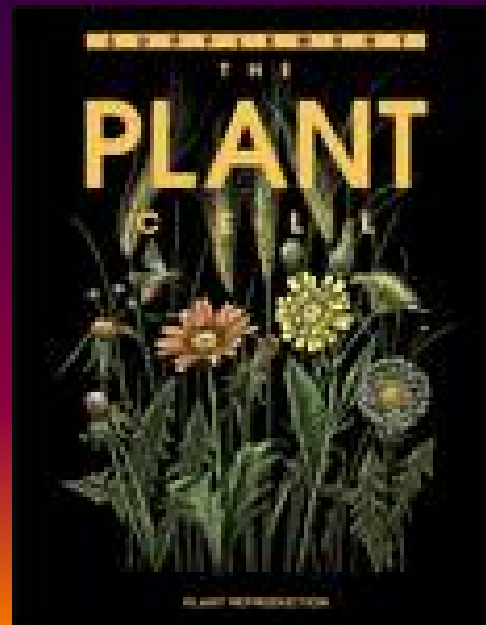


## 3) Plant reproduction

- g) Seed formation
- h) Embryo maturation
- i) Seed germination

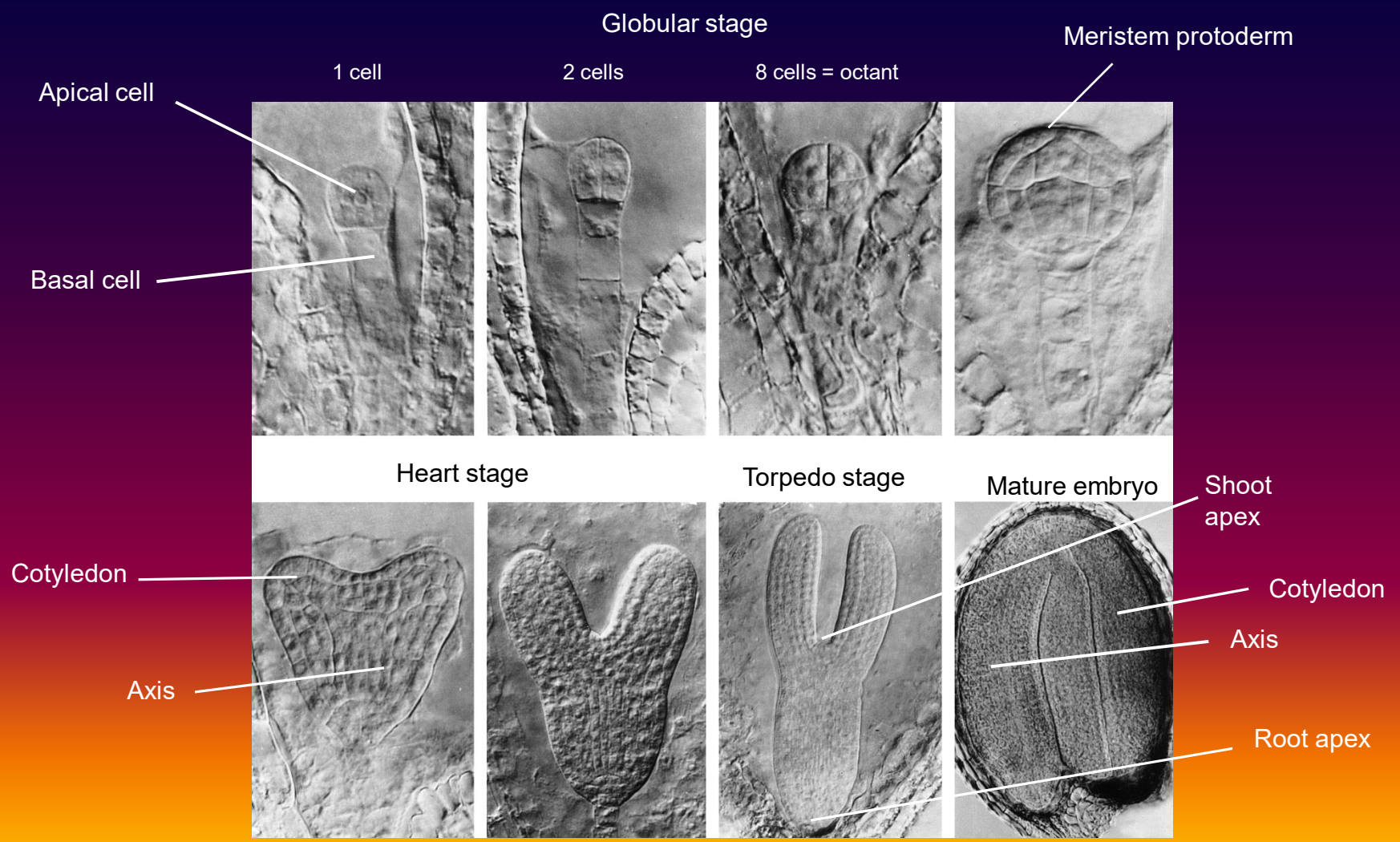


# g) Seed formation

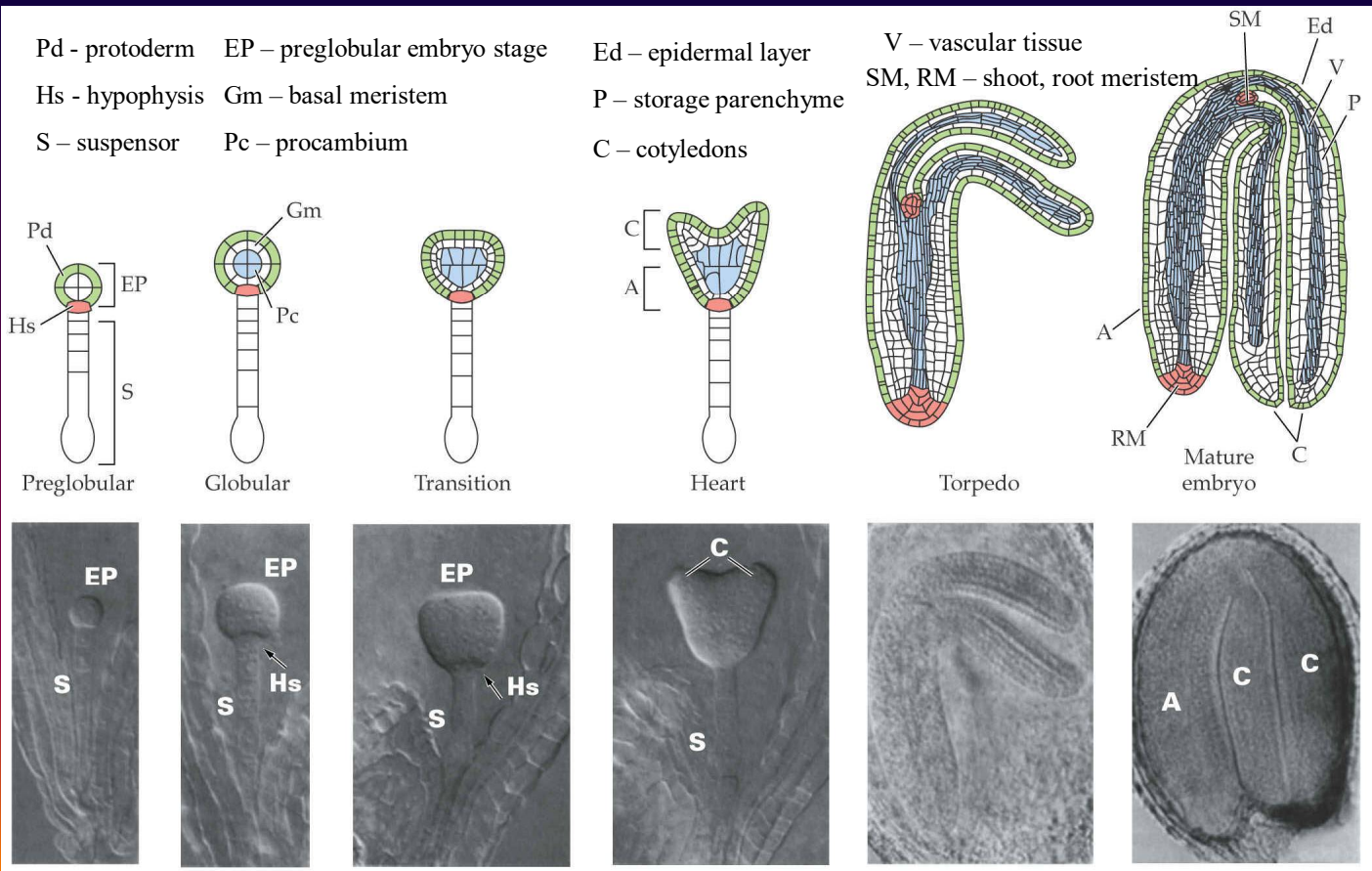
Seed of flowering plants (Angiosperms):

- embryo
- endosperm
- testum

## Embryo development



3 phases of embryogenesis on histological level: - tissue differentiation  
 - cell expansion  
 - embryo maturation



### **Tissue differentiation:**

- multiple division of fertilized ovule
- differentiation and formation of embryonic tissues (4 stages) including suspensor

