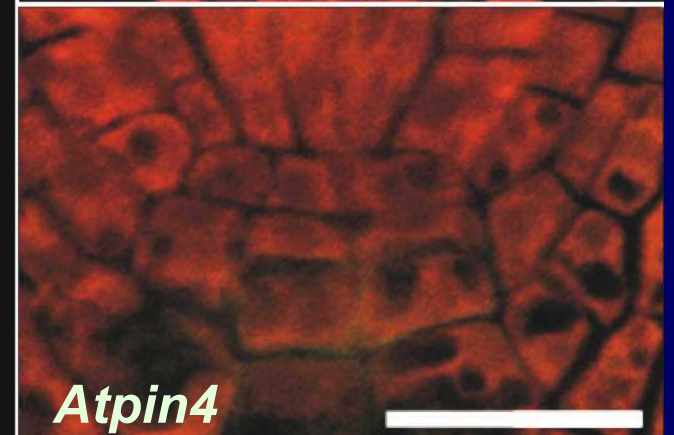
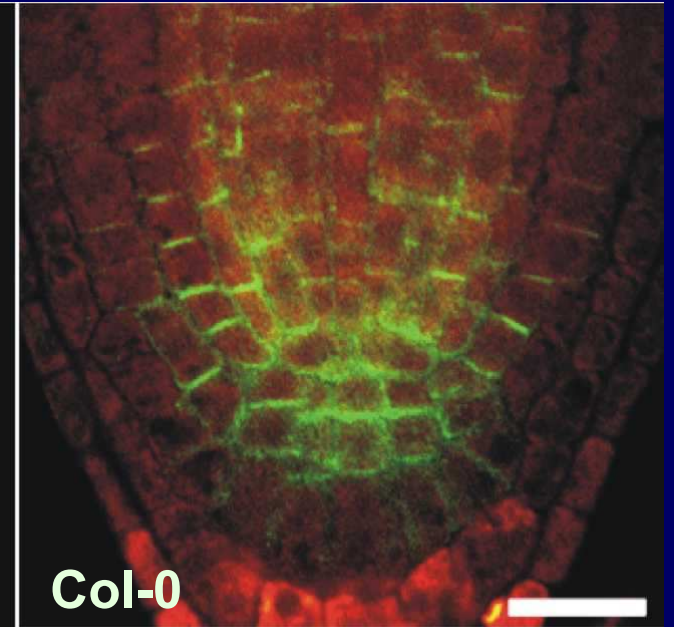
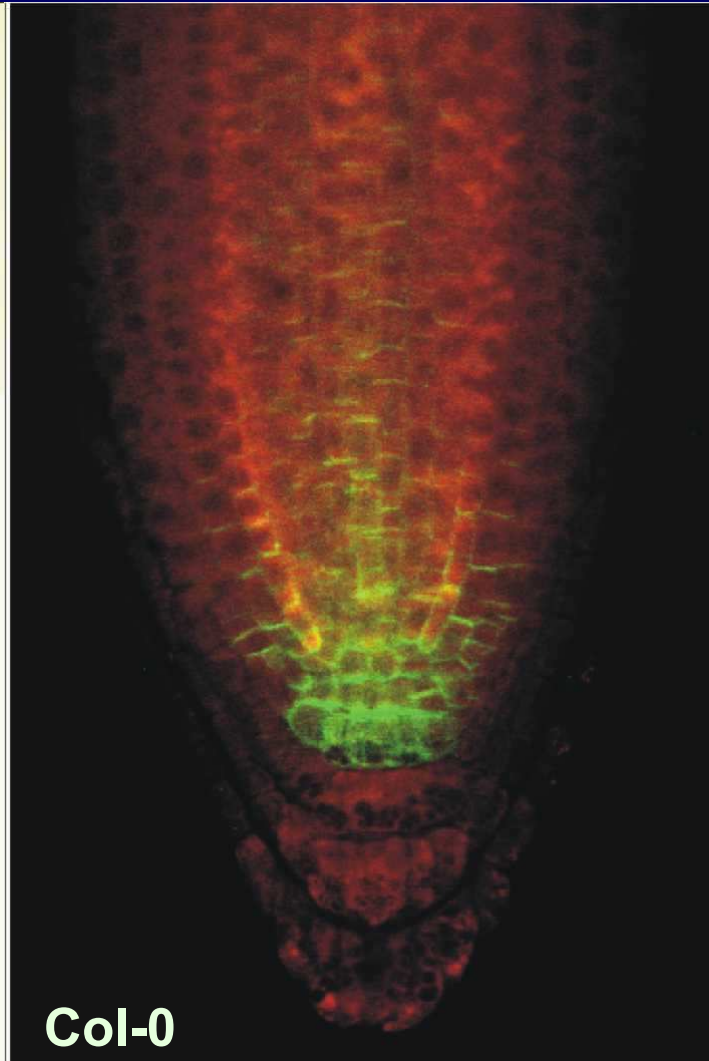
Auxin transport - *pin4*

