2.2 Identification des gènes régulateurs

Comment identifier les gènes régulateurs?

- mutations létales chez l'embryon
- mutations létales chez la jeune plantule (après la germination)
- mutations non létales, provoquant des malformations mineures
- approche bio-informatique

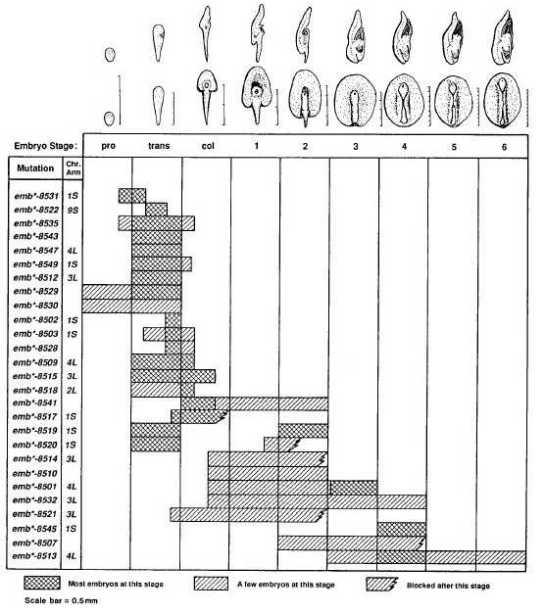
Chez Arabidopsis:

→ environ 4000 gènes essentiels pour l'embryogenèse
(mais pas nécessairement des gènes régulateurs)

→ environ 40 « embryonic patterning genes »

2.2.1 Mutations létales chez l'embryon

fournissent des information sur les gènes essentiels pour l'embryogenèse
p.e. maïs → mutants emb



Phénotype: arrêt précoce du développement, à un stade précis

Informations:

-nombre de gènes pour chaque stade

-progression du processus

Problème: les gènes mutés sont généralement des « gènes d'entretien » (« housekeeping gene »)

p.e. gène de synthèse de la biotine :

- embryon jeune: biotine fournie par la plante-mère
- embryon plus âgé: produit sa propre biotine

arrêt du développement : au moment où l'embryon devrait commencer à produire lui-même ce co-facteur

→ mutation d'un gène métabolique et non de développement

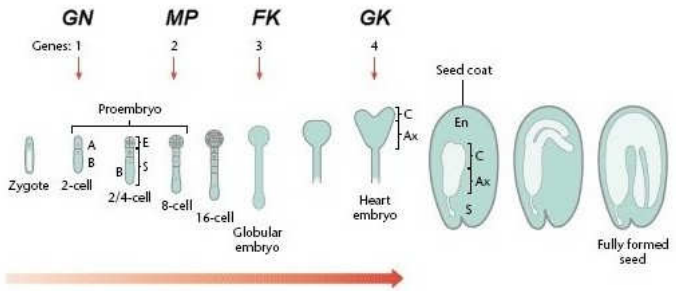
2.2.2 Des mutations létales chez la plantule ont permis d'identifier des gènes intervenant dans la mise en place de l'axe apical-basal de l'embryon

Chez ces mutants:

- l'embryogenèse est complétée (donc: gènes d'entretien OK)
- la germination est normale
- la plantule a des malformations (absence de certaines parties) et meurt quelques jours après la germination

L'expression des gènes sauvages intervient à un stade précis:

exemples



Les gènes identifiés par cette approche interviennent dans des phénomènes de **signalisation**

→ les mutants présentent un développement désorganisé

Exemple 1: signalisation par l'**auxine**

Gènes *MP* (*Monopteros*), *BDL* (*Bodenlos*) et *AXR6* (*Auxin resistant 6*)

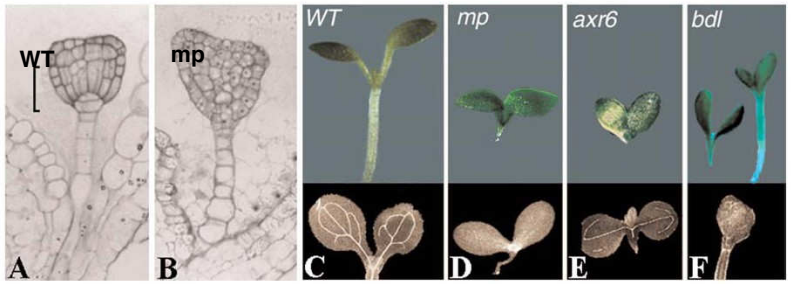
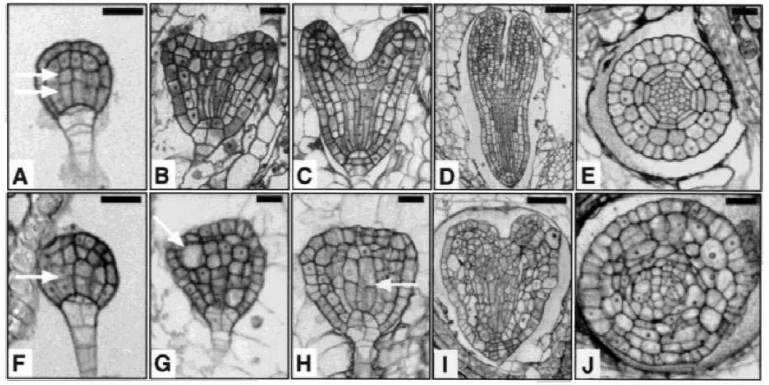