### **Cell expansion:**

- storage of reserves during torpedo embryo stage

### **Embryo maturation:**

- termination of developmental processes; embryo is ready for desiccation

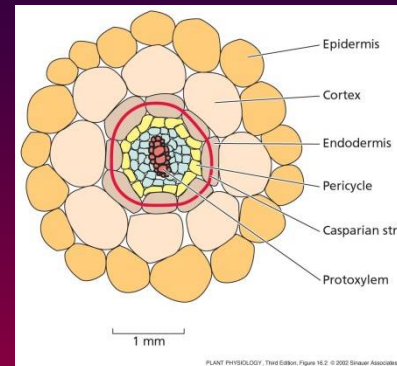
## Identification of mutants with defects in regulation of embryo development

Genetic and molecular characterization of mutants

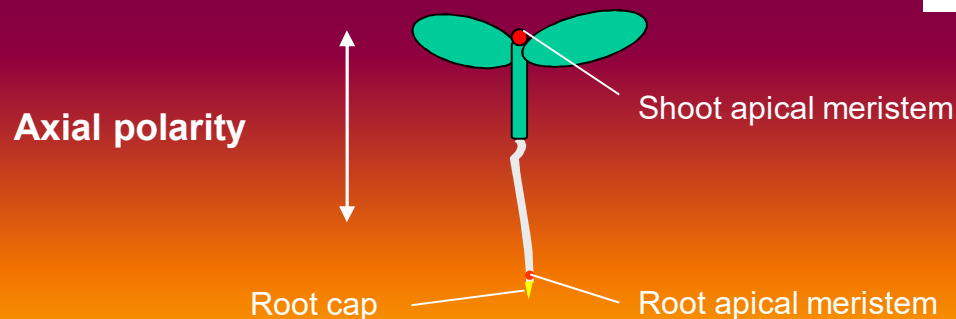
Identification of genes controlling: **early embryo development**  
**embryo maturation**

### Embryogenesis determines:

- axial (apical-basal) plant orientation
- radial shape of plant
- meristems – active after germination



Radial polarity



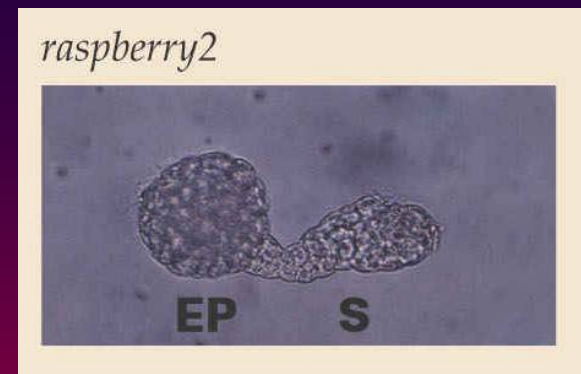
## Genes necessary in early stages of embryo development

Mutant *raspberry2* – morphological arrest of embryo development in globular stages; meristems are developed, which complete cell differentiation; abnormal development of suspensor



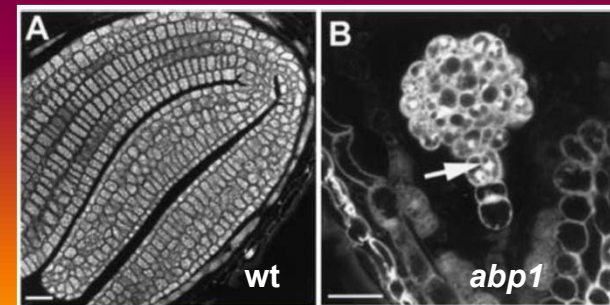
**RASPBerry2** – controls differentiation tissues and morphological development

Yadegari R (1994) Plant Cell 6: 1713-1729

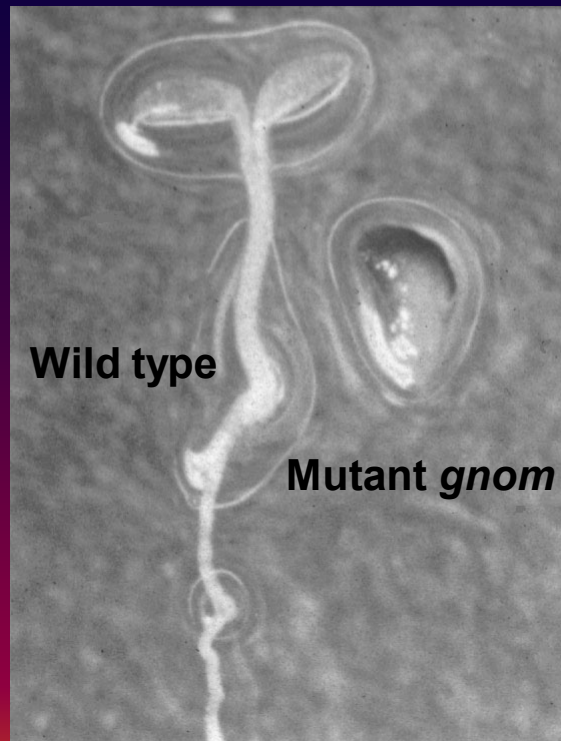


Loss of function of **ABP1 (Auxin-Binding Protein1)**: arrest of embryo development in *Arabidopsis* in globular stage

Chen JG (2001) Genes & Development 15: 902-911



## Genes involved in axial polarity



*gnom* - mutant

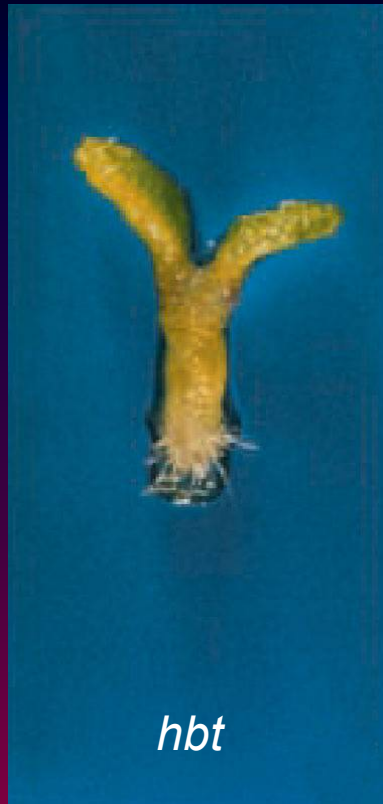
*GNOM* - gene

*GNOM* - protein

Mutant *gnom* does form cotyledons and root = axial polarity is missing



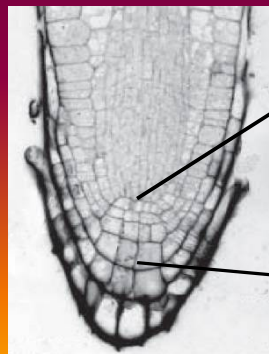
Gene *GNOM* controls apical-basal polarity



In mutant **hobbit (hbt)**, cells of hypophysis precursor divide vertical instead horizontal => hypophysis is not formed => root system does not have **quiescent center** and columela = root does not elongate.

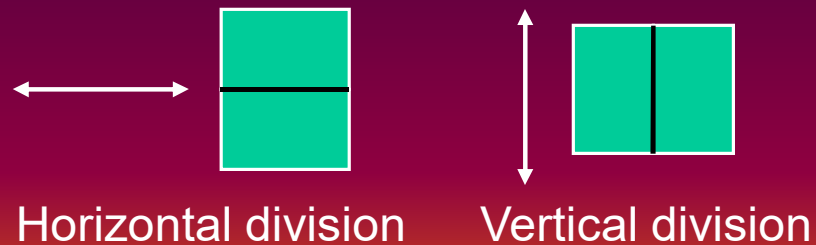


Gene **HOBBIT (HBT)** plays a role in formation of apical root meristem



Quiescent center

Columela



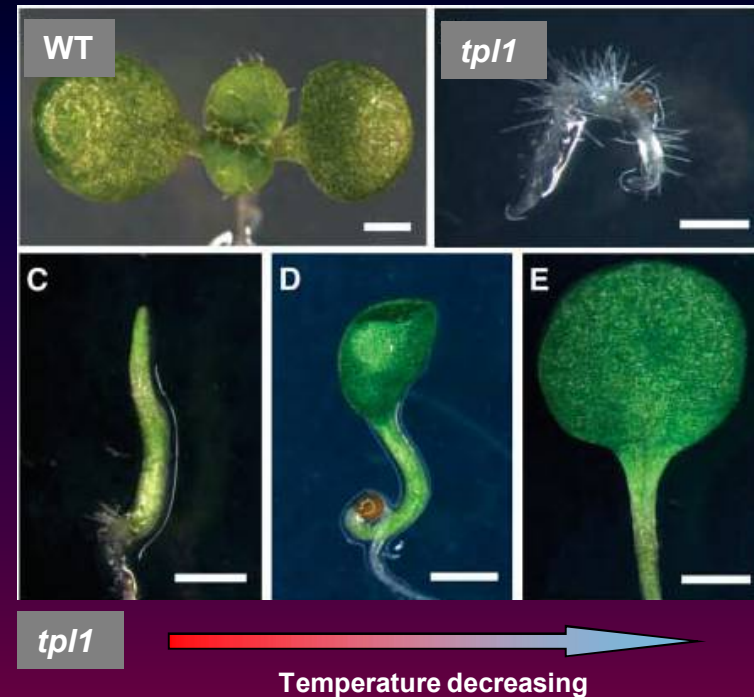
**Quiescent center** = central cells of meristem, which divide slowly than surrounding cells.