# AtPIN4 in Arabidopsis Root Tip

*in situ* RNA hybridisation

The AtPIN4 protein

The AtPIN4 protein



# DR5 Auxin Response in Roots

Col-0



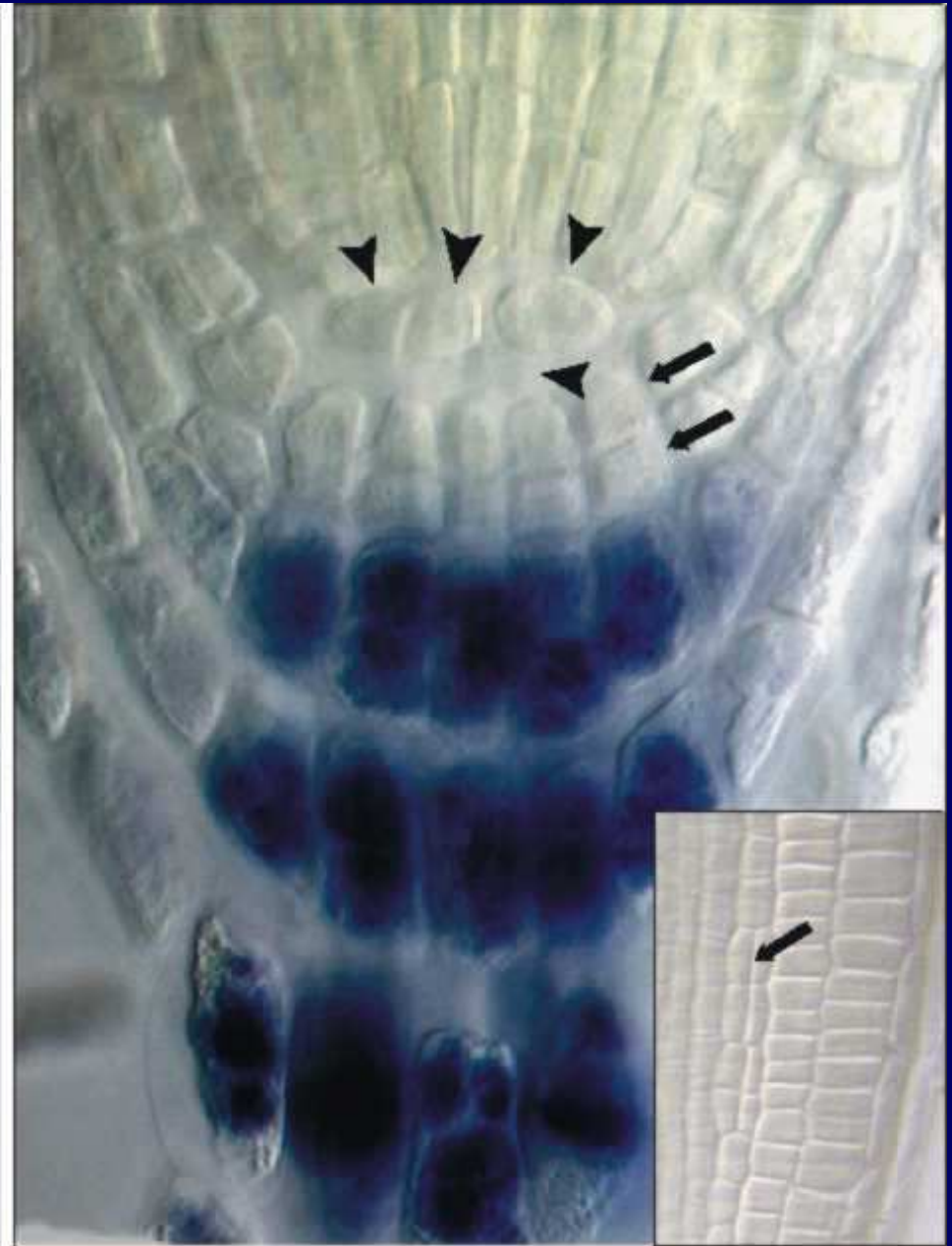
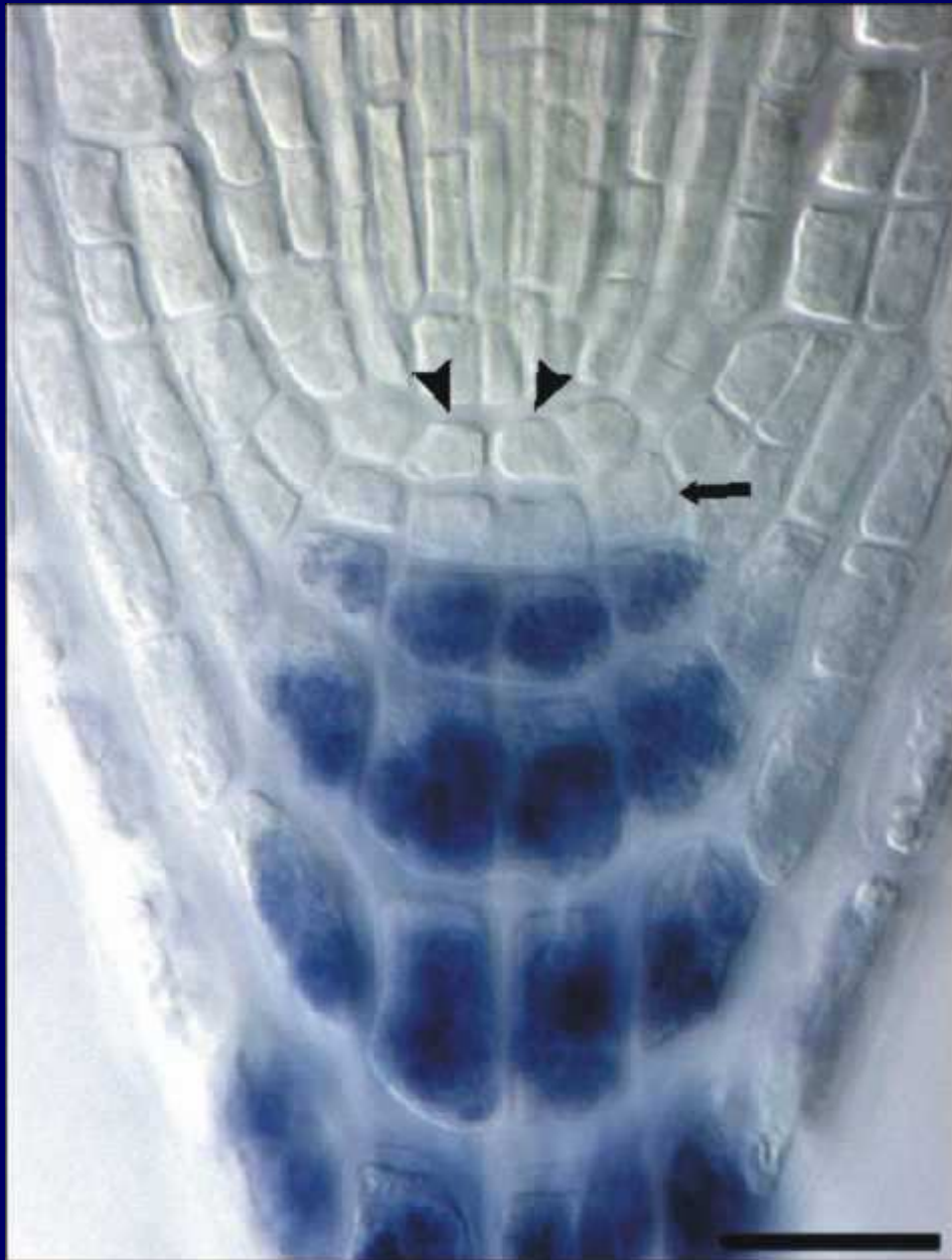
*Atpin4*