Figure 6: Embryo axis formation and auxin signal transduction
A: Basal domain of the wild type triangular-stage embryo (marked by bracket) comprises files of narrow cells in the center (embryonic stele) and a characteristically dividing uppermost suspensor cell ('hypophyseal' cell), whose derivatives form the QC and columella initials of the primary RAM. **B**: Triangular-stage *mp* mutant, cell divisions are not oriented along the apical basal axis, narrow cells of the embryonic stele are missing and the uppermost suspensor cell divides abnormally. **D-F**: Seedlings mutant for the presumed auxin signal transduction genes *MONOPTEROS (MP), AUXIN RESISTANT 6 (AXR6)* and *BODENLOS (BDL)* are defective in embryo axis formation and vascular differentiation. Hypocotyl and root are missing in *mp* and *axr6* mutants (**D** and **E**) and are variably reduced in *bdl* mutants (**F**).
A and **B**: from Berleth and Jürgens, 93. **C-E**: Seedling phenotypes and dark field view of xylem strands of indicated genotypes reproduced from Berleth et al., 2000; Hobbie et al., 2000 and Hamann et al., 1999, respectively.

NB Mutants: pas de méristème racinaire

Exemple 2: signalisation par des **dérivés de stérols** (brassinostéroïdes et autres)

Gène *FK* (*Fackel*)



WT

fk

Figure 3. Embryogenesis is abnormal in *fk* mutants. (*A–E,K*) Wild type. (*F–J,L–O*) *fk*. (*A,F*) Globular stage embryo. In wild type, the central vascular precursor cells divide asymmetrically to produce small apical and elongated basal cells (two arrows). In *fk*, the central cells divide to give cells of similar sizes (single arrow). (*B,G*) Early heart stage embryo. In wild type, the cotyledon primordia become visible and the central cells are elongated. In *fk*, cotyledon primordia are not visible and cells in the center of the embryo fail to elongate. Some cells are grossly enlarged (arrow); (*C,H*) Heart stage embryo. In wild type, the cotyledon primordia mark the bilateral symmetry of the embryo. *fk* embryos exhibit abnormal cell morphologies and incomplete cell walls (arrow); (*D,I*) Torpedo stage embryo. Wild-type embryo shows a characteristic torpedo shape. The *fk* embryo displays an abnormal heart shape and cell elongations leading to longitudinal outgrowth are defective. (*E,J*) Transverse sections of torpedo stage embryos. Wild-type embryo shows radial organization of tissue layers. The *fk* embryo lacks an organized radial pattern. Cells are misshapen and either larger or smaller than cells of the corresponding layer in wild type.

2.2.3 Des mutations provoquant des malformations mineures de la plantule ont permis d'identifier les gènes contrôlant l'initiation du développement embryonnaire

Les gènes LEC1* et *LEC2 (*Leafy Cotyledons*) sont nécessaires et suffisants au développement embryonnaire (de l'induction jusqu'à la maturation)

- **Mutants**:
  - poils épidermiques sur les cotylédons
  - pas de maturation de l'embryon
- **Produits**: régulateurs de la transcription
- **Expression**:
  - embryon, cotylédons et albumen
  - du début à la fin de l'embryogenèse

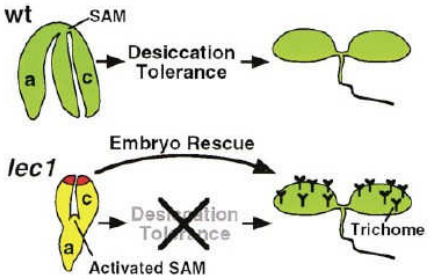
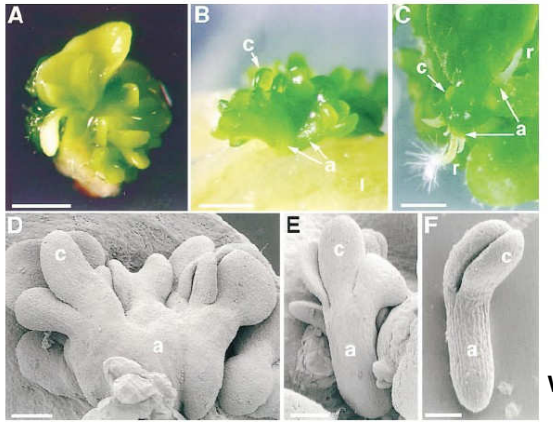