Mutant *topless1* – root is formed instead cotyledons

- expression of genes associated with development of apical part of embryo is missing
- expression of genes associated with development of basal part of embryo expands into apical part



**TOPLESS1 (TPL1)** – necessary for suppression of expression of genes inducing development of roots in apical part of embryo = it allows correct development of shoot pole of the embryo (plant)



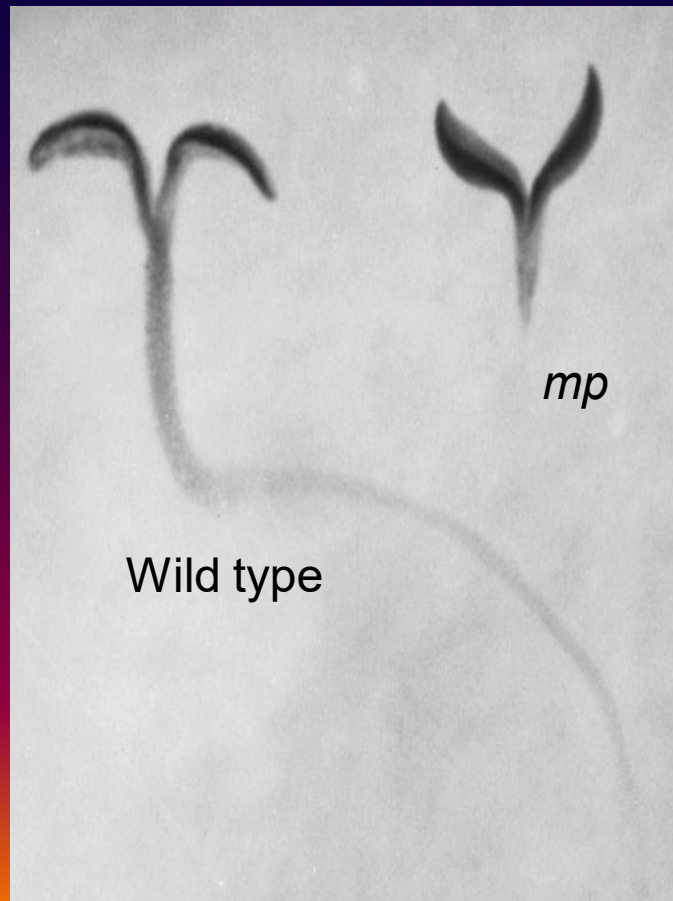
ARF5/MP - transcription factor (auxin-responsive factor)

IAA12/BDL - repressor

TPL - co-repressor



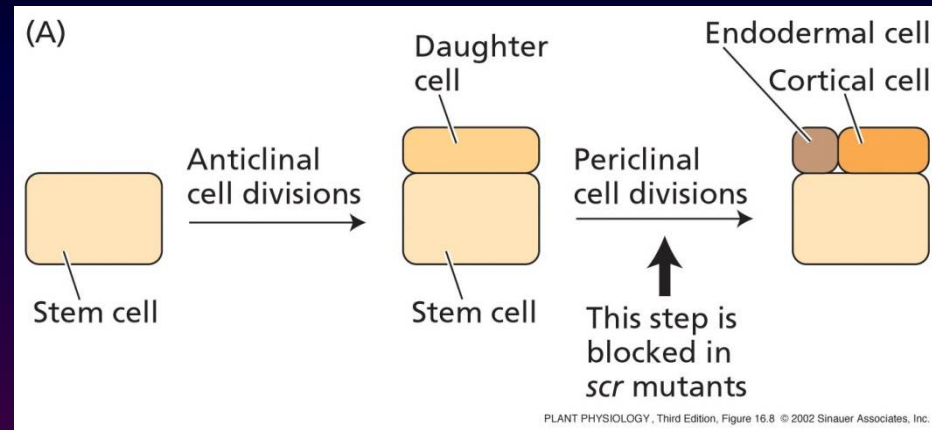
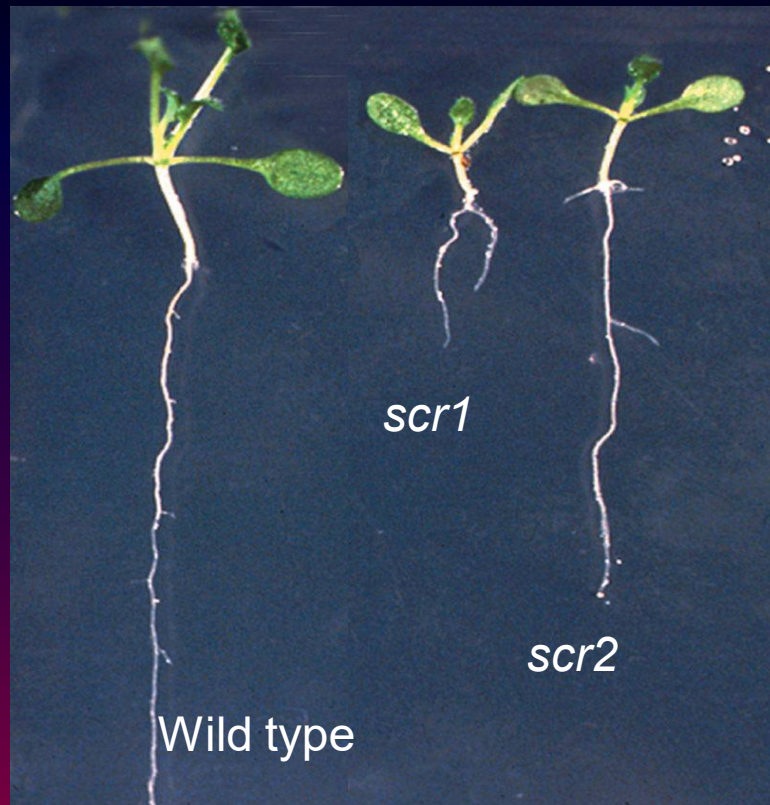
## Genes involved in radial polarity



Mutant ***monopteros (mp)*** does not form a root; globular embryo does not form procambium = part, which gives origin of embryonic root



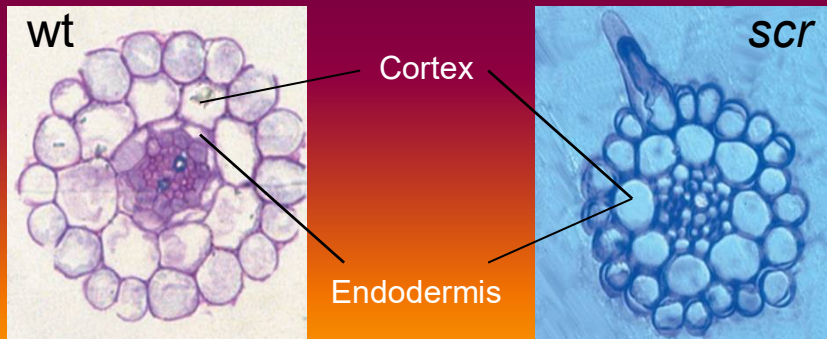
Gene ***MONOPTEROS (MP)*** is necessary for formation of embryonic root



Mutants **scarecrow (scr)** and **short root (shr)** shows a blockade of periclinal division of stem cells in root => cortical and endodermal cells merge => root is fragile => it grows slowly



Genes **SCR** and **SHR** are necessary to establishment of radial arrangement of the root during embryogenesis and post-embryogenesis.



## Other genes determining embryo development

***PLETHORA (PLT)*** – functionally completes *SCR* and *SHR*; transcription factor – plays a role in determination of the position of root stem cells

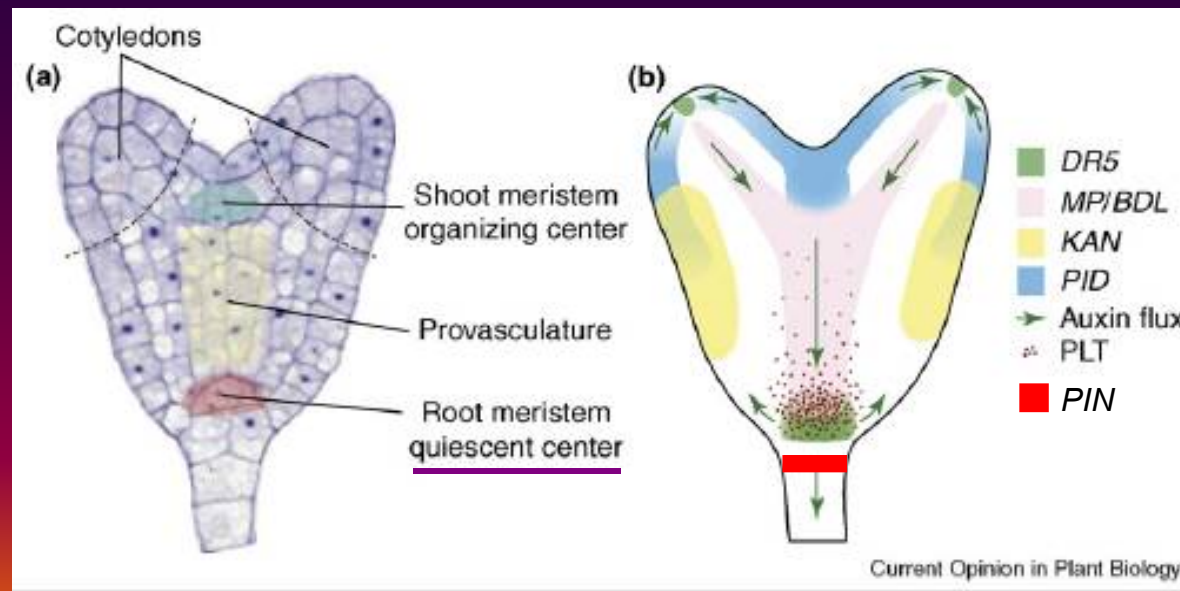
Proteins PLT1 – PLT4 interact each to other – power of interaction (= gradient of expression) determines fate of the stem cells

