Bi8940 Developmental Biology
Lesson 7

Plant Embryogenesis

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Outline of Lesson 7

Plant Embryogenesis

- Overview of the embryo formation in *Arabidopsis*
- Mechanism of the apical-basal axis formation
  - female gametophyte prespecification, invariant cell division or positional information?
  - differential gene expression
  - auxin gradients formation
  - the role of auxin signalling
- Roor meristem formation
  - auxin and hypophysis identity
  - differential gene expression and root meristem patterning
  - auxin-cytokinin interaction and the root meristem organization centre formation

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Plant Embryogenesis

- Patterning of the apical embryo pole
  - generation of cotyledons and shoot apical meristem
  - proper spacing of lateral organs
  - adaxial-abaxial axis formation
- Radial embryo patterning
  - epidermal layer specification
  - separating vascular and ground tissue

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- Overview of the embryo formation in *Arabidopsis*

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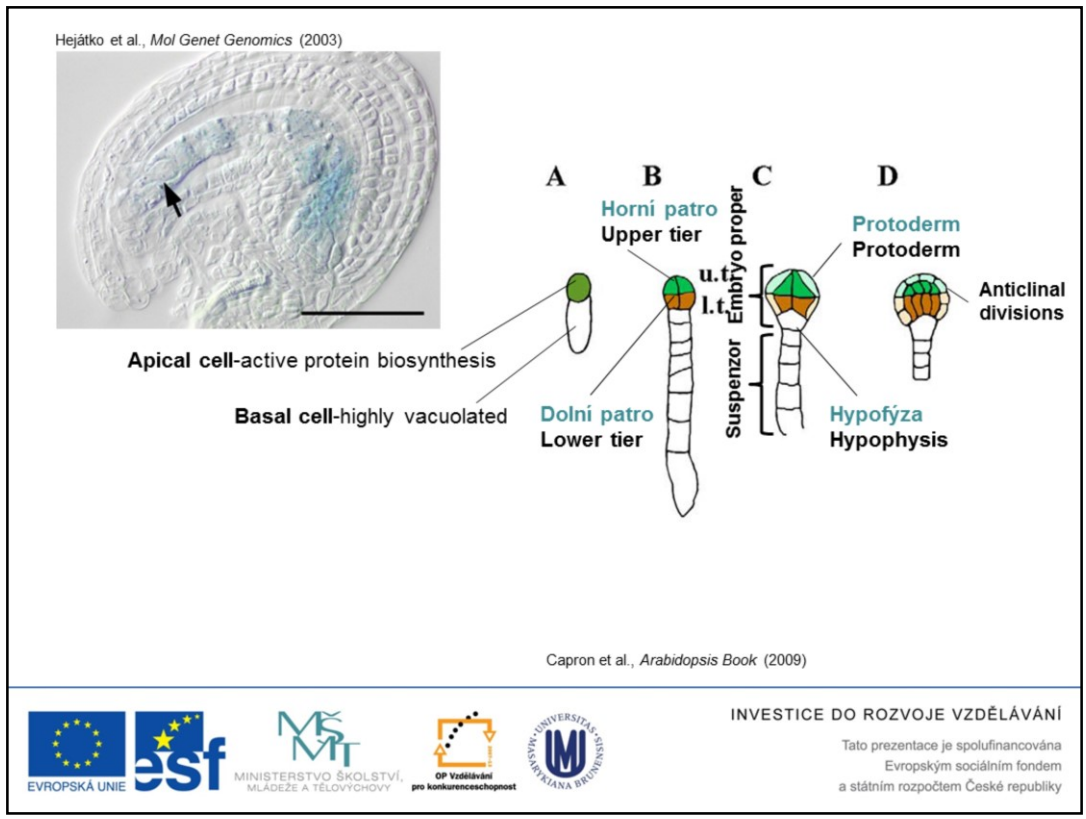


In contrast to animals, plant embryogenesis does not include dramatic movements of cells, which identity has been specified.

In plant embryos, the cells are tightly connected through the cell walls and "move" and acquire their identity via *directed* (or *oriented*) *cell divisions*.

In *Arabidopsis*, embryogenesis starts in a moment of fertilization (karyogamy of egg and sperm cell nuclei). The zygote elongates and undergoes an *asymmetric cell division*.

That allows formation of two cells, **apical** and **basal cell**. Further developmental fate of these two cells dramatically differs – only the apical cell develops into embryo proper, while the basal cells develops mostly into extraembryonic tissue. There could be seen a partial homology with the ICM formation during early embryogenesis in amniotes.

The apical cell contains dense cytoplasm and is the site of very active protein synthesis. In contrast, the basal cell and its descendants are highly vacuolated.

Similarly to that, when ICM leads to the embryo proper formation, apical cell develops in the embryo proper while the larger, lower cell develops into supporting structure called **suspensor**. The only exception is the upper most cell of the suspensor, the hypophysis that is of basal cell origin but become a part of the *root meristem*, as will be discussed later.

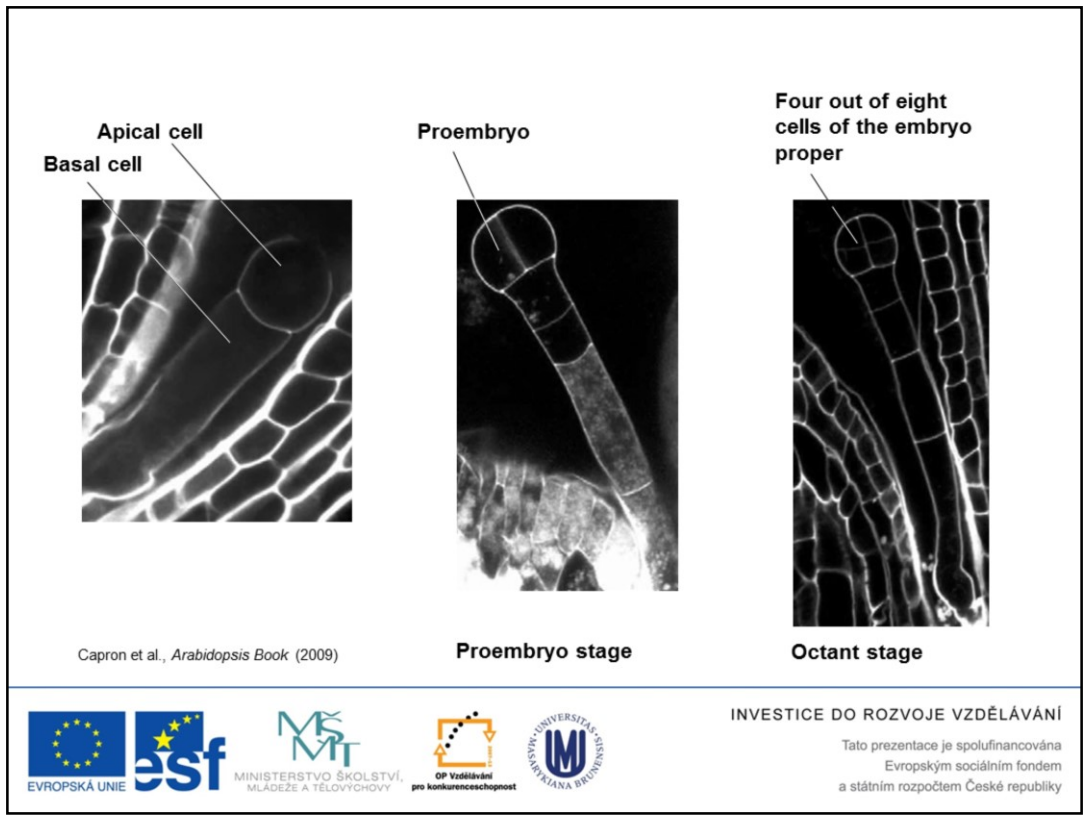
The apical cell undergoes set of oriented cell divisions. It divides two times vertically and ones horizontally, allowing thus formation of **octant**, comprising eight cells.