Figure 1. Pleiotropic Effects of the *lec1* Mutation on Embryo Development

Major differences between wild-type and *lec1* mutant embryos are as follows. Embryo shape: the axes of mutant embryos are short, and their cotyledons are round and do not curl. Anthocyanin generally accumulates at the tips of mutant cotyledons. Precocious germination: the shoot apical meristems of *lec1* embryos are activated in that they are domed and possess leaf primordia, unlike their wild-type counterparts that are flat and do not contain leaf primordia. Defects in seed maturation: *lec1* mutant embryos are intolerant of desiccation and normally die if dried on the plant. However, *lec1* embryos isolated before desiccation can be germinated to produce fertile homozygous mutant plants. The promoter of a 7S storage protein gene that is normally active during wild-type embryogenesis is not active in the *lec1* mutant. Incomplete specification of cotyledon identity: *lec1* seedlings possess trichomes on cotyledons. Trichomes are present on *Arabidopsis* leaves and stems but not on wild-type cotyledons. a, axis; c, cotyledon; SAM, shoot apical meristem.

- **Vérification** : plantes transgéniques exprimant gènes *LEC* de façon ectopique (promoteur 35S: expression forte, dans toute la plante)
  - 35S-*LEC1*
  - 35S-*LEC2*

  } embryons à partir de cellules de feuille (fonctions partiellement redondantes)



WT

Figure 7. Embryo-like Structures on Transgenic Plants Ectopically Expressing the *LEC1* Gene

(A) *35S/LEC1* seedling that grew vegetatively and produced multiple cotyledon-like organs.
(B) Embryo-like structures on the leaf of a *35S/LEC1* plant that grew vegetatively.
(C) Axes of embryo-like structures that "germinated" to produce roots.
(D and E) SEM analysis of embryo-like structures. Structures resemble fused cotyledon-stage embryos with multiple cotyledons.
(F) SEM of wild-type cotyledon-stage embryo.
a, axis; c, cotyledon; l, leaf; r, root. Bars, 1 mm (A and C), 0.5 mm (B), 0.1 mm (D and E), and 0.05 mm (F).

2.2.4 Approche bio-informatique

- Définir les stades de développement
- Identifier les gènes (mRNAs) exprimés à chaque stade…

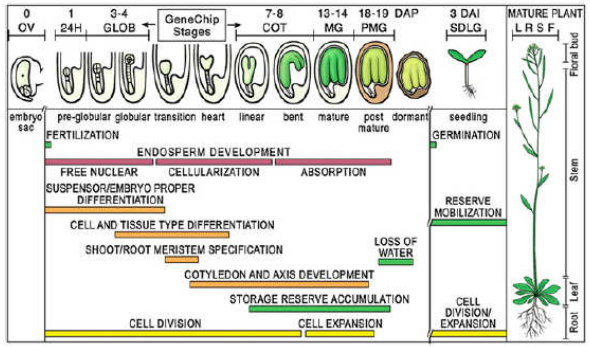
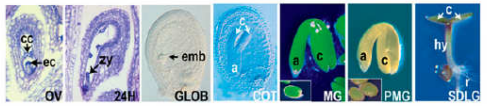


Fig. 1. Schematic representation of *Arabidopsis* seed development and stages of the life cycle used for GeneChip analysis. Seed cartoons were adapted from Bowman and Mansfield (57) and are not drawn to scale. Developmental events were modified from Goldberg et al. (1). Stages used for GeneChip analysis are described in *SI Materials and Methods*. Numbers correspond to days after pollination (DAP) or days after imbibition (DAI). Brackets mark the range of embryo stages included in each GeneChip seed sample. OV, unfertilized ovule; 24H, 24-h postpollination seed; GLOB, globular-stage seed; COT, cotyledon-stage seed; MG, mature-green-stage seed; PMG, postmature-green-stage seed; SDLG, seedling; L, leaf; R, root; S, stem; F, floral buds.

Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. Brandon H. Lea et al. PNAS (2010) 107, 8063–8070

- Déterminer la spécificité d'expression de chaque gène
- Identifier les gènes codant des régulateurs transcriptionnels
- Résultats
  - gènes déjà connus (*LEC*…)
  - gènes dont la fonction était encore inconnu

A



B

	OV	24H	GLOB	COT	MG	PMG	SDLG
No. of mRNAs Detected							
Biological Replicate #1	12,973	13,833	14,265	13,699	11,869	10,036	14,231
Biological Replicate #2	13,879	12,901	14,248	13,956	12,098	9,874	13,754
No. of mRNAs Detected in Both Biological Replicates	12,591 (987)	12,421 (963)	13,722 (1,078)	13,103 (1,039)	10,875 (847)	8,779 (696)	13,185 (1,015)

C

No. of mRNAs Detected Specifically in Each Stage	22 (4)	16 (0)	100 (17)	50 (9)	26 (3)	31 (6)	505 (56)

D

	+OV	+ 24H	+ GLOB	+ COT	+ MG	+ PMG	+ SDLG
No. of mRNAs Detected Specifically in Pairs of Stages							
OV	22 (4)	49 (7)	5 (0)	4 (2)	0	0	8 (1)
24H	-	16 (0)	16 (1)	3 (0)	0	0	9 (1)
GLOB	-	-	100 (17)	108 (18)	0	2 (1)	31 (2)
COT	-	-	-	50 (9)	8 (3)	1 (0)	18 (2)
MG	-	-	-	-	26 (3)	98 (9)	17 (0)
PMG	-	-	-	-	-	31 (6)	16 (0)
SDLG	-	-	-	-	-	-	505 (56)

E

	Three-Stages	Four-Stages	Five-Stages	Six-Stages
No. of mRNAs Detected in Multiple Stages	268 (27)	224 (28)	470 (47)	1030 (68)

F

No. of mRNAs Detected in All Stages	6,937 (473) →

Fig. 2. Genes active before, during, and after *Arabidopsis* seed development.

Conclusion: les différentes parties de l'embryon correspondent à des domaines d'expression spécifique de plusieurs gènes (détermination)

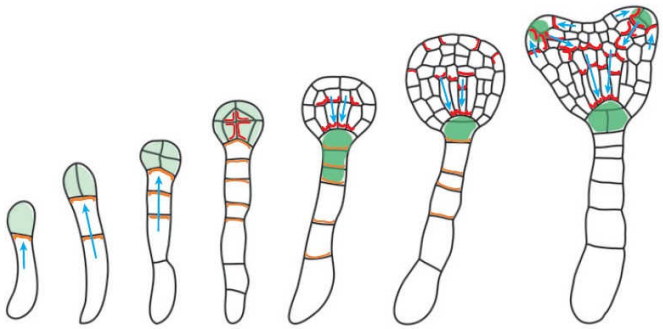
Transcriptional patterns in *Arabidopsis* embryos.

Gene	mRNA expression	Reference(s)	Gene	MRNA expression	Reference(s)
AtML1		[6]	*WOX5*		[7**]
PIN1		[30]	*WOX8*		[7**]
MP; BDL		[16,18]	*WOX9*		[7**]
*PIN7; ASK*η *ptrak276S*		[8**,10**,31]	*ANT*		[33]
PIN4		[32]	*REV*		[34]
WUS		[5]	*PHV*		[34]
WOX2		[7**]	DR5::GFP		[10**]

2.3 Mise en place des axes de symétrie et des méristèmes

2.3.1 Axe apical basal

Observation: transport de l'auxine au cours de l'embryogenèse (transporteurs trans-membranaires)



Auxin flux in the embryo (*blue arrows*) is initially directed upward and mediated by PIN7 (*orange*). At the globular stage, apical-to-basal auxin transport becomes established and persists throughout the life cycle. This is marked by the localization of PIN1 (*red*) at the basal membranes of the provascular cells. Auxin perception maxima (*green*) in the embryo can be visualized by the use of a DR5 construct that consists of a synthetic ARF/auxin-responsive promoter fused to GFP. In the epidermal cells of incipient cotyledon primordia, PIN1 becomes redistributed to direct auxin flux toward the emerging tips.

Transport polarisé de l'auxine:

phénomène qui s'auto-amplifie, à partir d'une source (▼): « canalisation »

permet la différenciation de files de cellules (système vasculaire, p.e.)

▼

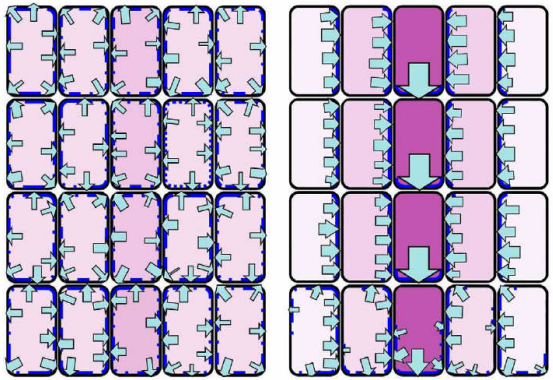


Figure 6. Integration of cell polarity through auxin transport.

A highly schematic view. Rectangles represent cells and arrows of different strength represent the intensity of auxin flow. For simplicity it is assumed that intensity and direction of auxin flow is solely controlled through the quantity and distribution of auxin efflux carriers (dark blue) in the plasma membrane. Routes of preferred auxin transport have been associated with sites of vascular differentiation (dark purple).