**Expression level of *PLT* genes:** **High** – maintains the identity of stem cells  
**Low** - stimulation of mitotic activity of daughter stem cells  
**Lowest** - differentiation of stem cells

*PLT1 - PLT3* control the expression of *PIN* genes    ***PLETHORA*** → ***PIN***

Maximum accumulation of proteins **PLETHORA** in the **quiescent center** – the gradient of the accumulation determines fate of the of the stem cells

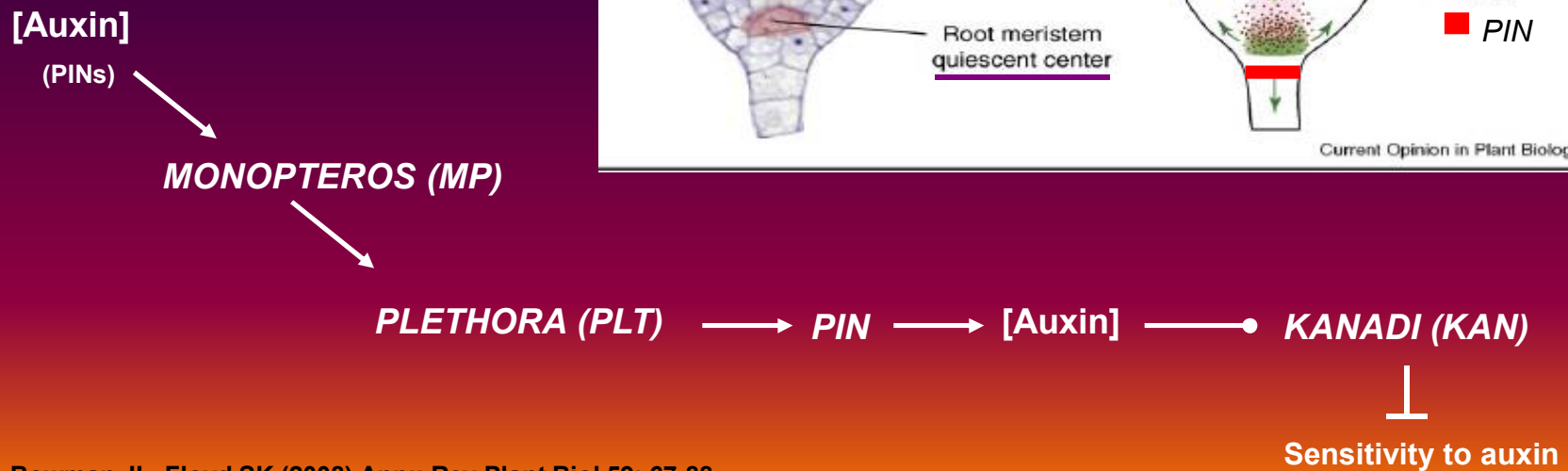
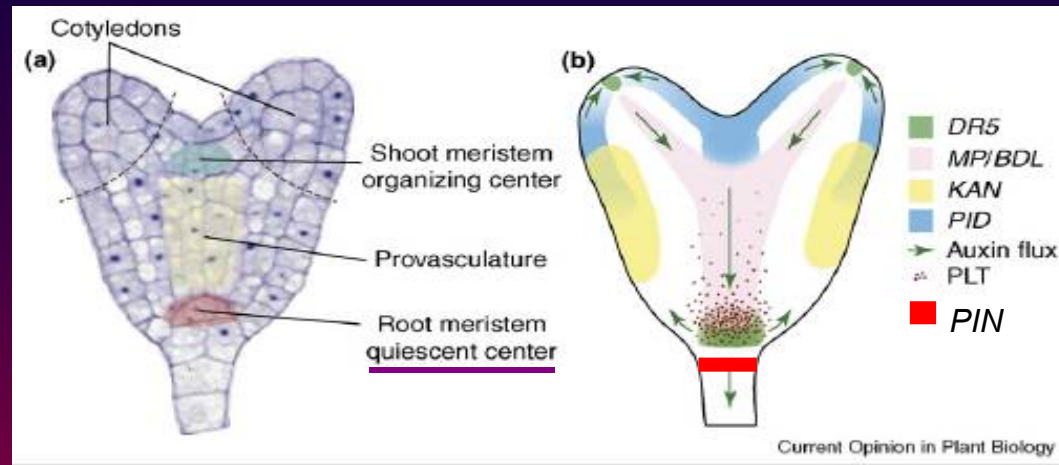
Expression of *PLETHORA* – likely determined by auxin via *MONONOPTEROS*



**KANADI (KAN)** – controls radial polarity of the embryo

Temporal and spatial expression of *KANADI* is controlled by auxin via PIN proteins

*KANADI* suppresses the sensitivity to auxin at the cotyledons edges



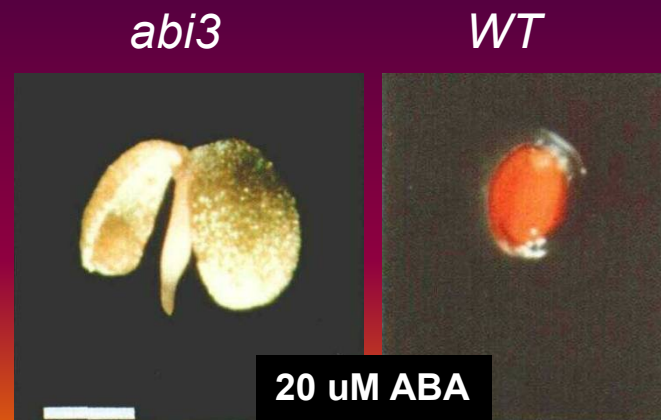
## Genes controlling late phase of embryo development - maturation

Embryo becomes dormant, when it contains 20 thousands cells.

Dormancy occurs at the water loss and stopping of transcription and protein synthesis in the embryo and the seed.

In order to reach dormancy, some genes have to be expressed:

***ABI3* (*abscisic acid insensitive 3*)** – controls expression of storage proteins that store in the cotyledons during maturation phase of embryogenesis; the mutant is insensitive to ABA



Rohde A et al. (2000) Plant Cell 12: 35-52

***FUSCA3 (FUS3)*** and ***LEAFY COTYLEDON1 (LEC1)*** – code for proteins, which function as critical component in the transmission of external and internal signals during embryo development.

Mutations in these genes result in lack of the synthesis of storage proteins and lipids => inability to withstand drying => premature germination

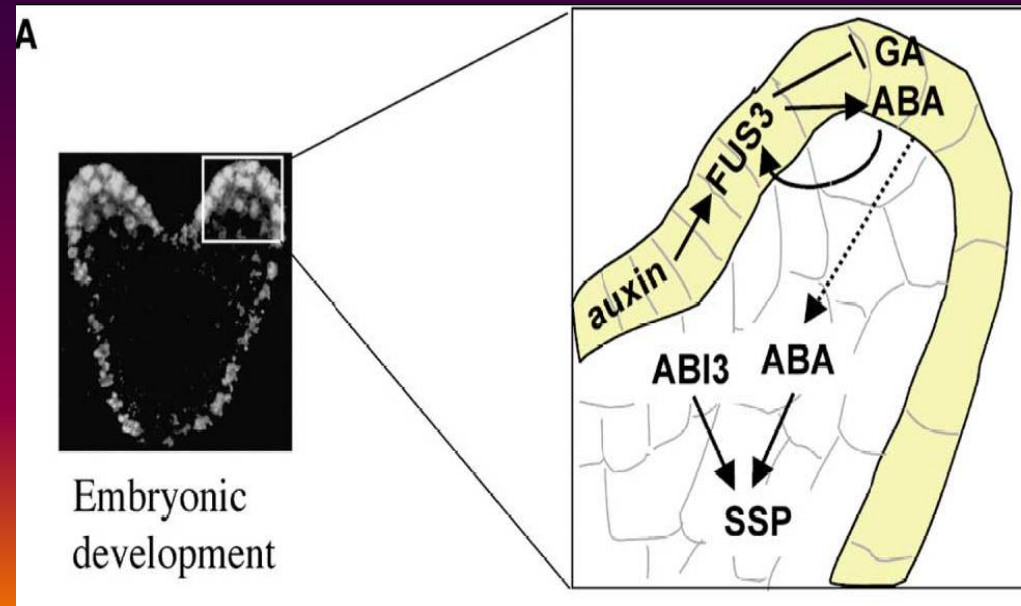
Gene ***FUS3*** – sensitive to ABA, gibberellins and auxins; defect in the gene *FUS3* results in wrong embryo maturation => wrong cotyledon formation. Leaves develop instead cotyledons.