# *Atpin4* Root Pattern (4 days)

Col-0

*Atpin4*





# *Atpin4* Root Pattern (10 days)

*AtPIN4* antisense



*Atpin4*



*Atpin4*



# Changes in Cell Fates in *Atpin4* Mutant

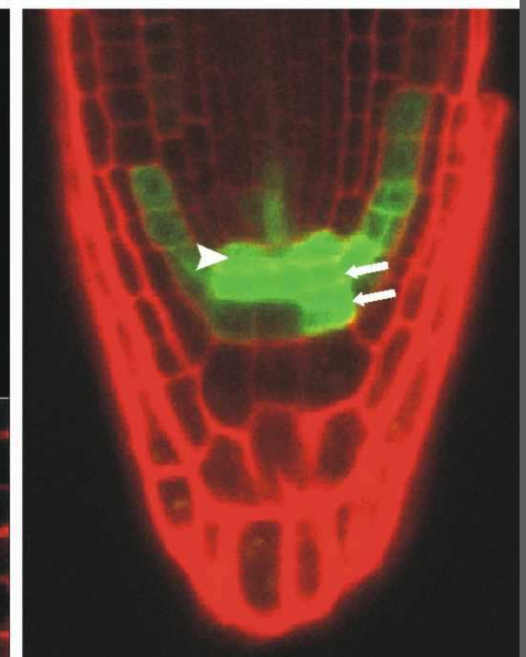
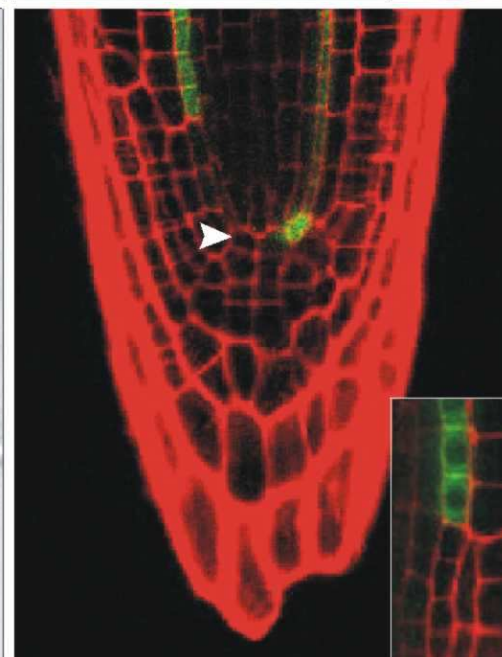
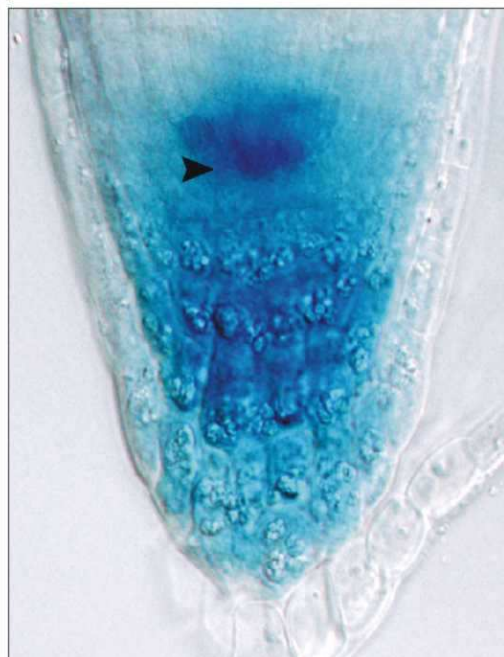
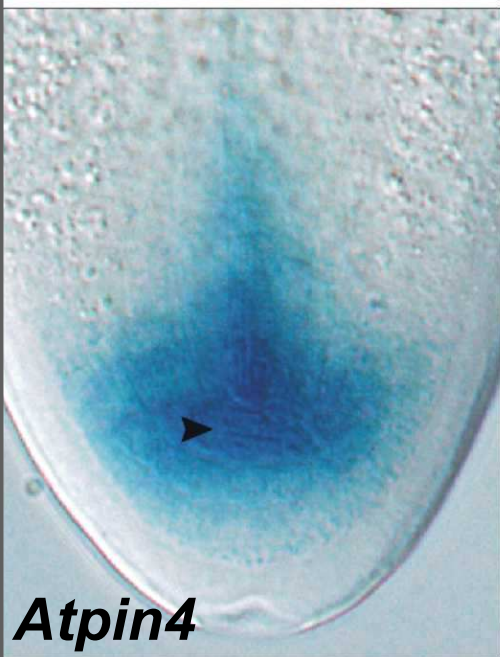
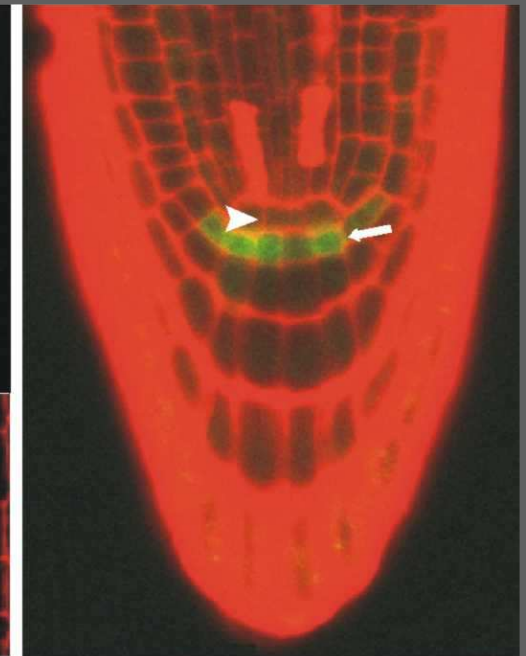
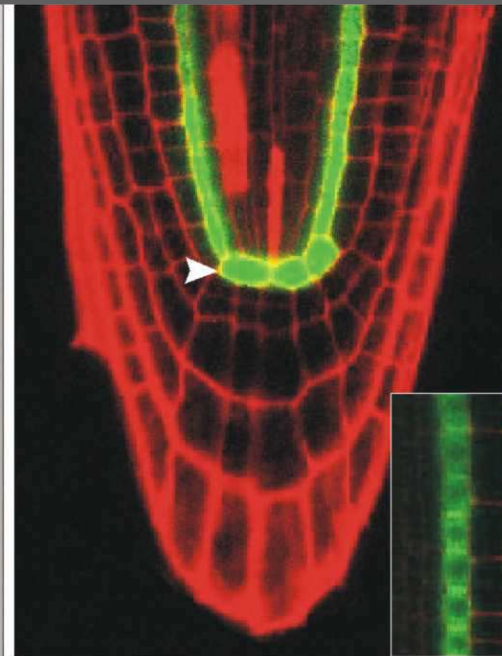
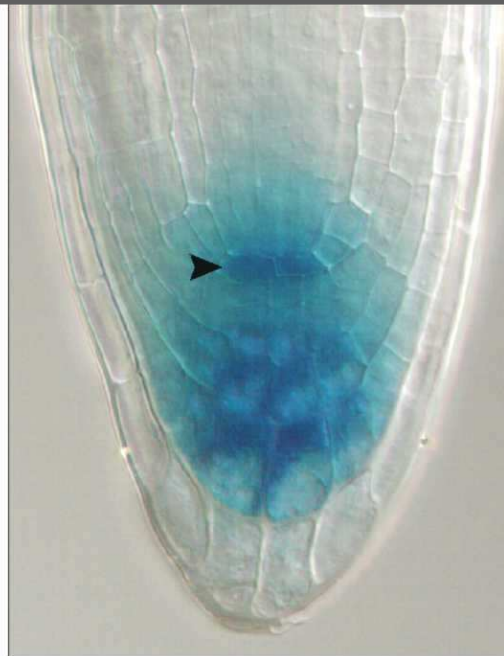
QC