The central proposition is that auxin flow and cell polarization are connected in a positive feedback loop, illustrated here by restricting auxin efflux to the basal side of each cell as an expression of cell polarization. Thereby, cells in a given region, including cells newly formed by division, would integrate polarity. The feedback system could further include the stabilization of auxin sources or sinks. Note that the same cellular feed-back mechanism would progressively enhance initial differences in auxin conductivity leading to the specification of different cell types in the radial dimension. Drawn after Sachs (1991).

Le transport polarisé de l'auxine dépend d'une série de transporteurs transmembranaires: les protéines PIN

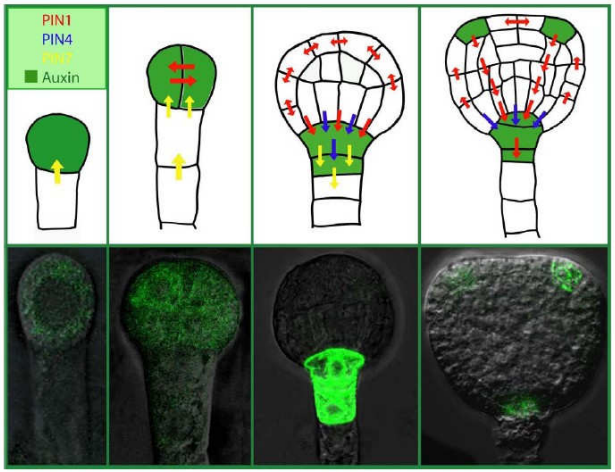
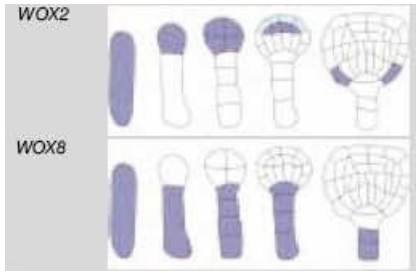
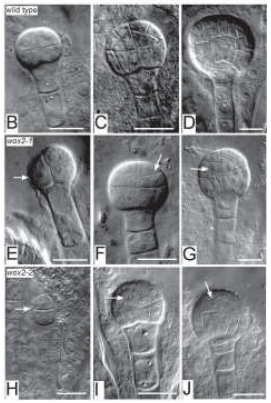


Figure 5. PIN-mediated auxin transport and distribution during Arabidopsis embryo development. The upper panel illustrates embryo development, auxin transport and distribution from the one-cell stage to the triangular-stage. PIN7 become expressed from the earliest stages of embryo development (marked by yellow arrow), later also PIN1 and PIN4 become present (marked by red and blue arrows, respectively). Notice that during embryo development the polarity of PIN localization changes. PIN expression correlates with the accumulation of auxin (highlighted in green) first in the apical cell and developing proembryo, and later at the basal part of the embryo with a maximum in the upper suspensor cell. The lower panel shows pictures of auxin accumulation as indirectly visualized by DR5rev::GFP. Images adapted from Friml et al (2003b) and Benkova et al (2003).

- Les **gènes *WOX*** (*Wuschel-related Homeobox*) participent à l'établissement de la polarité (segmentation) de l'embryon, p.e.





Mutants wox2

- Produits des gènes *WOX*: régulateurs de transcription à **homéodomaine** (HD; définissent segmentation chez les embryons animaux)

Expression des gènes *WOX* au cours de l'embryogenèse

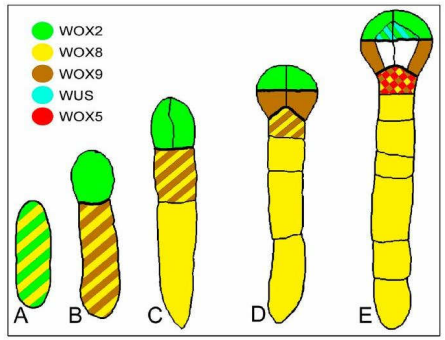


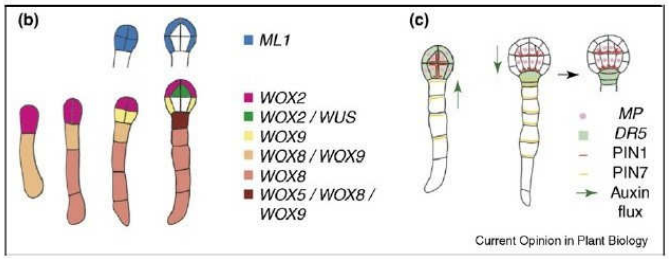
Figure 5. Apical-basal *WOX* expression domains.