The upper portion of octant forms what is called **upper tier (UT),** accordingly, the lower portion is called **lower tier (LT).**

The sequence of these early cell divisions of apical cell is highly conserved (reproducible) in *Arabidopsis*. The basal cell divides horizontaly for several times, resulting into formation of suspensor.

At the stage of transition from the octant stage, series of tangencial divisions separate eight prospective epidermal cells, the **protoderm**.

Protoderm remains histologically separated through the predominantly *anticlinal* (perpendicular to surface) *cell divisions*.

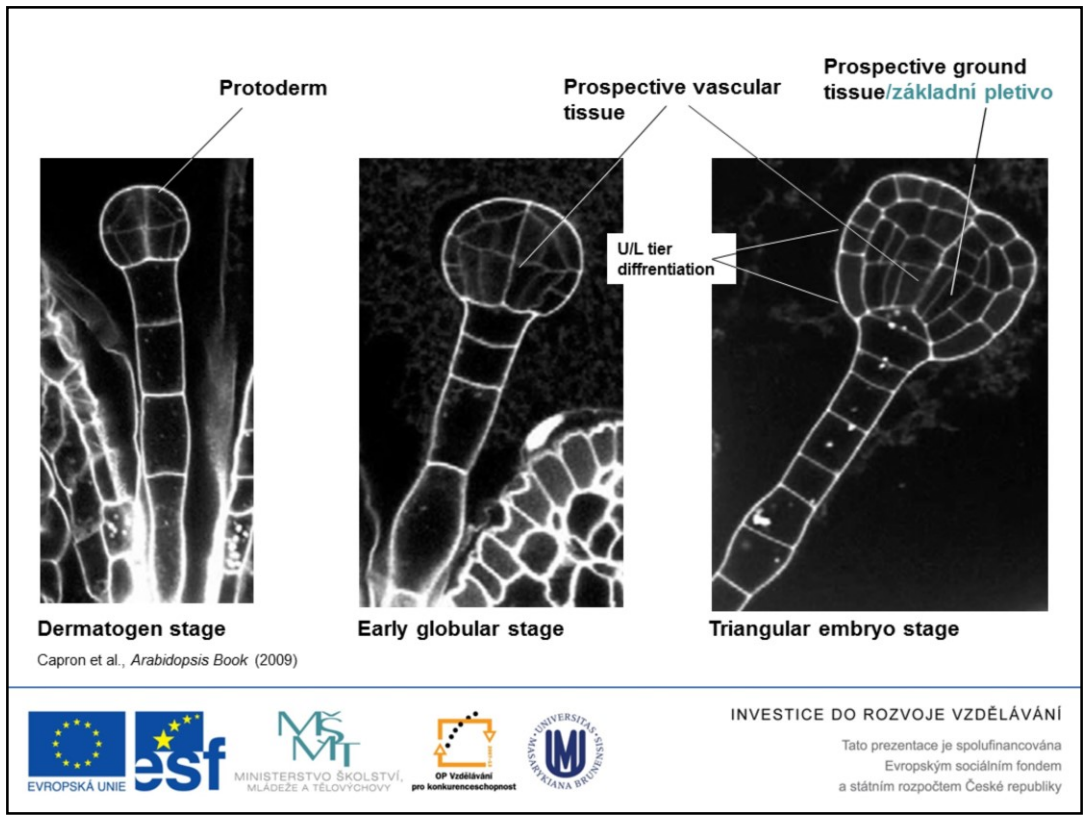


Micrographs from the confocal microscope showing the embryo development in *Arabidopsis*.

Early embryo, just after the first asymmetric division, with a single cell in the embryo proper (left).

Early embryo with two cells in the embryo proper as a result of vertical division of the apical cell, forming what is called **proembryo** (middle).

Octant stage with four out of eight cells in the embryo proper are visible. These eight cells together with upper most suspensor cell (not specified yet at this stage) will develop into entire embryo proper.

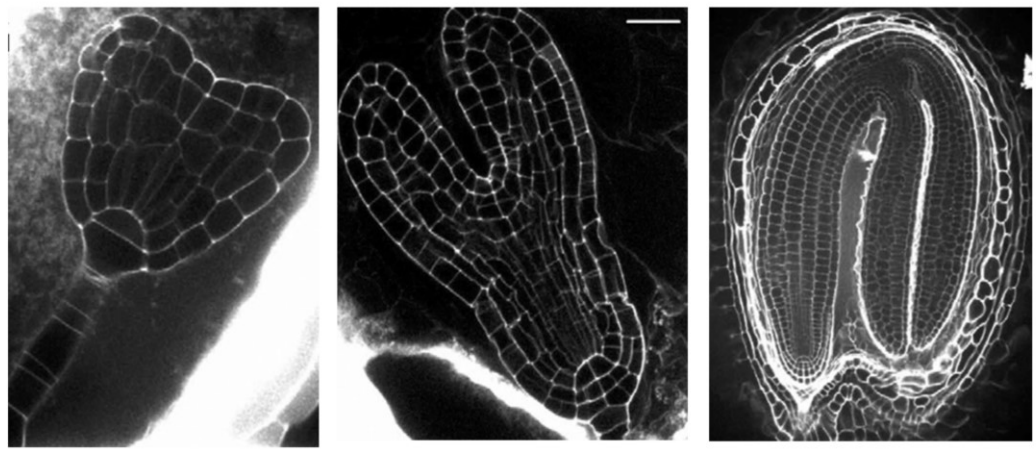


At the transition form the octant stage, when the protoderm is specified (**dermatogen stage**, left), the embryo proper has still a globular shape. However, the cell divisions of the inner cells start to be oriented along the apical-basal axis.

All inner cells adopt a common orientation of cell divisions, however, morphologically, the upper and lower tier are still rather homologous (**early globular stage**, middle).

During the next round of cell divisions, the cells of lower tier further divide in a radial pattern, leading thus to the formation of narrow cells in the center of the lower tier, the prospective vascular and surrounding ground tissue. At that moment, the morphology of upper and lower tier descendants differentiates, reflecting thus the forming apical-basal axis.