QC + columella

QC + endodermis

columella initials

Col-0

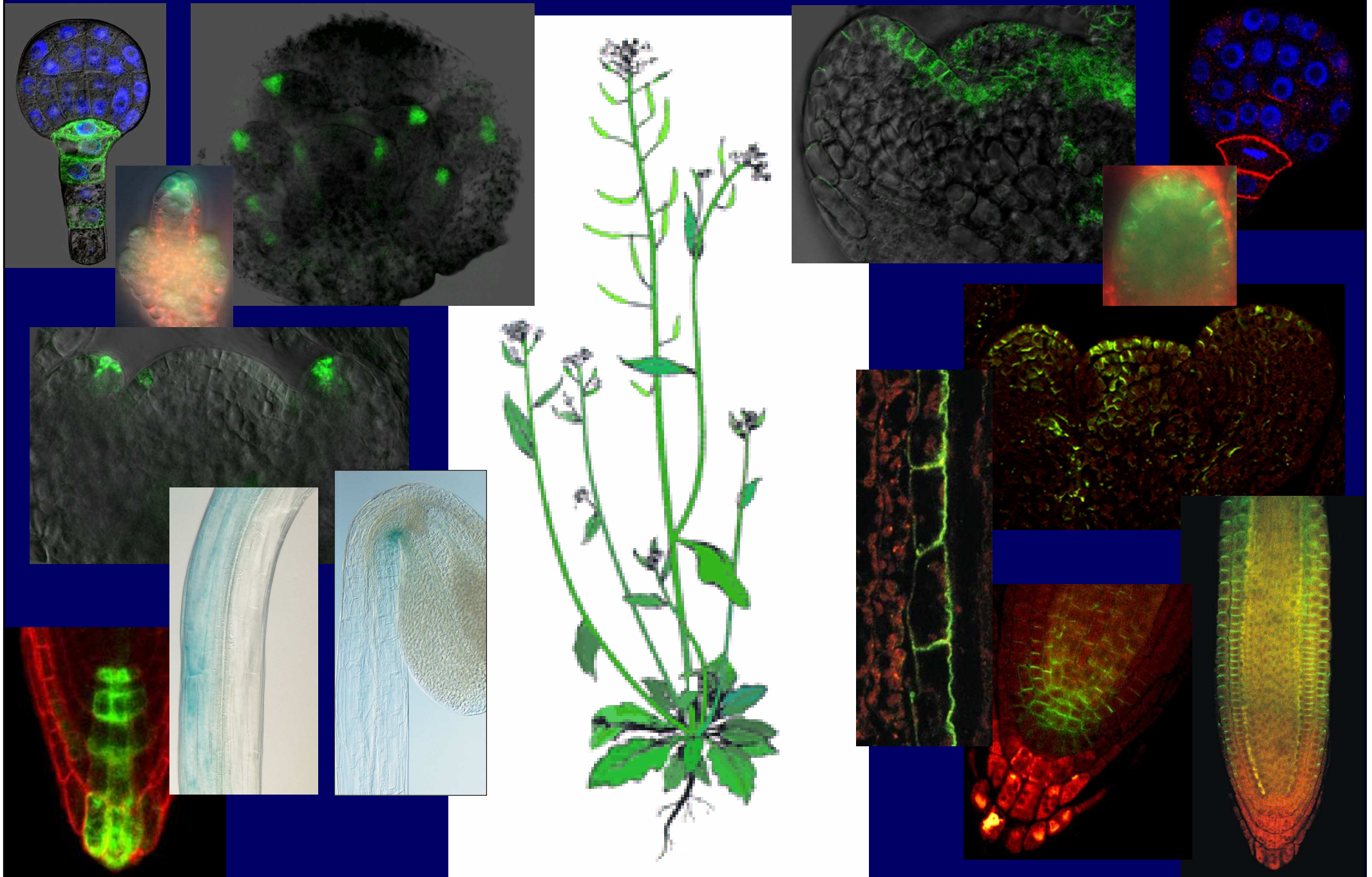


*Atpin4*

# Primary Root Meristem

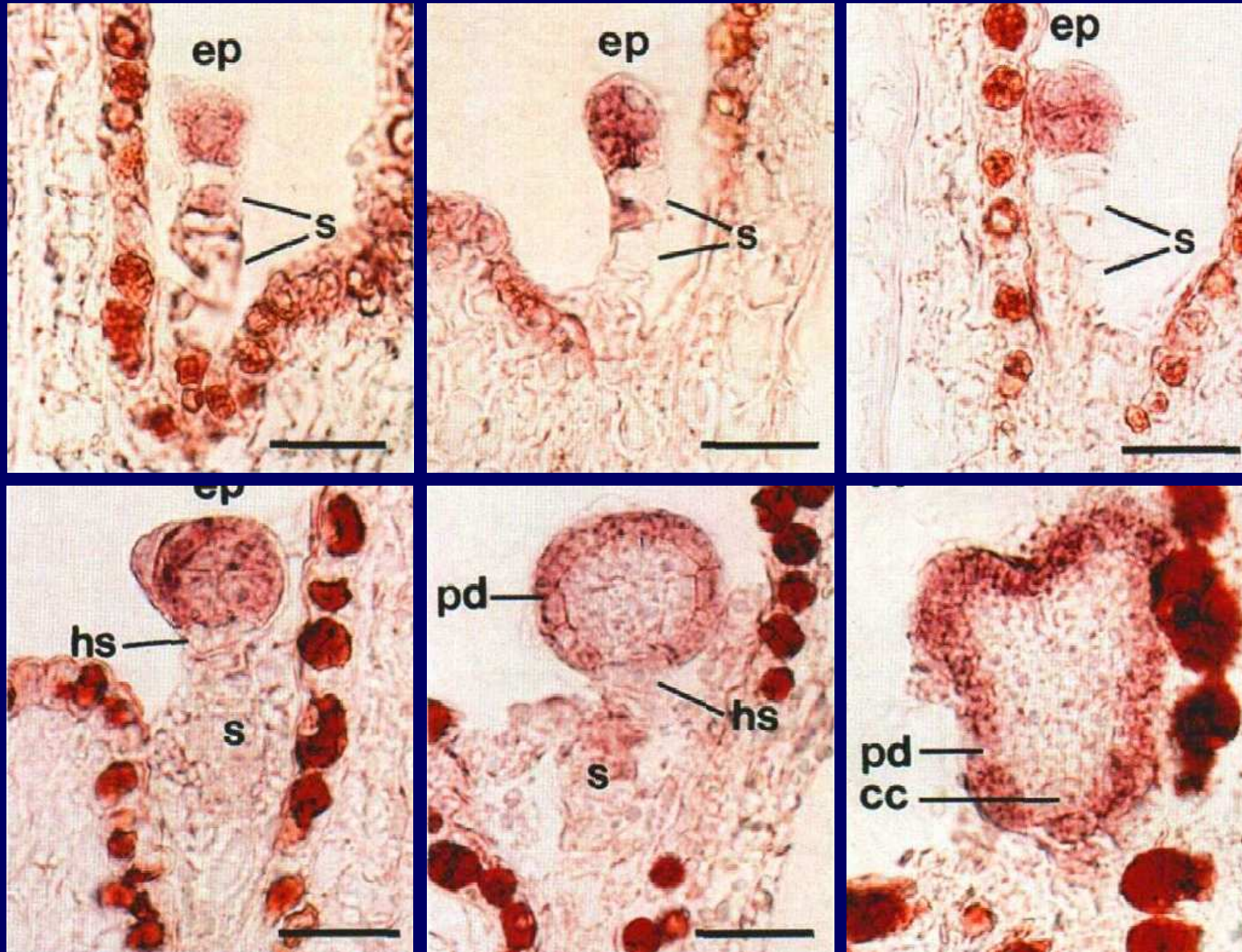
- Simple, highly invariant cell anatomy.
- The QC in the root meristem centre keeps the surrounding initials undifferentiated.
- Positional signal (probably auxin) instruct cell to differentiate into respective cell types.
- The auxin gradients instructive for meristem patterning are maintained by polar auxin transport system.

# PIN-dependent Auxin Gradients in Plant Development



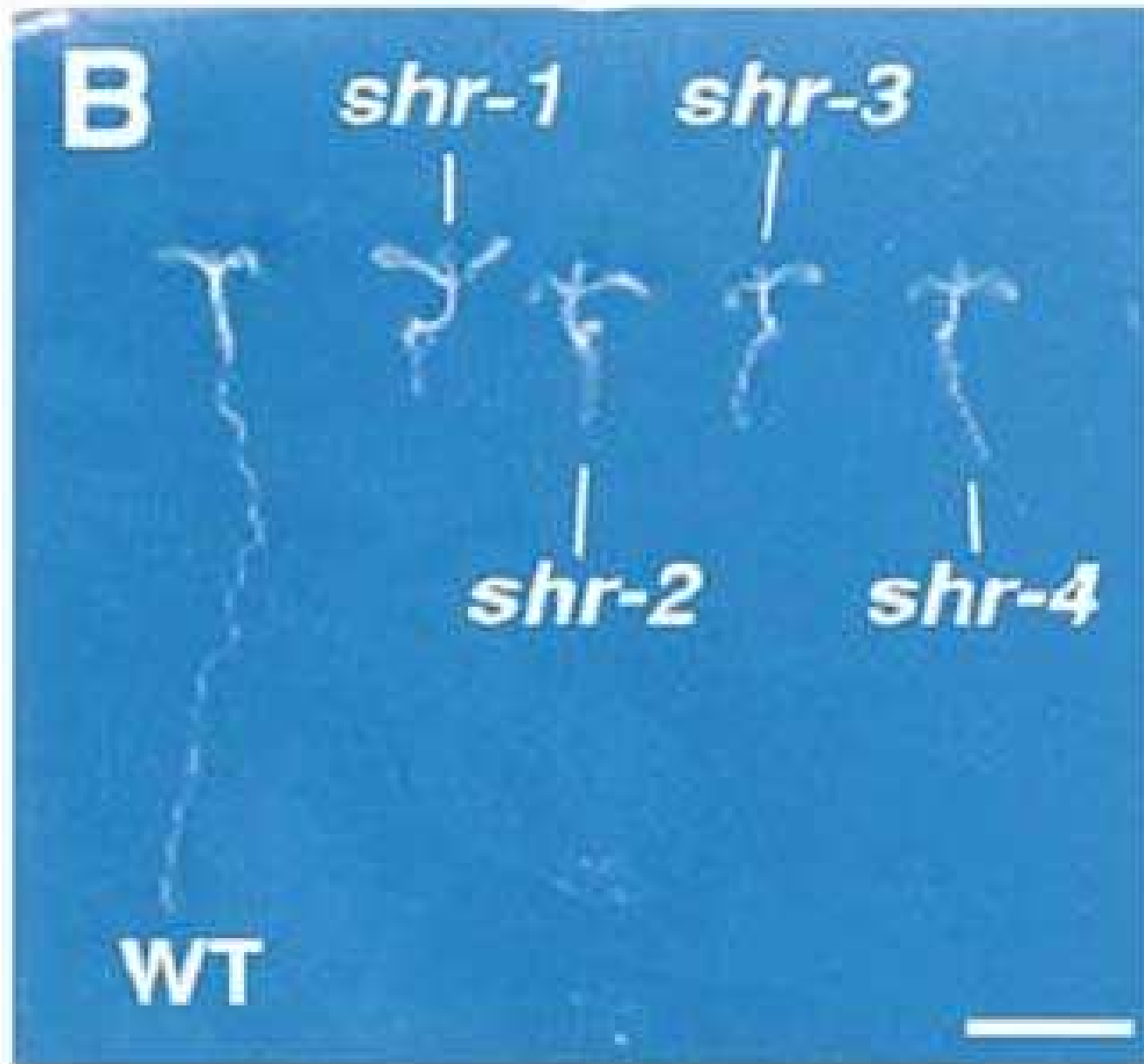
# Radial Patterning of *Arabidopsis* Root