Transgenic plant overexpressing gene *FUS3* (8 weeks)

## Interaction of *FUS3*, *ABI3* and hormones during embryonic development

- Expression of *FUS3* in epidermis is dependent on auxin gradient
- *FUS3* inhibits function mediated by gibberellins => inhibition of germination programme
- *FUS3* induces synthesis of ABA => feedback = ABA stabilizes the function of *FUS3*
- ABA migrates into mesophyll and induces together with *ABI3* accumulation of storage proteins (SSP – seed storage proteins)

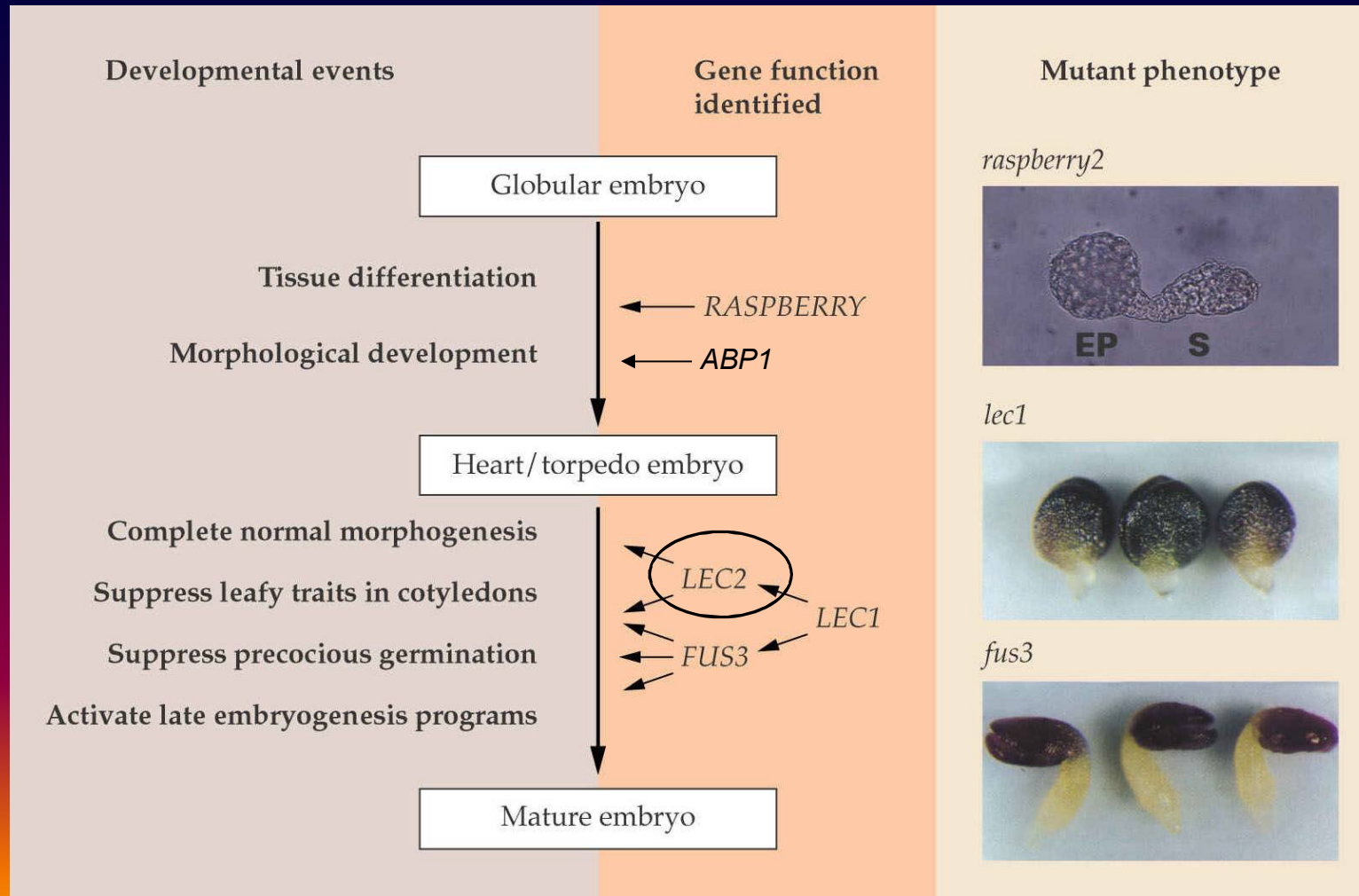


**LEC1** – embryo with the mutation in this gene does not survive drying and therefore is not able to get dormancy; embryo cultivated *in vitro* is able to develop into a plant, which develops leaves instead cotyledons and lacks storage protein 7S.

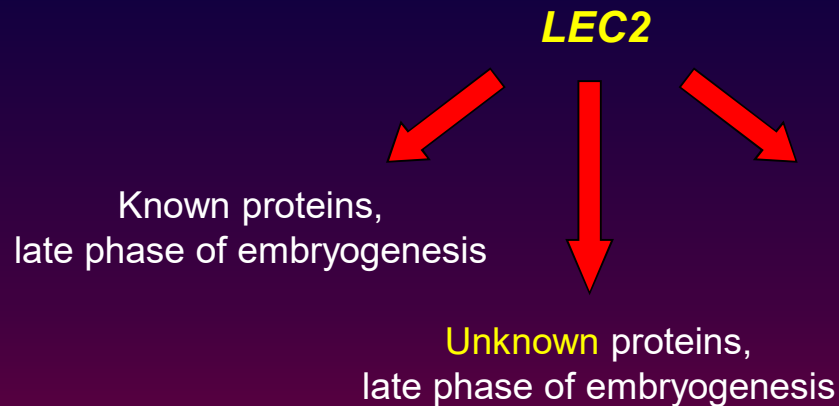
Gene **LEC1** is essential for embryogenesis, and functions as a repressor of vegetative development.



## Genes controlling early and late embryo development



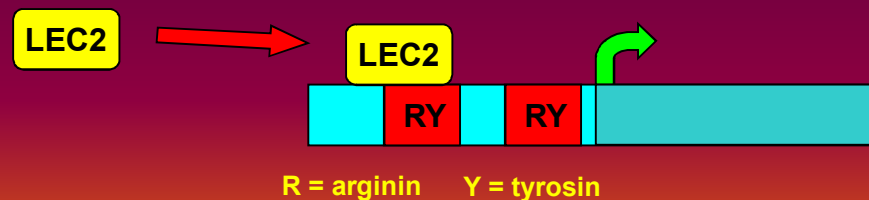
***LEAFY COTYLEDON2 (LEC2)*** – necessary for embryogenesis in the late phase of embryo development



Proteins accumulated in **early phase** of embryogenesis (zygote rises)

***AGL15*** – in tissues passing through zygotic and somatic embryogenesis

***IAA30*** – codes a group of proteins involved in auxin signaling and contained in the cells of root apical meristem



***LEC2*** likely plays a role in somatic embryogenesis mediated by auxins

## Endosperm development

**Endosperm – tissue accumulating reserves until the developing embryo is able to accumulate them into its cotyledons**

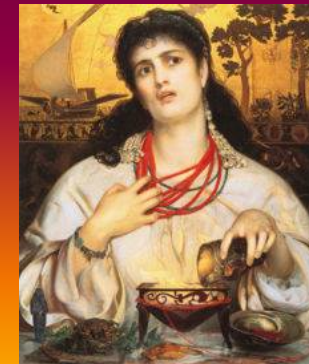
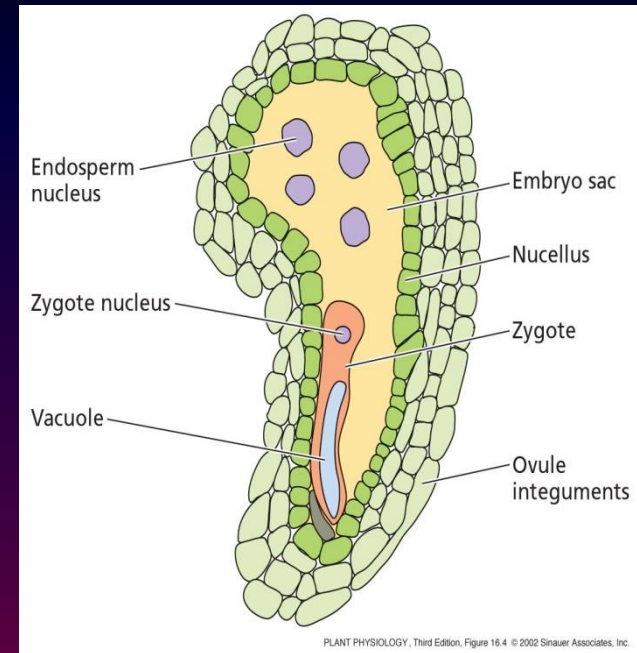
Seeds with **featureless endosperm** (lettuce, tomato) – minimum storage function of endosperm