(A-E) Expression domains of *WUSCHEL-RELATED HOMEOBOX* (*WOX*) genes during the early stages of embryogenesis and development from the single-celled zygote. Images **A–E** are redrawn after Haecker et al. (2004) and Nawy et al. (2008).
(A) The zygote expressing *WOX2* (green) and *WOX8* (yellow), which subsequently mark the apical and basal daughter cells of the first division **(B)**.
(B) *WOX9* is upregulated in the basal cell at this time.
(C) After division of the basal cell, *WOX9* is expressed only in the more apical cell, while *WOX8* is expressed in both daughter cells.
(D) At the octant stage, the upper and lower tiers of embryo proper are marked by *WOX2* and *WOX9* expression, respectively. Both *WOX8* and *9* are expressed in the hypophysis.
(E) At the dermatogen stage *WOX9* expression is downregulated in the embryo proper, except for the outer cells of the lower tier. Simultaneously, *WUSCHEL* is turned on in the inner cells of the upper tier and *WOX5* within the hypophysis.

Segmentation de l'embryon → accumulation différentielle des ARNm et protéines WOX dans le zygote (mécanisme?) puis dans les différentes parties de l'embryon

→ détermine le développement différentiel des deux cellules issues d'une même cellule-mère

Conclusion: le transport polarisé de l'auxine est le signal orchestrant la polarité (segmentation) de l'embryon

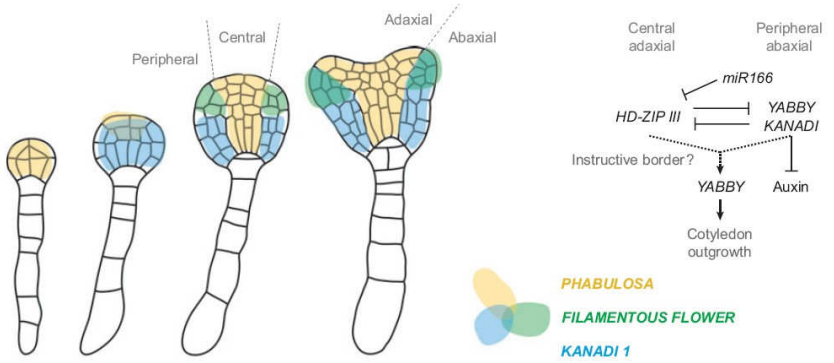


(b) Dynamic patterns of gene expression closely parallel the anatomical pattern of cell divisions. Expression of *WUSCHEL*-type homeodomain (*WOX*) genes reflects subdivision of the apical–basal axis, while the distantly related homeodomain gene *MERISTEM LAYER1* (*ML1*) marks formation of the protoderm. **(c)** Auxin flux is organized in two phases. In the early embryo, localization of the auxin transporter PIN-FORMED7 (PIN7; orange) to the apical membranes of suspensor cells presumably mediates transport (green arrow) towards the proembryo, where PIN1 (red) is present but not localized in a polar fashion. In the globular embryo, PIN1 and PIN7 localization to basal membranes establishes apical-to-basal auxin flux, as it persists throughout the life cycle. Auxin accumulates in the hypophysis, triggering robust expression of the synthetic reporter *DR5* (green). Asymmetric division of the hypophysis produces a lens shaped cell, the presumptive QC, and is dependent on an inductive signal mediated by the ARF MONOPTEROS (MP; blue dots) in the provasculature.

Talk global, act local - patterning the Arabidopsis embryo. Tal Nawy, Wolfgang Lukowitz and Martin Bayer. Current Opinion in Plant Biology 2008, 11:28–33

2.3.2 Signalisation pour l'établissement de la symétrie radiale (domaines central/périphérique de l'embryon)

Par l'effet antagoniste de deux groupes de régulateurs



The origin of central versus peripheral domains and the breaking of radial symmetry. (*a*) Class III homeodomain leucine-zipper (*HD-ZIP III*) genes, such as *PHABULOSA* (*PHB*), and *KANADI* (*KAN*) genes, such as *KAN1,* are initially expressed throughout the early embryo, but their expression soon becomes limited to the central (*PHB*) or peripheral (*KAN1*) domain. In adult plants, the *HD-ZIP III* and *KAN* genes show mutually antagonistic interactions. In addition, the *miR166* family of microRNAs plays a role in delimiting *HD-ZIP III* expression to the central/adaxial domain. *KAN* activity restricts expression of the auxin transporter PIN1 in the periphery of the hypocotyls, negatively regulating auxin responses. The *YABBY* (*YAB*) gene *FILAMENTOUS FLOWER* (*FIL*) is first expressed at the late globular stage, marking the abaxial side of cotyledon primordia.