At the early heart (also called **triangular embryo**, right), the prospective vascular and surrounding ground tissue is clearly detectable.

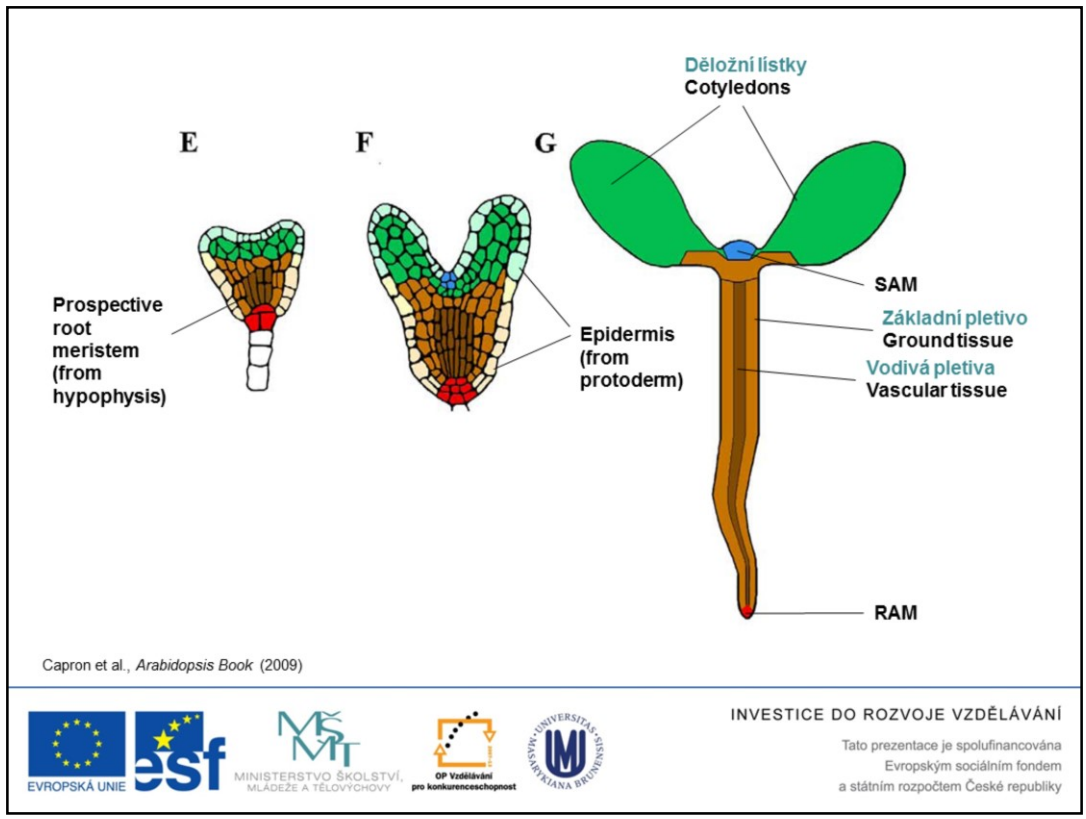


Heart stage **Topedo stage** **Bended cotyledon stage**

Capron et al., *Arabidopsis Book* (2009)

Further divisions of the LT descendants are strictly parallel (periclinal) or perpendicular (anticlinal) to the apical-basal axis, allowing thus increase of concentric cell layers or formation of continuous cell files, respectively.

This together with further refinement of the embryo shape leads to the **heart stage** (left), **torpedo stage** (middle) and **bended cotyledon stage** (right) formation.



Fate map of the developing embryo in *Arabidopsis*.

At the *heart stage*, apical basal axis is further pronounced, with different cell fate specification of the LT and UT descendants.

Protoderm develops into **epidermis** of both shoot and root.

The upper most cell of the suspensor**, hypophysis**, undergoes a series of cell divisions, leading thus to the **root apical meristem (RAM**) (in particular its organizational centre) formation.

Shoot apical meristem (SAM) differentiates somewhat later (heart stage) in the middle between the newly formed embryonic leaves, the cotyledons.

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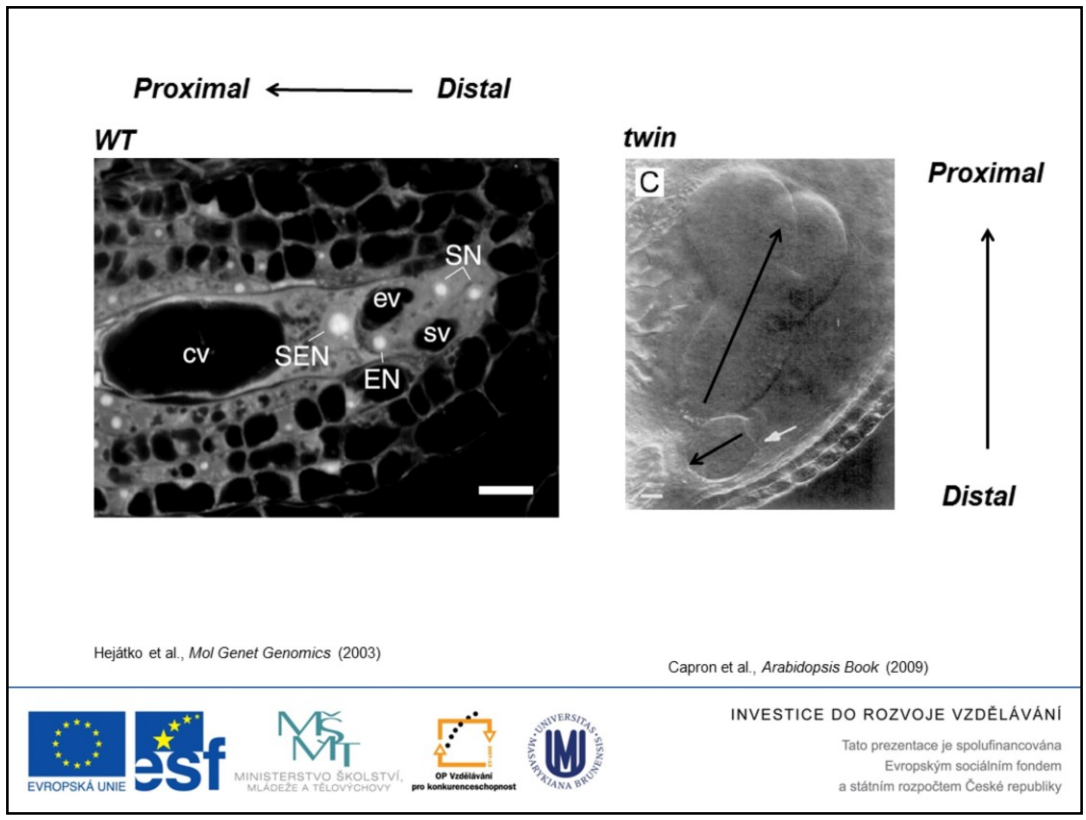
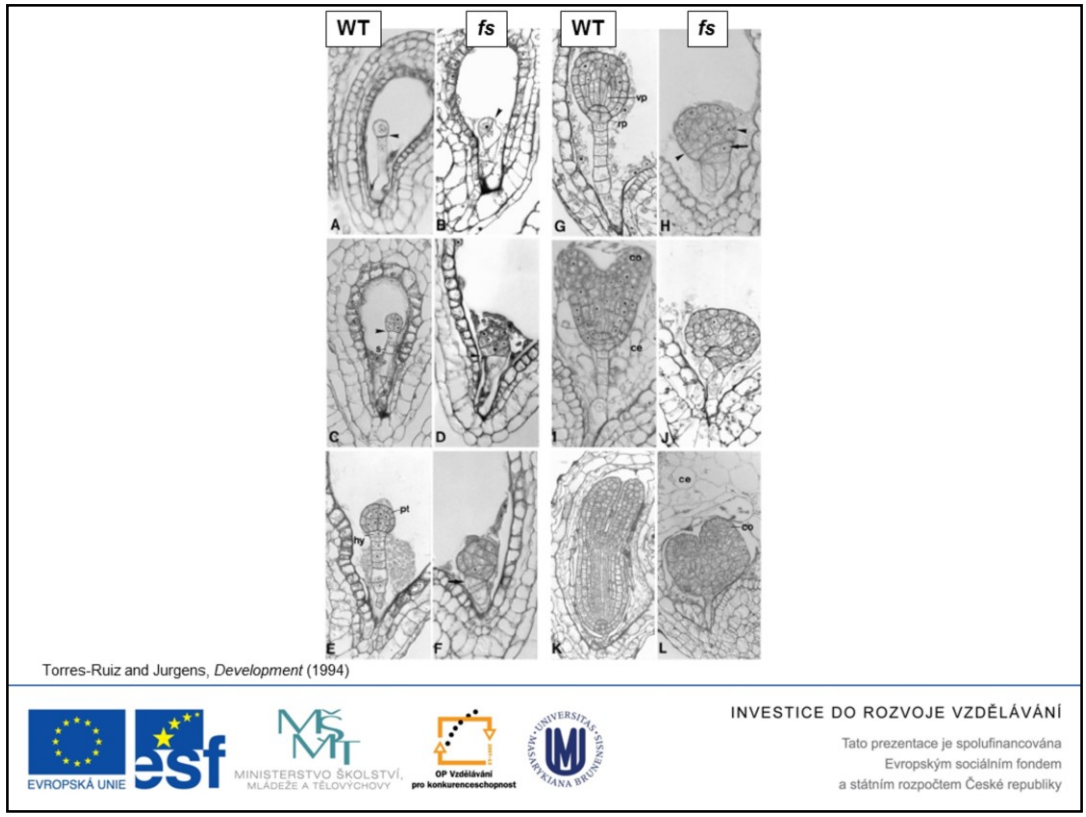