# *Example for radial patterning – the AtML1 gene*

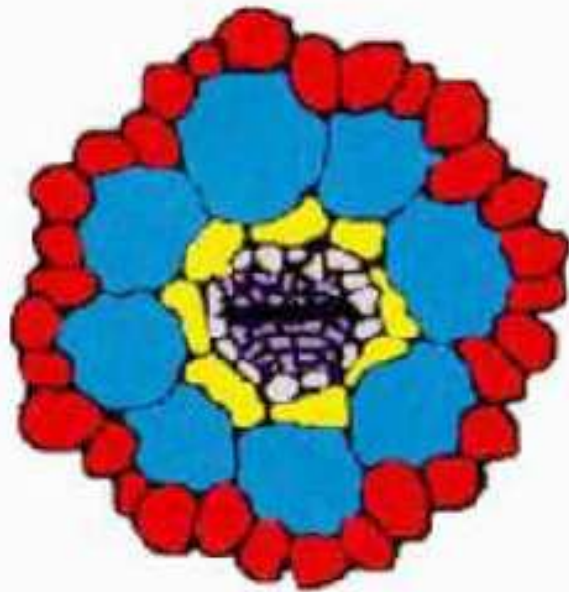
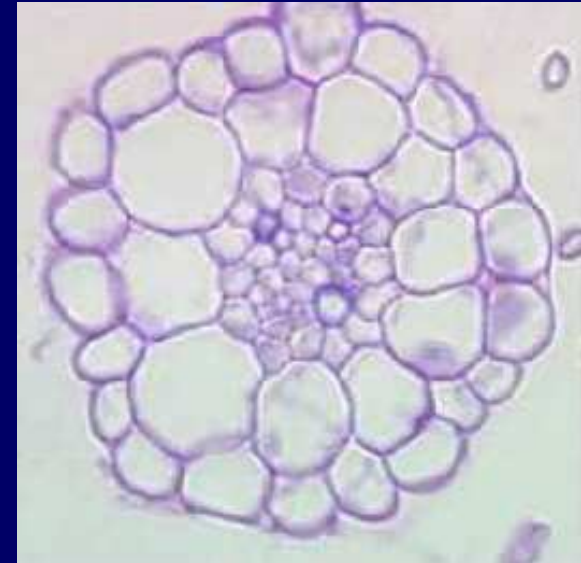
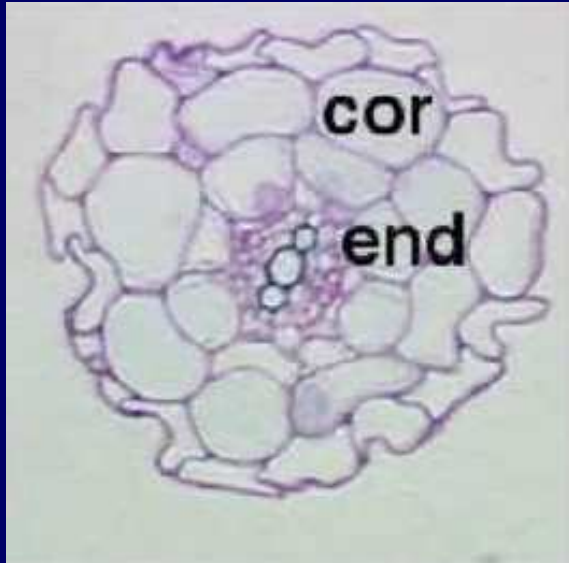


Lu P et al.  
Plant Cell,  
1996

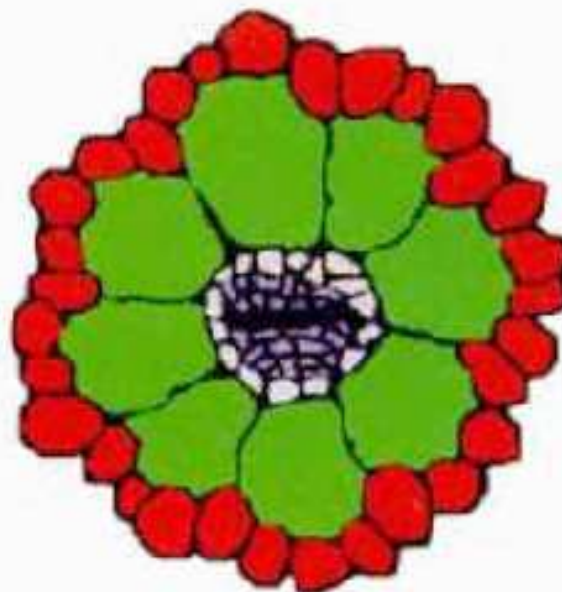
# Short-root mutant alleles



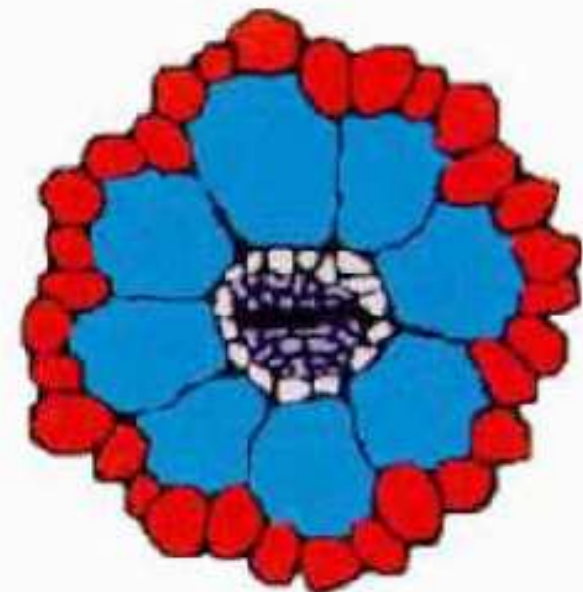
# Radial Mutants with Defects in Ground Tissue