Le cas particulier de l'épiderme:

- gènes *ATML1* (*Arabidopsis thaliana Meristem Layer1*) et *PDF2* (*Protodermal Factor2*)
  - expression spécifique: protoderme
  - produits: régulateurs de transcription à **homéodomaine** (HD)
  - double mutant: plantule sans épiderme, non viable

2.3.3 Symétrie bilatérale: les cotylédons sont formés AVANT le SAM et déterminent la position de celui-ci

Principe: mise en place d'un réseau de régulateurs de transcription pour partager la région apicale en 2 partie:

- bande centrale: répression des gènes de formation d'organes (maintien d'une population de cellules-souches)
- zone périphérique: formation des primordia de cotylédons

Le gène ***ANT*** (*AINTEGUMENTA*) définit les primordia de cotylédons

→ maintient un état méristématique → mitoses

Antagonisme avec gènes *CUC*…

ANT ⊣ *CUC*

Les gènes ***CUC1, CUC2* et *CUC3***
(*CUp-shaped Cotyledons*) établissent la frontière SAM-cotylédons (BCM)

- **Mutants**: croissance anormale entre les primordia de cotylédons:
  - – fusion des cotylédons
  - – absence de SAM
- Participation de l'**auxine**:
  - – mutants mp, bdl, axr6 (et pin1) OU
  - – inhibiteur du transport d'auxine

  → fusion des cotylédons

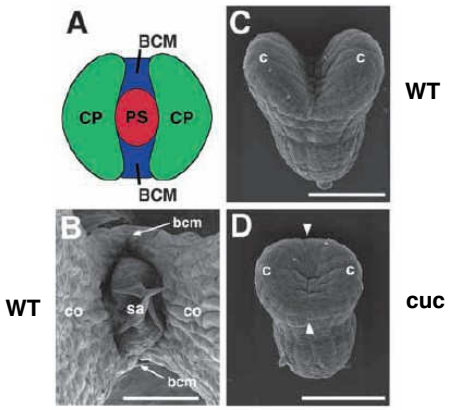
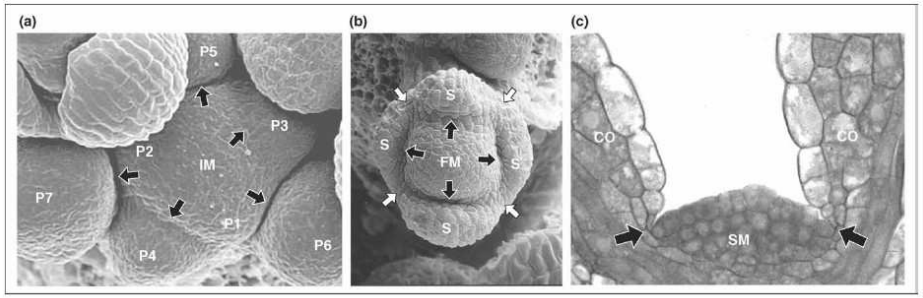


Fig. 1. Development of the apical region in the wild type and *cuc1 cuc2* embryos. (A) Schematic diagram of the apical region of the wild-type embryo viewed from above. CP, cotyledon primordia region; PS, presumptive SAM region; BCM, boundary region of cotyledon margins. (B-D) Scanning electron micrograph (SEM) images of (B) wild-type seedling at 3 days postgermination viewed from above; (C) wild-type embryo at the heart stage; (D) *cuc1 cuc2* embryo at the heart stage. Arrowheads indicate ectopic bulging of BCM. Scale bars, 100 μm (B) and 40 μm (C,D). c, cotyledon primordia; co, cotyledons; sa, SAM; bcm, boundaries of cotyledon margins.

Les frontières méristème-organe (M-O) et organe-organe (O-O) sont composées de cellules particulières:

cellules-frontières (« boundary cells »)



Examples of shoot organ boundaries in *Arabidopsis*. **(a)** Top view of the inflorescence meristem. **(b)** Floral meristem. **(c)** Longitudinal section of the seedling apex stained with Toluidine blue. The M–O boundary (black arrows) corresponds to the region between a meristem and an organ primordium, whereas the O–O boundary (white arrows) is the region between adjacent organ primordia. Growth is generally suppressed in both types of boundary, as evident by the formation of a hollow or groove. Note that in (c), the shoot meristem (SM) consists of densely stained small cells, whereas the cotyledons (CO) contain vacuolated and elongated cells. The border of these cell types coincides with the location of the M–O boundary (black arrows). FM, floral meristem; IM, inflorescence meristem; P1–P7, flower primordia; S, sepal primordia.