What are the factors affecting patterning and proper development of the plant embryo?

In contrast to animals, where the individual axis (anteroposterior and dorsoventral) are pre-specified in the female gametophyte, the maternal role seems to be reduced, if there's any, in the plant embryogenesis.

In spite of that the apical-basal axis corresponds to the proximodistal axis specified during female gametophyte development (see the Lesson 6 and the figure on the left), the apical-basal axis is established also during somatic embryogenesis that takes place outside the female gametophyte (see later).

Another evidence about the limited contribution of the maternal control is provided by the phenotype of *twin* mutants, which develop two embryos of opposite polarity. White arrow points at the basal end of a second embryo developing from a suspensor cell, the black arrow shows basal-to-apical direction in both embryos.

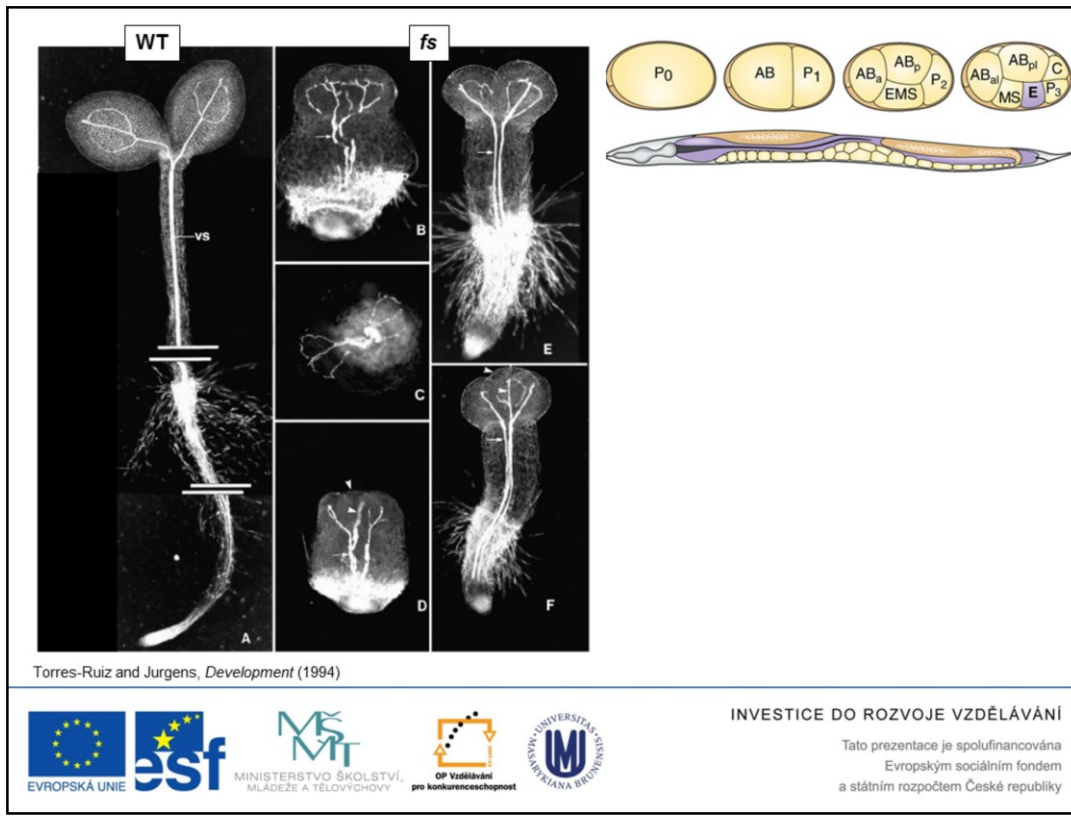


In plants, the **positional information** seems to be decisive for the **specification of the cell fate** during embryogenesis.

In *Arabidopsis*, the first cell divisions are invariant. However, there are species that are not revealing any invariant cell division during embryogenesis, e.g. cotton.

Thus, another information must be involved in the specification of the cell pattern during plant embryogenesis. Very probably, the **positional clues** have dominant role in the specification of the major elements of the body pattern differentiation.

This could be show in e.g. *fass (fs)* mutants affected in the cell division pattern. *fass Arabidopsis* embryos have defective pattern of cell divisions.



fass mutants are strongly retarded in their growth. However, in spite of that, the overall structure of *fass* seedling is maintained.