Wildtype



*scarecrow*



*short root*

Cortex + Endodermis

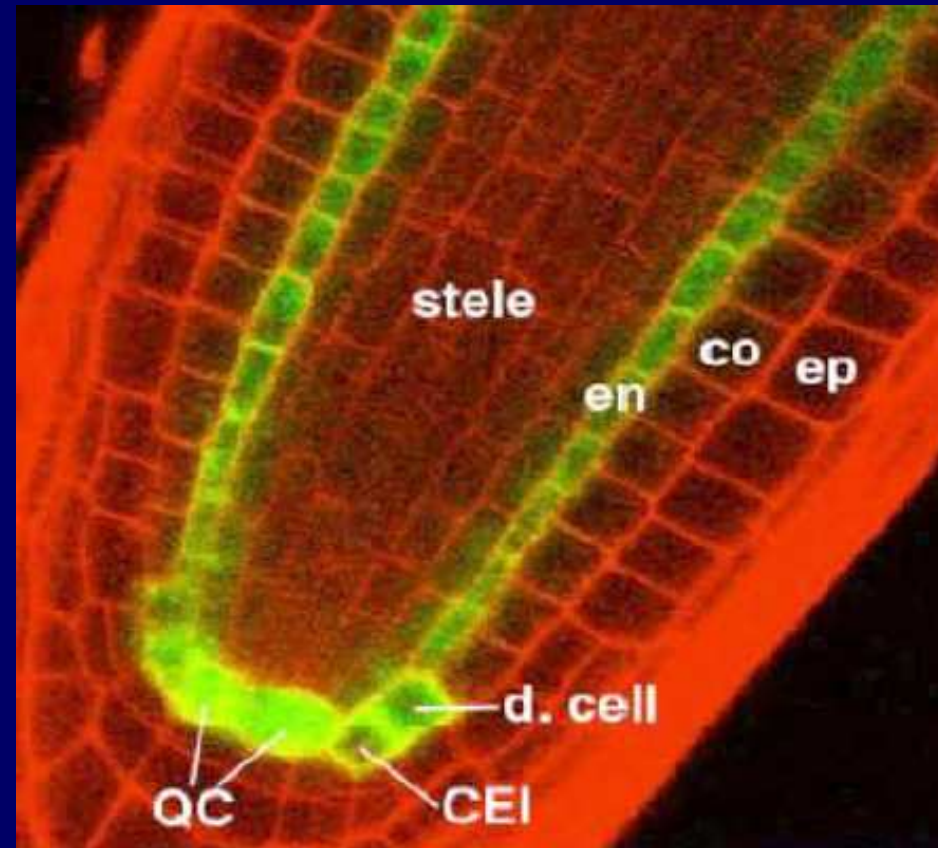
Mixed (Cx+En)