Principaux caractères des cellules-frontières

- Peu ou pas de mitose
- Morphologie particulière
- Très grande sélectivité au niveau du transport par les plasmodesmes (limite échange de signaux entre organes différents)
- Source de signaux pour le développement de tissus adjacents

- **Expression des gènes *CUC*:**

  régulation négative par l'auxine (primordia) et par un miRNA

  p.e. surexpression de miR164 → même phénotype que mutant cuc

2.3.4 La conservation d'un groupe de cellules-souches entre les primordia de cotylédons permet la formation du SAM

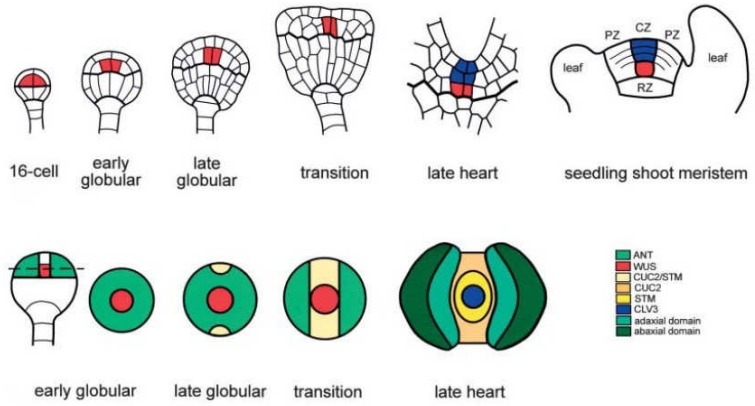


Figure 2. Development of the Apical Embryo Domain.

The top row shows schemes of longitudinal median sections. The upper and lower thick lines represent clonal boundaries between the descendants of the apical and basal daughter cells of the zygote and between the apical and central embryo domains, respectively. The bottom row shows cross-sections of the same stages as indicated by the dashed line at left. CZ, central zone; PZ, peripheral zone; RZ, rib zone. The expression domains of early genes in the apical region are shown in color as indicated. See text for details.

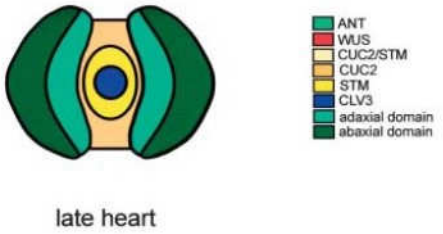
Gènes: *WUS (WUSCHEL), STM (SHOOT MERISTEMLESS)*

Position du SAM:

- Déterminée par signaux provenant de la face adaxiale des cotylédons

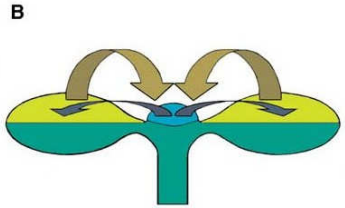
  NB **adaxial**: situé du côté de l'axe

  abaxial: situé du côté opposé à l'axe



late heart

- **Observations**:
  - Mutation transformant la face adaxiale en face abaxiale: problème de mise en place du SAM
  - Mutation favorisant identité adaxiale: SAM de plus grande taille



Signalisation SAM ↔ cotylédons

puis

SAM ↔ feuilles

2.3.5 La position du RAM est déterminée par l'auxine

Inhibition du transport de l'auxine

1- Mise en place des cellules du centre quiescent (QC) sous le contrôle de l'**auxine**:

mutants mp, bdl, axr6

→ (pas de RAM)

2- Signaux provenant du QC déterminent l'identité des cellules voisines:

→ **cellules initiales,** à l'origine des différents tissus de la racine

3- Signalisation entre tissus adjacents

NB Columella root cap : coiffe à columelles

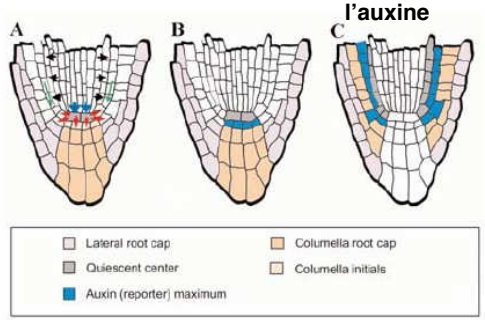


Figure 8: Cell fate specification in the root meristem

A: Organization of cell types in the root meristem. Centrally located QC cells (grey) are flanked by initials of various tissues: initials extending tissue layers in the growing root and, laterally and basally, initials replenishing cells in the lateral (violet) and central root cap (orange). Blue arrows indicate that the acquisition of QC cell fate seems to be dependent on signals from the shoot (compare Figure 2A); red arrows the dependence of stem cell fate on signals from the QC. Black arrows represent endodermis inducing signals from the stele (Figure 18) and green arrows the stabilization of tissue identity within each layer. B: An auxin-response reporter gene detects a maximum (blue) at the position of the columella initial cells. C: When the auxin response maximum is displaced (e.g. because of auxin transport inhibition), the positions of all three cell types in relation to the stele and auxin response maximum are maintained, suggesting an important role for auxin distribution in root meristem patterning. From Scheres, 2000.