Seeds with **distinct endosperm** (bean) – endosperm is the main reservoir; it contains live cells with proteins and lipids; it contains mobilization enzymes

**Nuclear development of the endosperm** – endosperm passes through free nuclear division before the new cell wall is formed - cereals

**Cellular development of endosperm** – no stage of the free nucleus

MEDEA – protein controlling development of endosperm and expression of itself (MEDEA in ♀ controls MEDEA in ♂ = keeps it at rest)



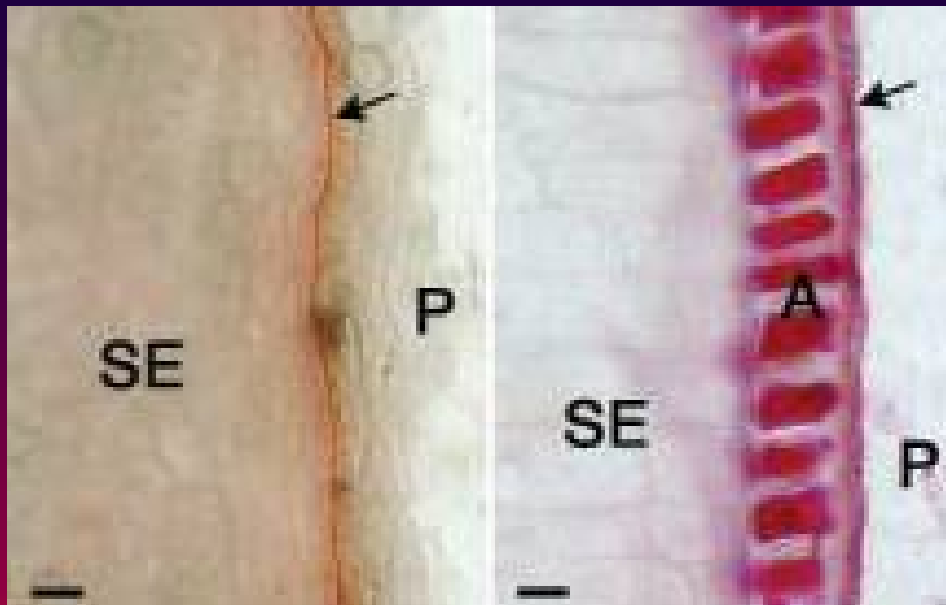
## Mutations in endosperm development:

- affect biosynthesis of reservoirs
- affect endosperm morphology

Mutant **defective endosperm (de)** – shows suppressed formation of zein protein in maize endosperm. **DE** gene controls synthesis of signal peptide in kD  $\alpha$ -zein protein.

Mutant **crinkly 4 (cr4)** – lacks aleurone layer; gene **CR4** codes for receptor-like kinase

Mutant **defective kernel 1 (*dek1*)** – does not form aleurone layer, instead it forms starch cells; gene ***DEK1*** maintains and inhibits fate of aleurone cells given by the gene ***CR4***.

*dek1*

WT

SE = endosperm cells

P = pericarp

A = aleurone cells

Mutant **supernumerary aleurone layers 1 (*sal1*)** – instead one it forms 7 aleurone layers; gene ***SAL1*** encodes protein homologous to human protein CHMP1 involved in transport of membrane vesicles.



Shen B et al (2003) PNAS 100: 6552-6557

Model of mutual cooperation of the genes ***DEK1***, ***CR4*** and ***SAL1*** in the development of aleurone and endosperm:

- ***DEK1*** receives and transfers the signal about the place where aleurone layer should be formed
- ***CR4*** mediates the movement of the signal between aleurone cells
- ***SAL1*** maintains required concentration of proteins ***DEK1*** and ***CR4*** in the plasma membrane by means of endosome recycling or degradation

## Saving reserves during seed development

### Carbon (C) and nitrogen (N)

There is no direct vascular connection between embryo or endosperm and mother plant. Vascular system terminates on the seed surface.

Transport of assimilates from parent tissues to apoplast and then to endosperm realizes by a passive transport.



### Storage proteins

**Albumins** – proteins soluble in water

**Globulins** – proteins extractable in salt solutions

**Prolamins** – proteins soluble in the mixture of water and alcohol

**Glutelins** – proteins soluble in acids and bases

## h) Embryo maturation

Maturation of embryo is typical by the fact that embryo becomes dormant. In order to reach dormancy, expression of genes and synthesis of proteins associated with tolerance to desiccation must occur in the cell. Expression of these genes is induced by ABA.

- heat-shock proteins
- LEA proteins
- LEA related proteins – genes containing ABRE motive

Mechanisms of tolerance desiccation?



LEA proteins can bind cell components by the formation of amorphous spirals (coils), preserving the cell content from damage.



Synthesis of the proteins increases in the torpedo stage embryo, when synthesis of storage proteins decreases.

Tolerance to desiccation could be induced by accumulation of oligosacharides – raffinoses. Ration sucrose : raffinose = 20 : 1 => tolerance to desiccation.

Drying seed: [ABA]  => Sensitivity to ABA 

Rehydration: dry seed switches the developmental program to germination program



mRNAs connected with germinating program   
mRNAs connected with developmental program 

Switching the programs is regulated by several ways, including the regulations of changes in the gene (promoter) sensitivity to ABA.

## Changes at the switching of the program from developmental to germinating

### Hormonal level

- Decline in ABA production
- Increase in ABA destruction/sequestration
- Decline in ABA sensitivity



### Genomic level

Storage protein, *Lea* genes, etc.:

- Transcriptional inactivation
  - Loss of promoter activity
  - Loss of trans-acting factors
- Germination/postgermination genes:
- Transcriptional activation
  - Activation of promoters
  - Synthesis of trans-acting factors



### Cytoplasmic level

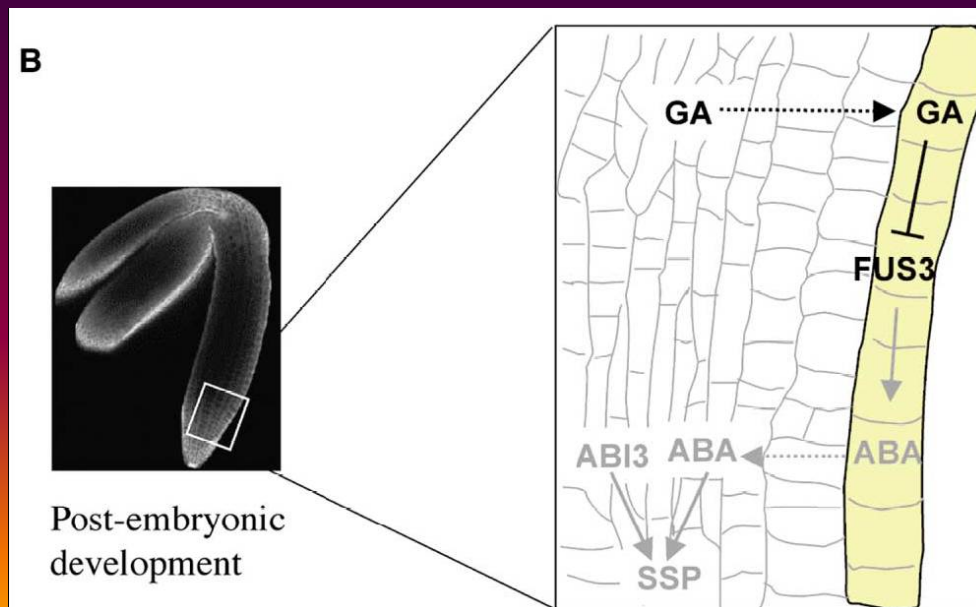
- Destruction of residual developmental mRNAs
- Translation of stored and newly synthesized germination-related mRNAs
- Expression of postgermination genes

## i) Seed germination

Germination – starts when quiescent and dry seed starts to absorb water = **imbibition**; elongation of embryonic axis ends