Recessive mutations in the *FASS* are affected in the cell division pattern. However, in spite of no primordia of the main seedling structures can be recognized in the *fass* embryos at the early-heart stage, all elements of the body pattern are differentiated in the seedling.

Adult *fass* plants are tiny, with strongly compressed apical-basal axis.

At the cellular level, *fass* mutations affect cell elongation and orientation of cell walls. However, in *fass* mutants, the cell polarity is retained, as evidenced by the unequal division of the zygote.

The results suggest that the *FASS* gene is required for morphogenesis, i.e. oriented cell divisions and position-dependent cell shape changes generating body shape, but not for cell polarity, which seems to be essential for pattern formation (Torres-Ruiz and Jurgens, 1994).

In the figure, there are whole-mount preparations of wild-type and *fs* seedlings.

(A) Wild-type with a simply reticularised vascular system in the cotyledons. The vascular strands run tightly together through the center of the hypocotyl into the root. The upper end of the root is marked by root hairs and a more intense birefringence, which is also seen in the apical and especially the basal end (root meristem zone).

The wild-type is not shown in full length (interruptions indicated).

(B-F) *fs* seedlings (same stage and same magnification as the wild type). The main features of the wild type are also visible in *fs* seedlings (note the epidermal layer in the cotyledons). Vascular strands are clearly separated from each other in the hypocotyl and the root (small arrows).

(B-D) Strong *fs* phenotype. Dicotyledoneous seedlings in B (side view) and C (top view): vascular system is sometimes interrupted. (D) *fs* seedling with three cotyledons: arrowheads point to the supernumerary vascular strand and cotyledon respectively.

(E,F) Weak *fs* phenotypes: compare with B and D. Dark-field optics.

FASS (allelic to *TONNEAU2*) was found to be important for the cytoskeleton-dependent positioning of the cell plate during cytokinesis.

However, the role of cell lineage specification (as discussed e.g. in case of *Coenorhabditis*, see the inset on the right) can not be completely excluded in plants, particularly in the later developmental events.

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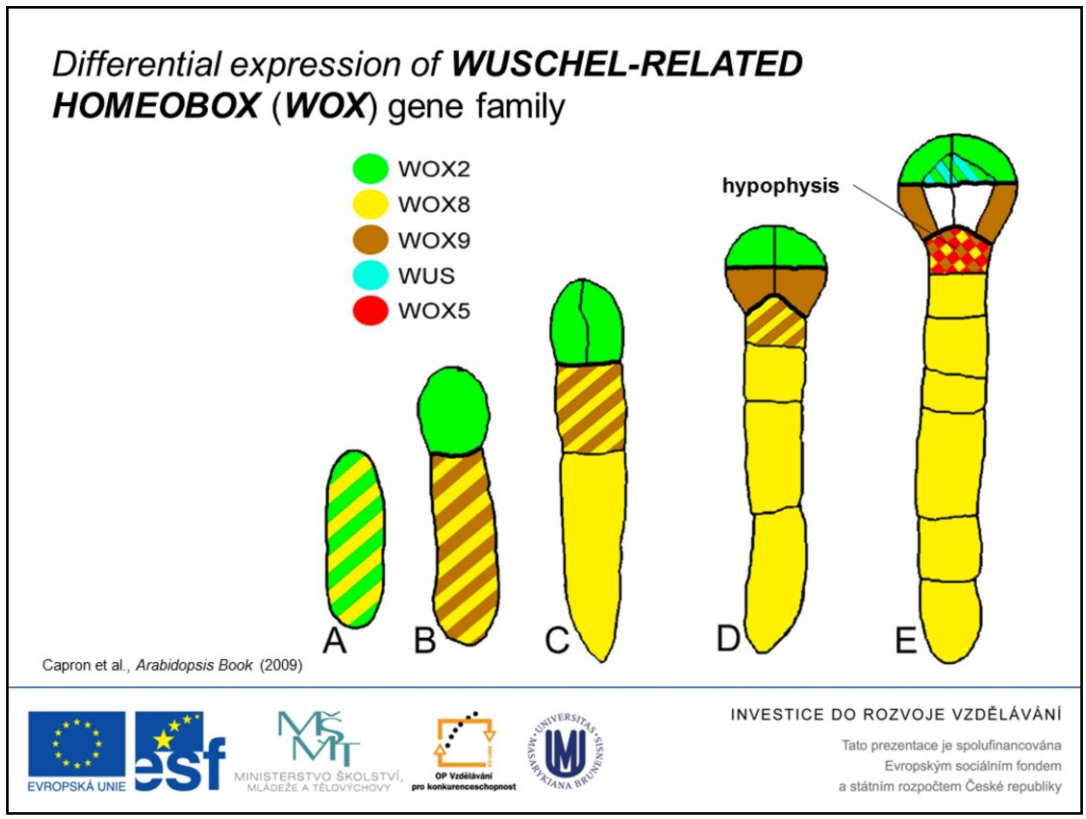


Thus, if that is not the invariant cell division, **what are the factors responsible for proper embryo patterning?**

In terms of the apical-basal axis, two important regulators were found.

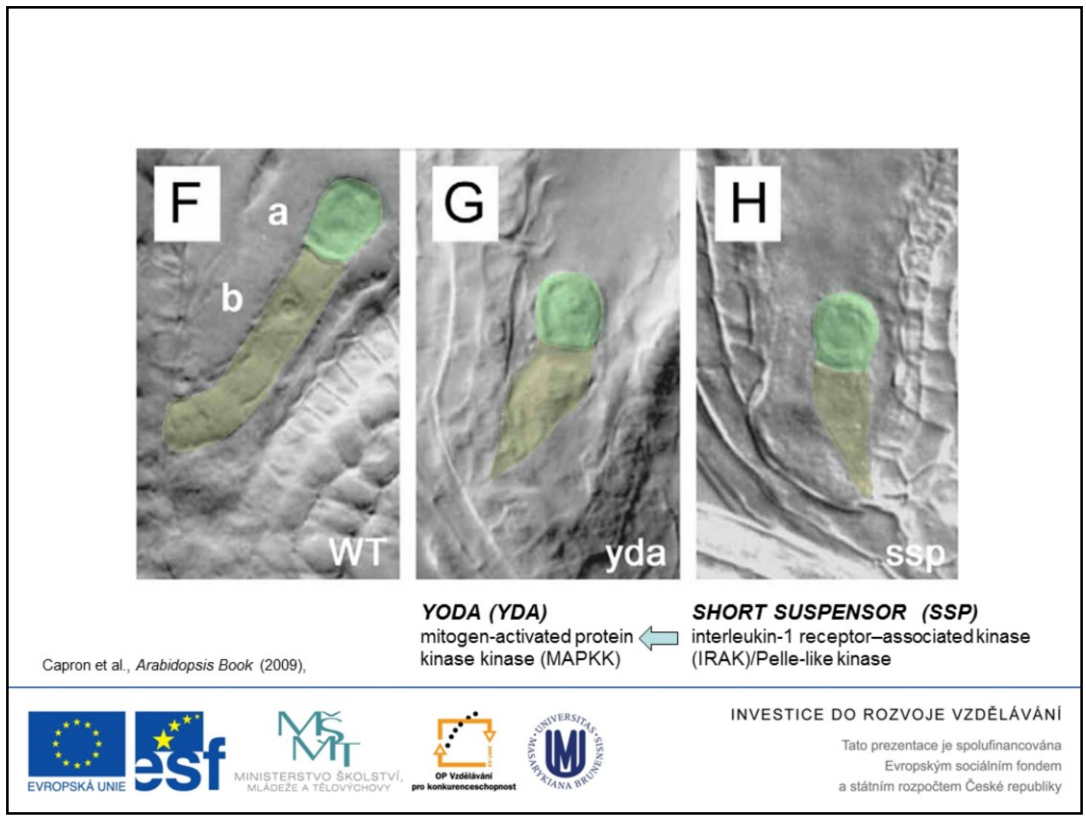
First, it is the **spatiotemporal specific gene expression** along the apical-basal axis.