Cortex



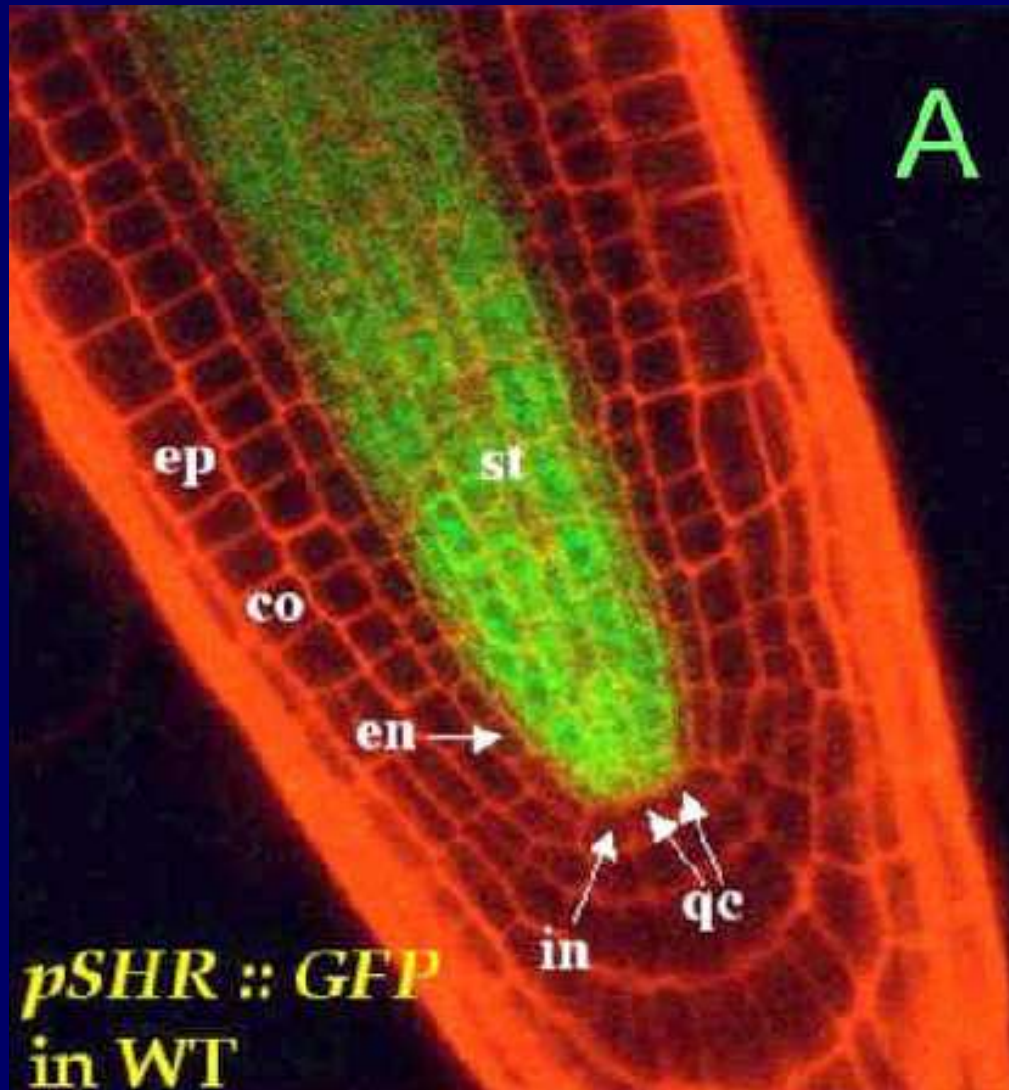
# SCR Expression in Endodermis

mRNA

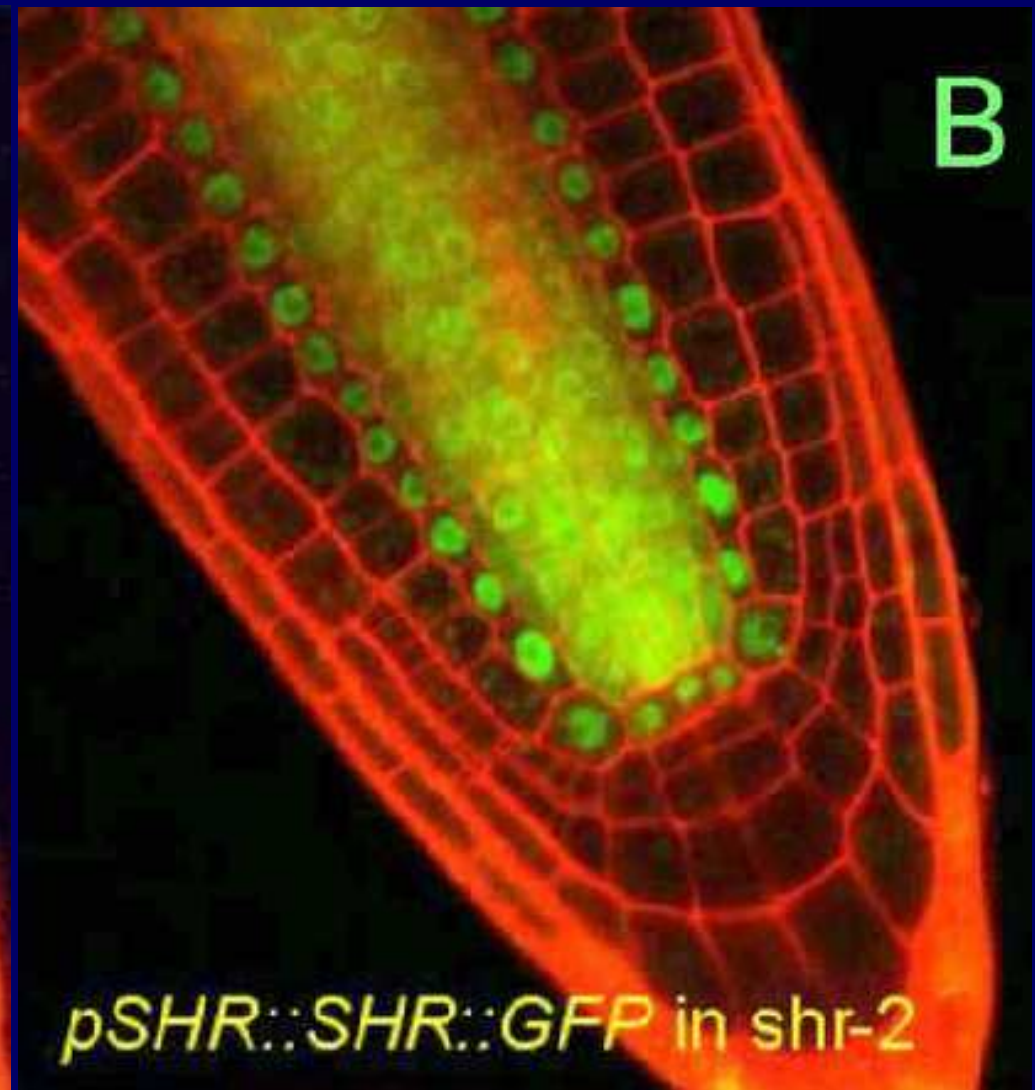


SCR::GFP

# SHR Expression + Proteintransport

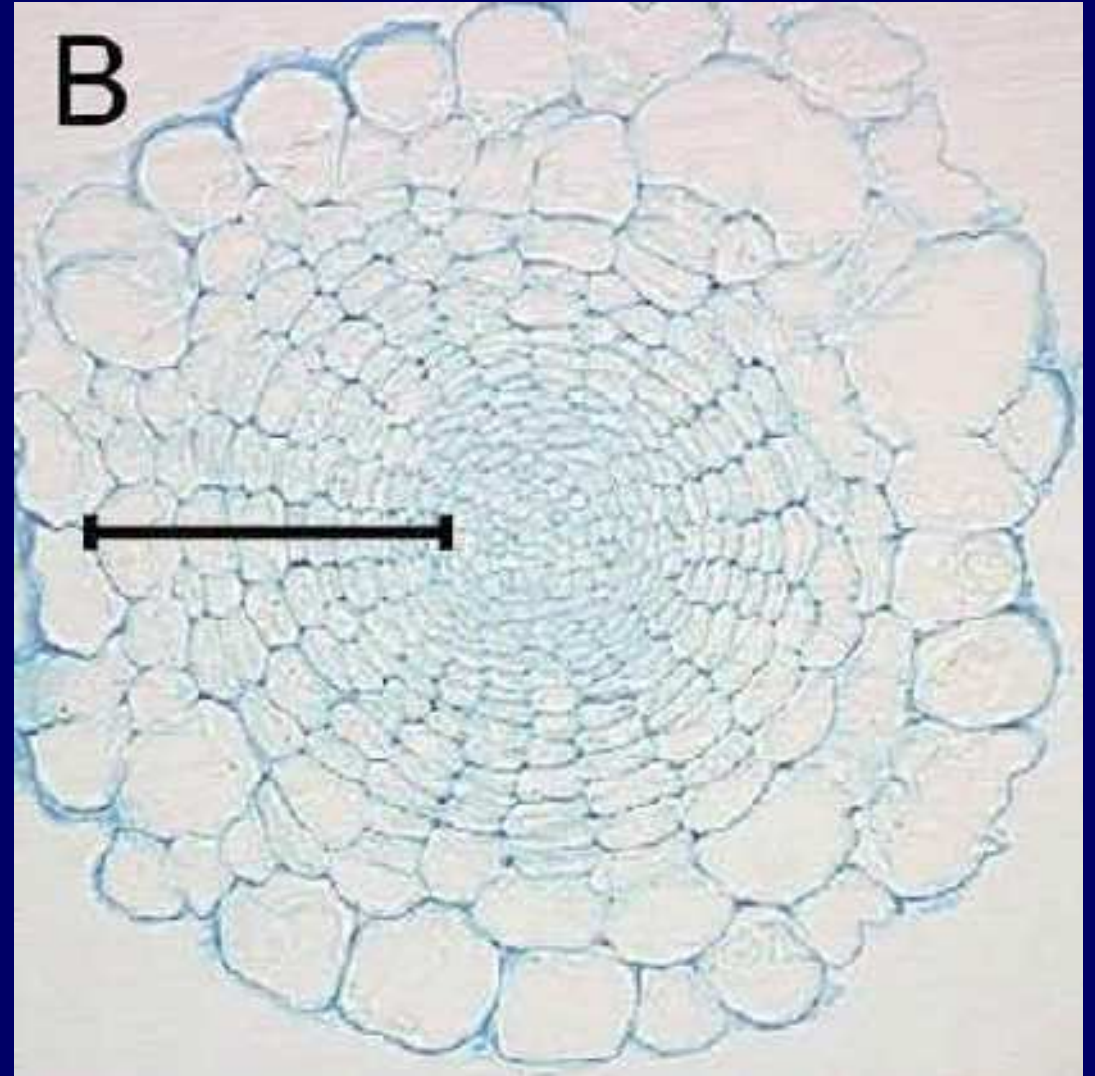
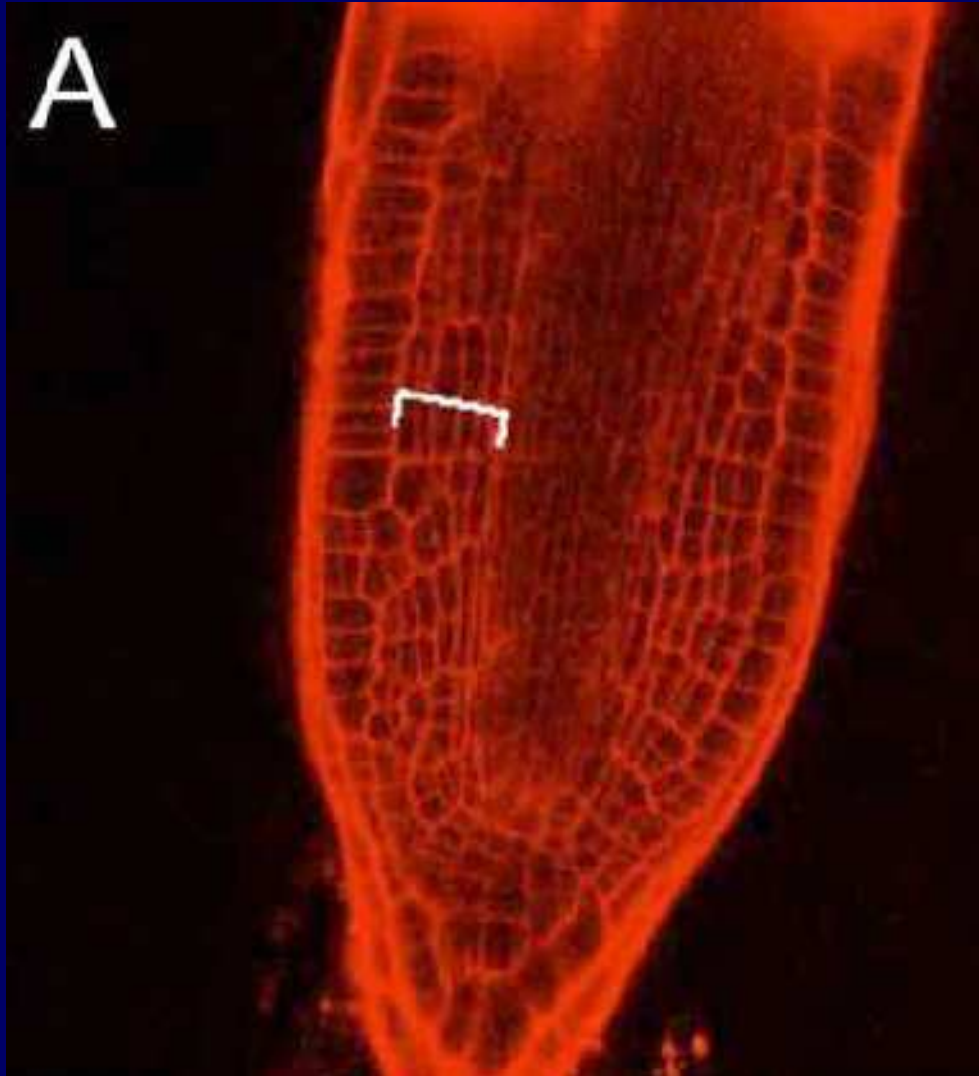