Function of **FUS3** during postembryonic development – germination of embryo

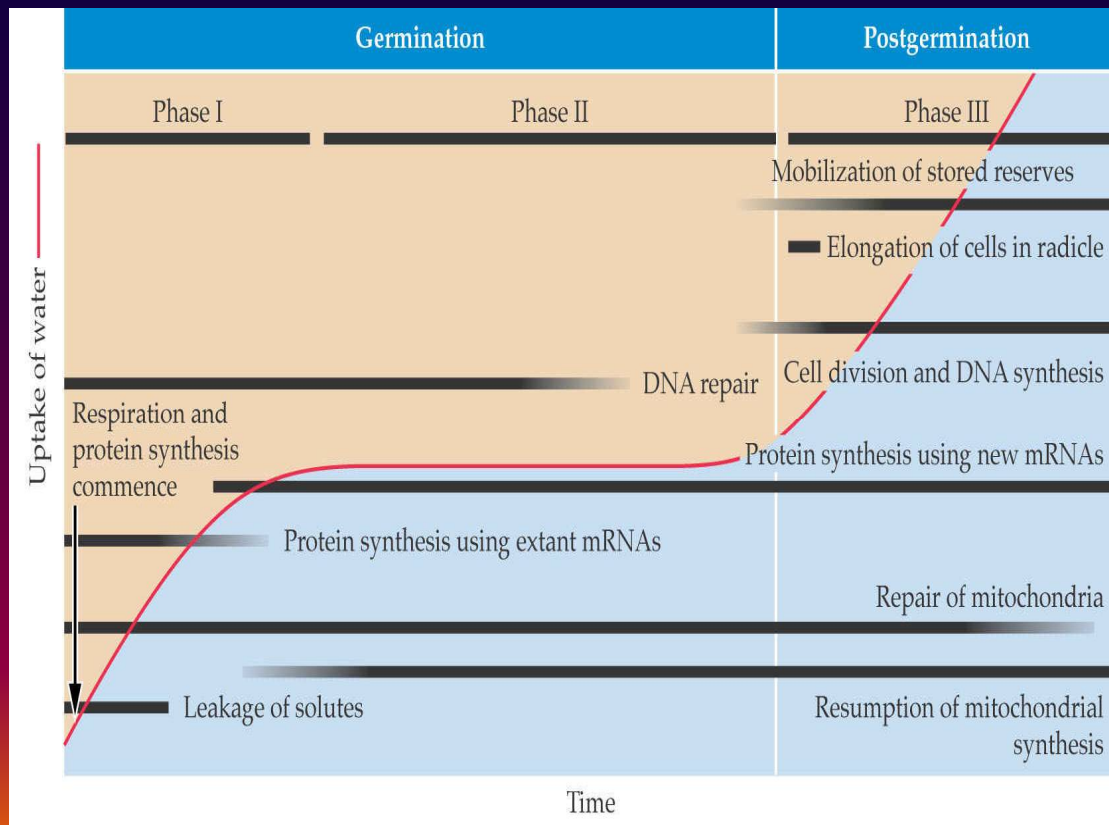
- After the imbibition, the level of gibberellins in the embryo increases
- GAs migrate to epidermis and arrest the function of **FUS3** => suppression of embryonic development controlled by the gene **FUS3** => vegetative development starts = germination (growth)



Visual germination terminates when the structures surrounding embryo are ruptured by the root.

After the germination terminates, the reserves in the storage tissues are mobilized – support for plant growth.

## Time sequence connected with germination and with the post-germination phase



### Phase I

Rapid water sucking by a seed => leakage of solutes and metabolites

### Phase II

Stabilization of the water amount in seed => leakage stops.

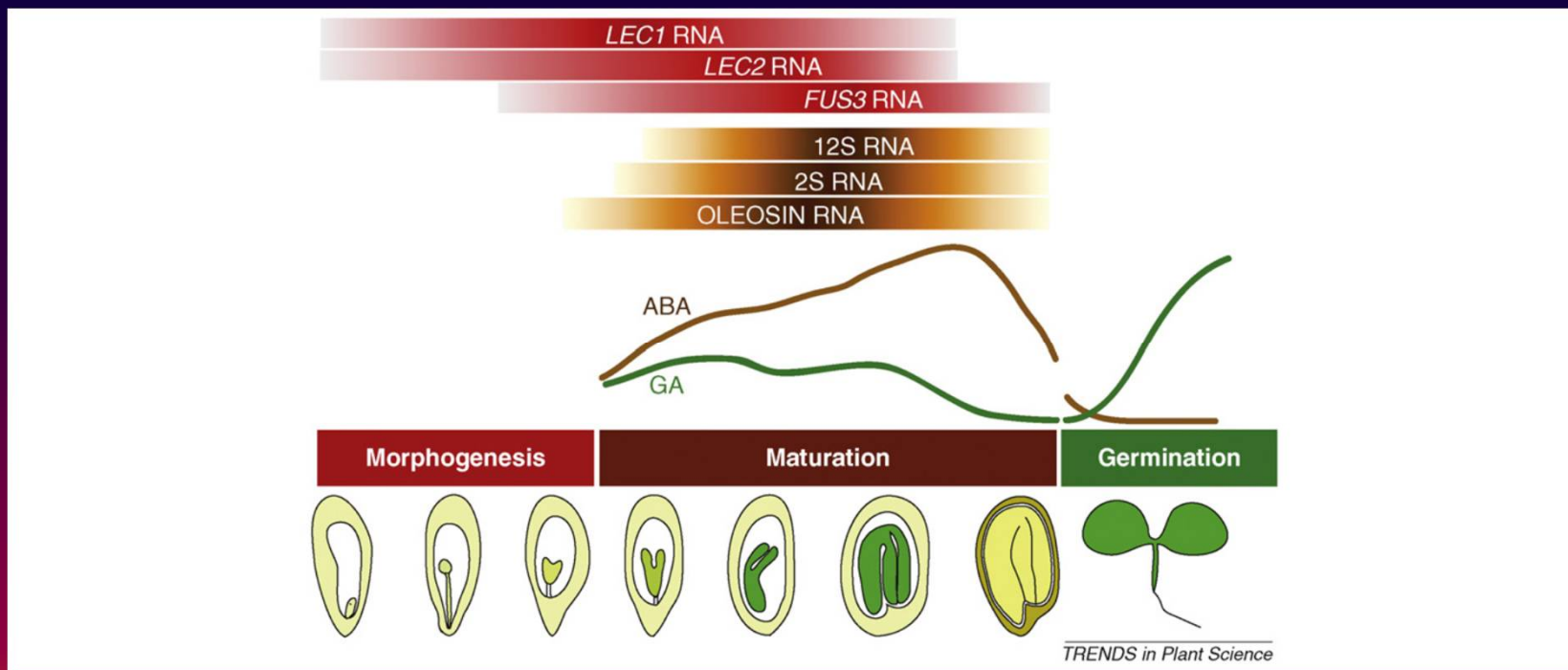
Repair of mitochondria and repeated start of their synthesis.

Synthesis of proteins in newly formed mRNA

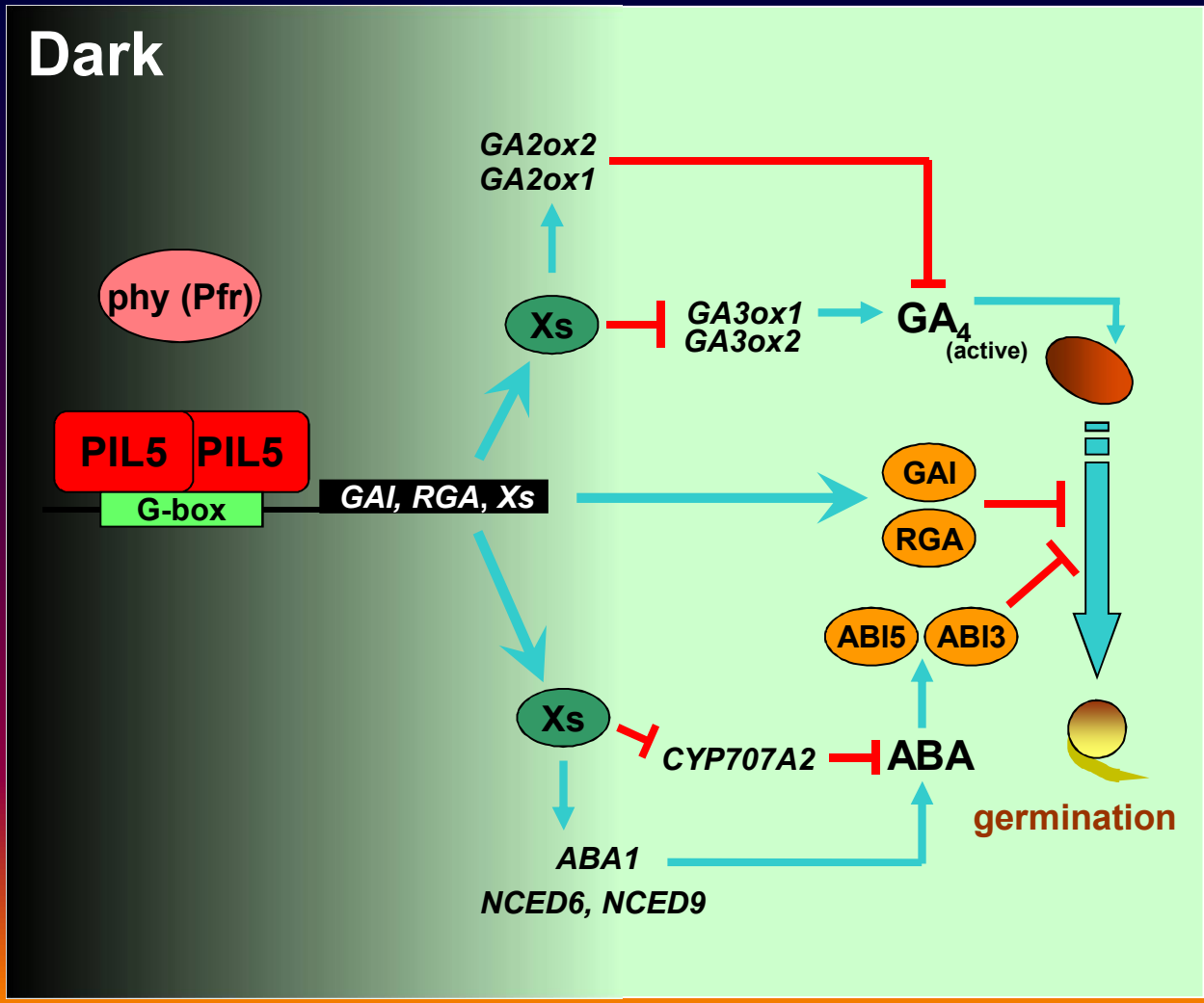
### Phase III

Cell division and elongation, DNA synthesis, reserve mobilization => increasing of seed FW