Second, **distribution of plant hormone auxin** seems to be very important for the initial establishing of the apical-basal axis.

WUSCHEL-RELATED HOMEOBOX (***WOX***) gene family was shown to be differentially expressed during early embryogenesis (on the topic of homeobox genes, see the lecture of prof. Vyskot in frame of his course "Bi0580 Developmental genetics".

While *WOX2* is expressed in the apical cell, WOX8 and WOX9 expression is specific for the basal cell after the first asymmetrical zygote division.

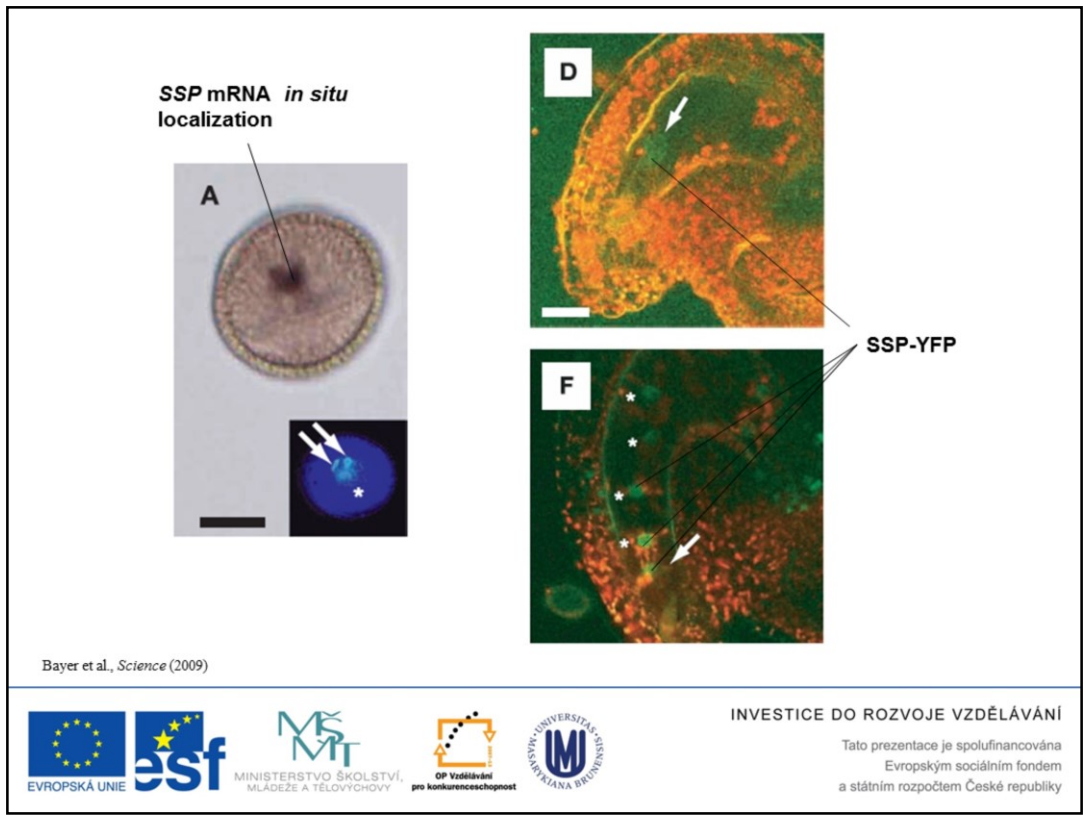
After further cell divisions, expression of *WOX2* occurs in the progeny of UT, while *WOX8* expression marks suspensor and hypophysis and *WOX9* is expressed in the outer layer of LT progeny and the hypophysis, where the activity of *WOX5* is also detectable (see above).



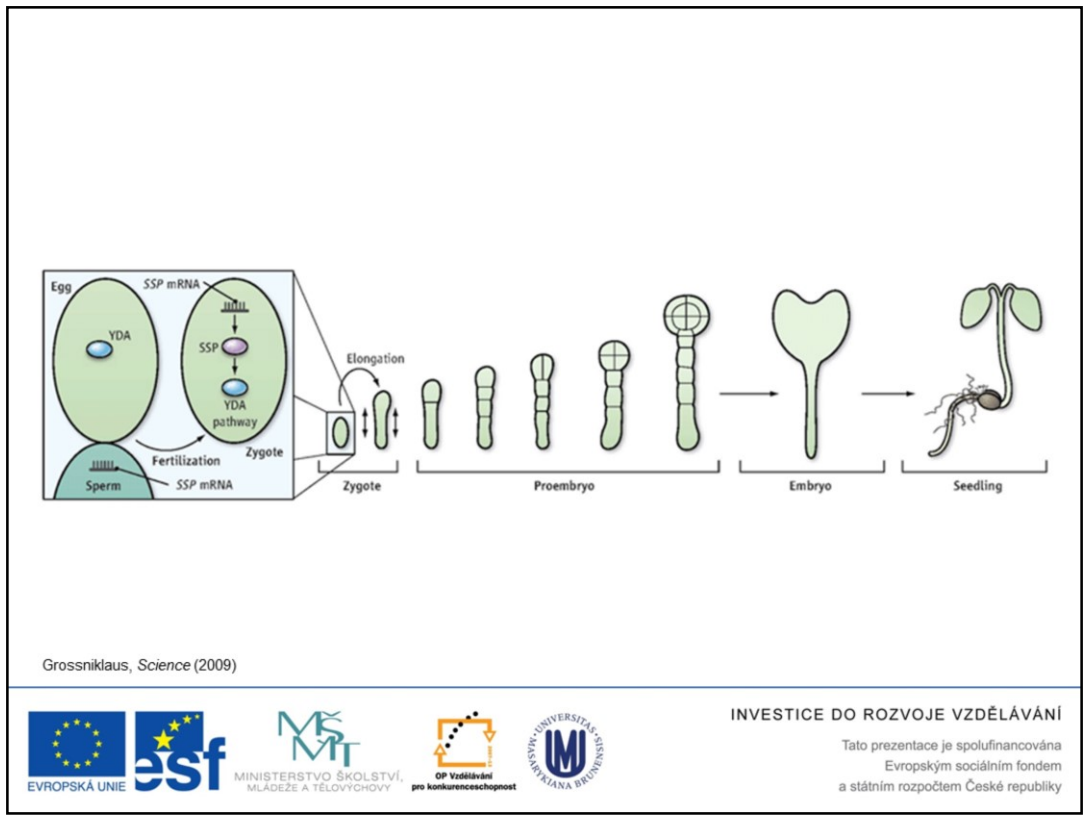
Another gene involved in the differentiation of apical and basal cell is e.g. ***SHORT SUSPENSOR (SSP)*** that regulates the activity of ***YODA (YDA***).