Genexpression in stele

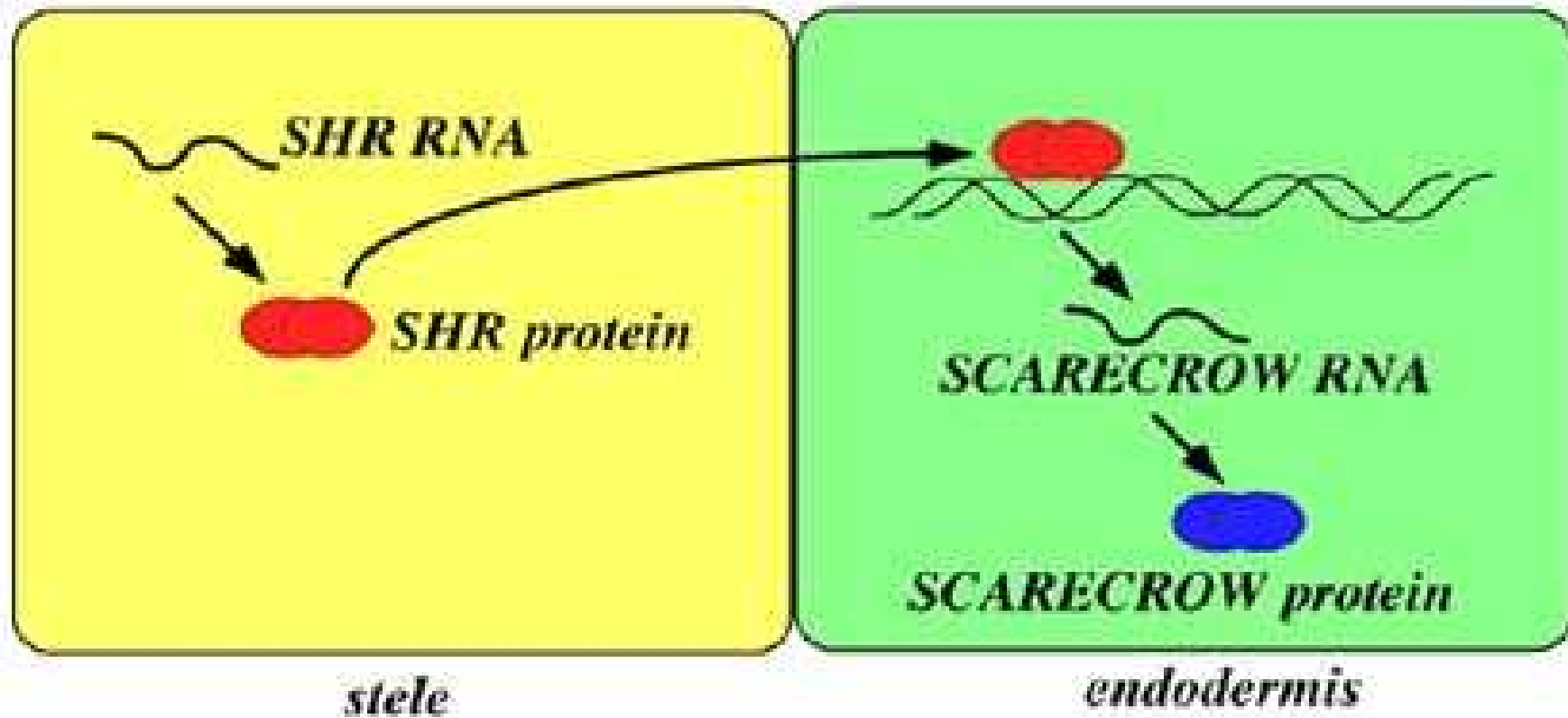


Protein in Endodermis + QC

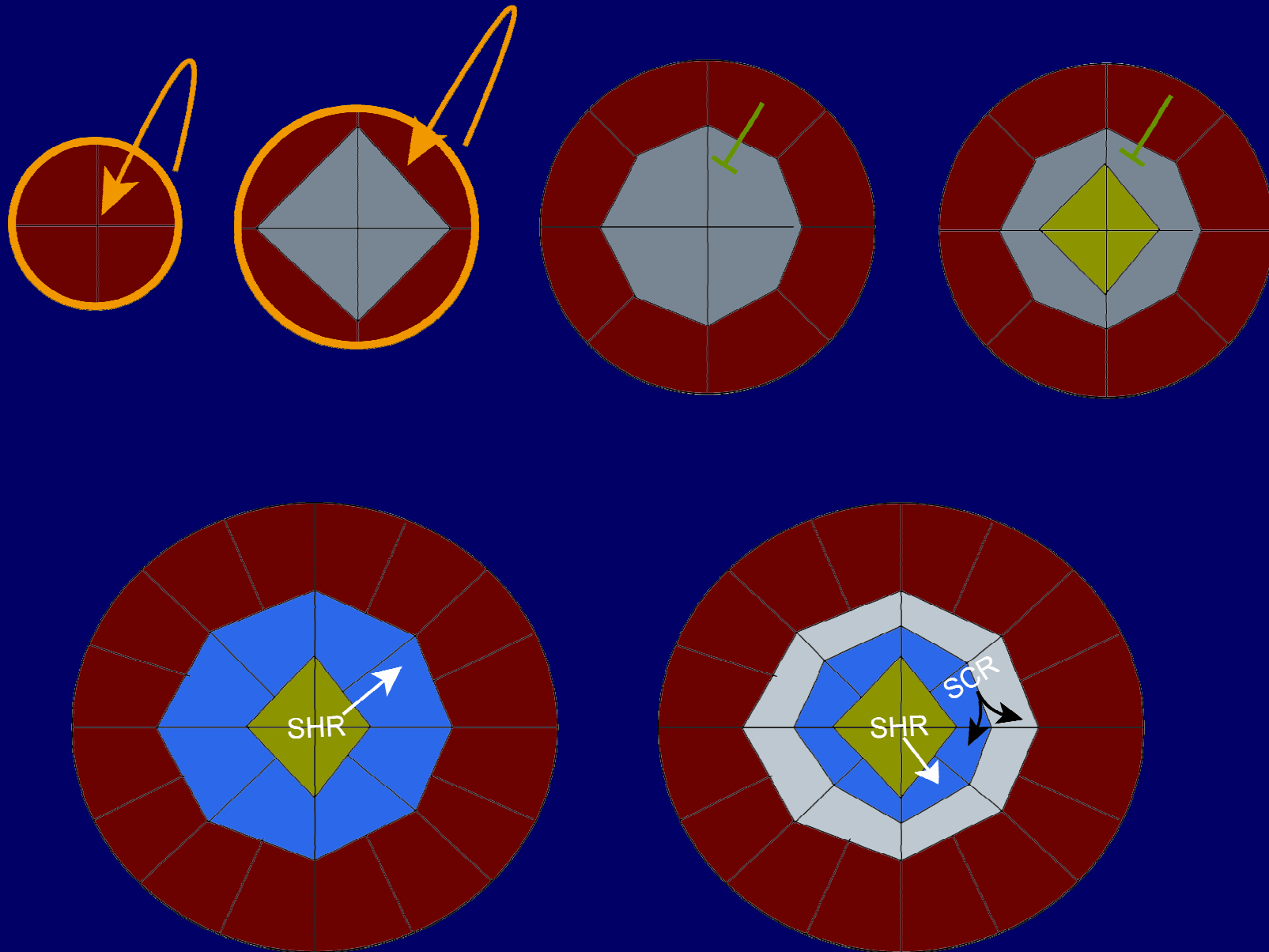
# SCR::SHR Expression: More Ground Tissue



# Model of SHR and SCR Action



# A model of radial patterning



# Root Radial Patterning

- Epidermis, cortex, endodermis, pericycle, stele cell types.
- Genetic analysis - shortroot (*shr*) and scarecrow (*scr*) mutants.
- SHR transcription factor is expressed in pericycle, moves into endodermis, activates SCR expression, which in turn properly specify endodermis.