# Role of transcription factors LEC in the embryo development



# Induction of germination by light – regulation of GAs and ABA biosynthesis and signaling (*Arabidopsis*)



**PIL5** Transcription factors (activators)

**Xs** Unknown factor X

GA2ox2 } GA deactivating genes  
GA2ox1 }

GA3ox1 } GA biosynthetic genes  
GA3ox2 }

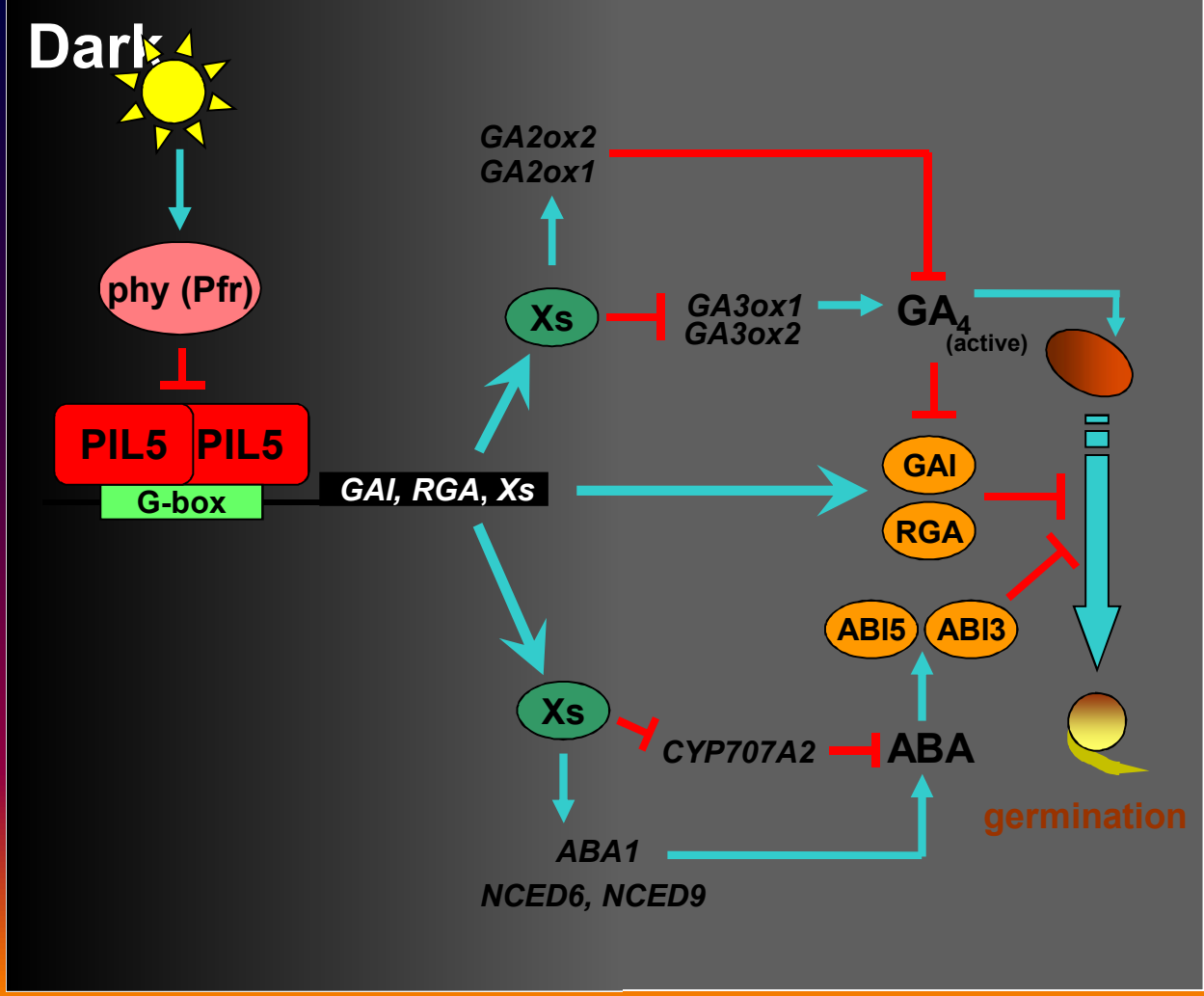
GAI } DELLA proteins  
RGA }

ABA1 } ABA biosynthetic genes  
NCED6  
NCED9 }

ABI5 } Transcription factors induced by ABA  
ABI3 }

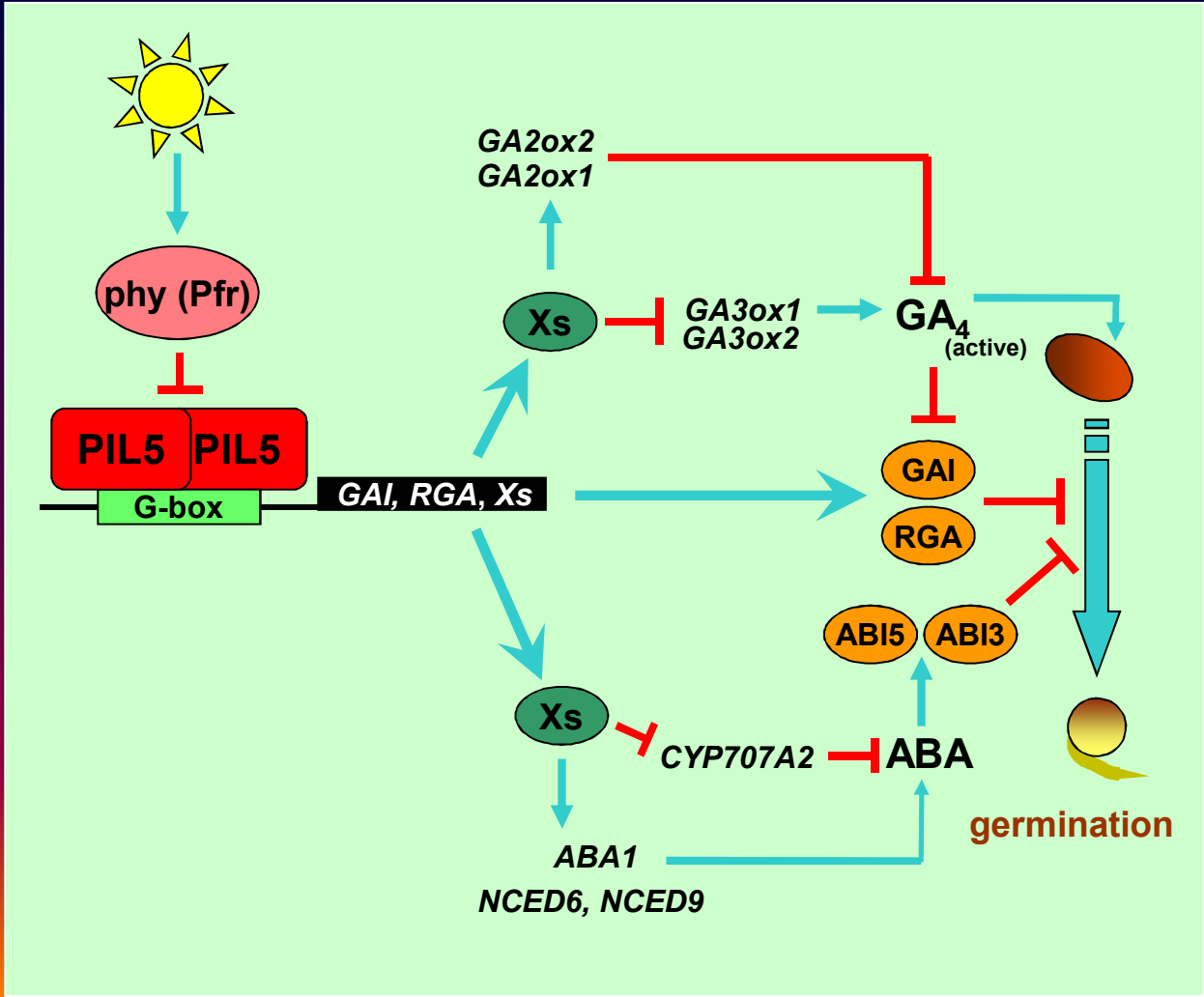
CYP707A2 ABA catabolic gene

Based on Oh et al. (2007) Plant Cell 19: 1192-1208



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