Both of these genes are necessary for the zygote elongation and first asymmetric division, that allows formation of basal cell of the proper size.

SSP encodes interleukin-1 receptor–associated kinase (IRAK)/Pelle-like kinase that regulates another kinase from the mitogen-activated protein kinase kinase (MAPKK) family, YDA.



Interestingly, *SSP* transcript accumulates in the pollen grain, in the sperm cells (the arrows in the figure A depict the DAPI stained nuclei of sperm cells) and is transferred to the zygote, where the translation is activated (see the green signal of SSH-YFP construct, paternally transmitted to the developing zygote and endosperm 24 hours after pollination (HAP), arrows in D and F; asterisk depict the micropylar endosperm with the YFP signal).

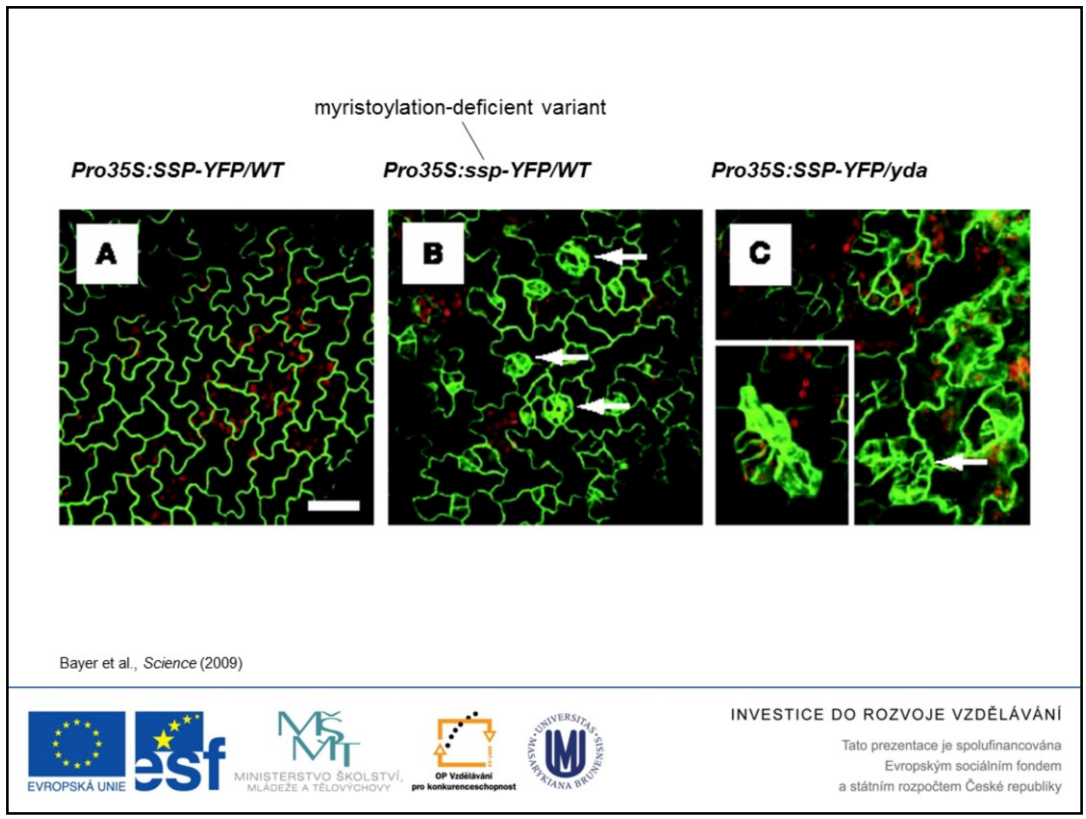


Thus, this mechanism very probably reflects **parent-of-origin effect**.

SSP mRNA is delivered to the egg cell via sperm cells and is translated there. SSP subsequently activates YDA MAP kinase pathway, leading to proper zygote elongation.

The paternal contribution of SSP that is important for the nourishing function of the suspensor via direct delivery of *SSH* transcripts probably partially compensates the maternal-negative regulation via imprinting of paternal genes.

More about the genetics of parent-of-origin regulations in the lecture of prof. Vyskot in frame of his course “Bi0580 Developmental genetics”.

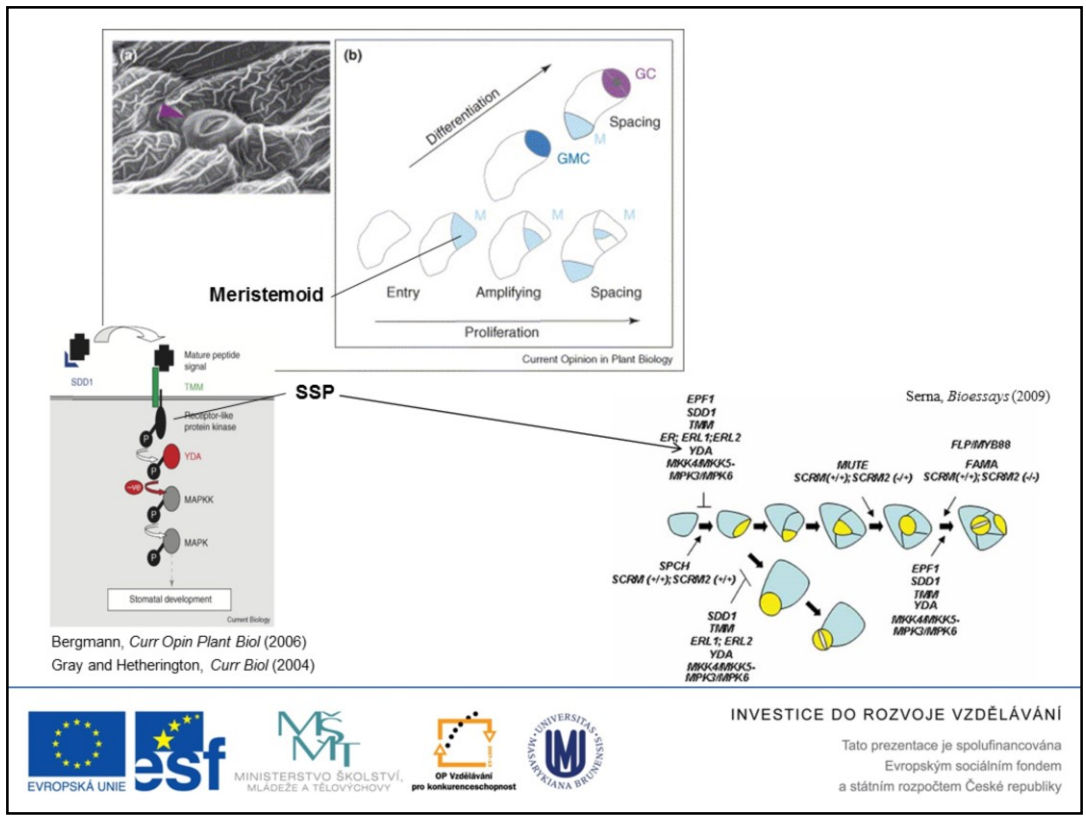


Interestingly, YDA controls also differentiation of guard cells in the leaf stomata.

Ectopic overexpression of functional *SSP* in a translational fusion with yellow fluorescent protein (YFP) leads to the absence of stomata formation, suggesting interference of SSP-mediated activation of YPD MAP kinase pathway with stomata formation.

In the figure B, there is a result of the overexpression of *ssp* myristoylation-deficient variant, in the figure C, there is overexpresion of SSP-YFP in the *yda* mutant.

In the Inset of figure C, there is apparent cluster of stomata progenitor cells, as typical for *yda*. These data confirm that functional YDA is necessary for the inhibition of stomata formation via *SSP* overexpression.



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In the process of stomata differentiation, the epidermal leaf cell asymmetrically divides, leading into formation of small cell called *meristemoid* (entry). The meristemoid cell might differentiate into *guard mother cell (GMC)* or further undergoes asymmetrical divisions, resulting into amplification of epidermal cells of the leaf.

Spacing divisions of the epidermal cells leading to formation of novel meristemoid are similar to entry divisions, but are oriented, leaving thus the large doughter cell (epidermal) between the future stoma and the new meristemoid.

YDA acts there together with he activation of the YDA MAP kinase pathway

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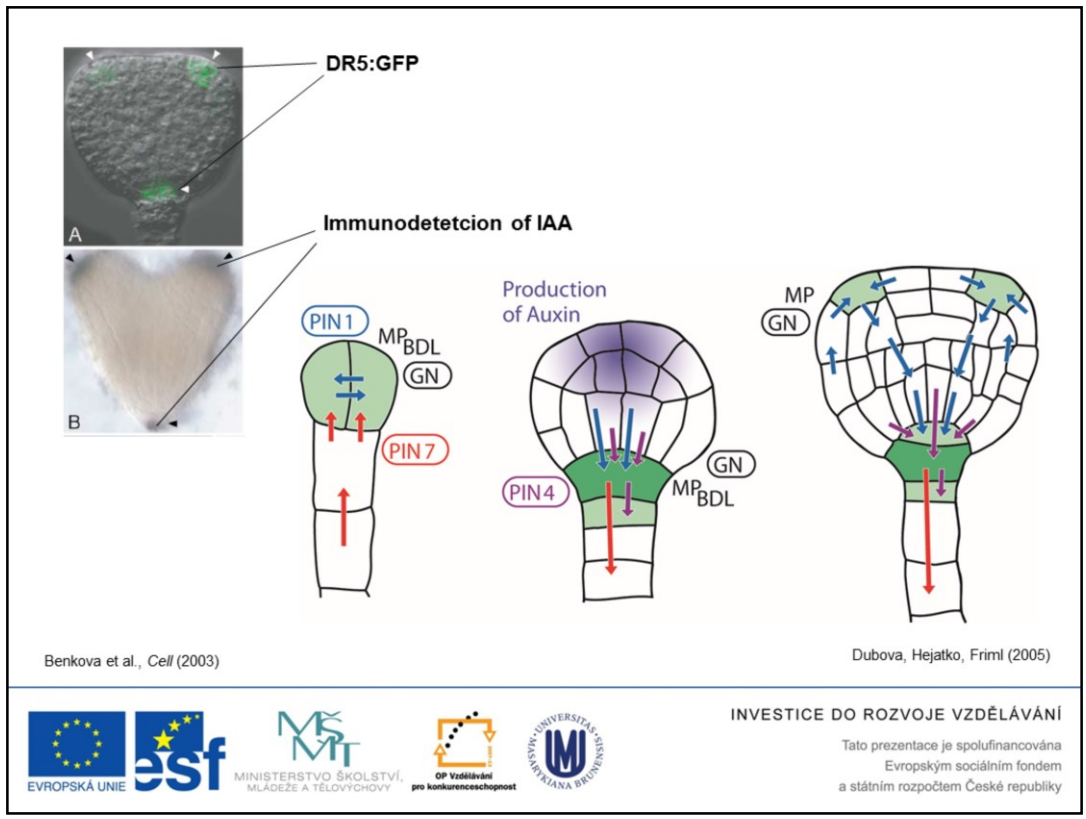


Besides the differential gene expression, another important mechanisms affecting formation of the apical-basal axis is the **auxin accumulation**.

Auxin is one of the important plant hormones (phytohormones) with large morphogenic and organogenic effects, as will be discussed later.

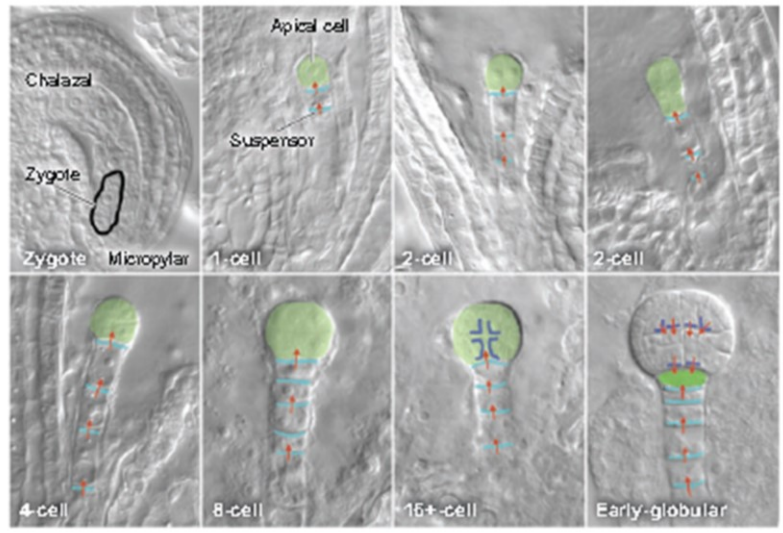
The main breakthrough in the understanding of the role of auxin in embryo patterning was the discovery of dynamic **auxin gradients** during embryogenesis.

Cellular distribution of auxin and its response was indirectly visualized using anti-IAA antibodies and an auxin-responsive reporter ***DR5::GFP*** (green signal in the figure above).

Immediately after the division of the zygote, auxin accumulates in the apical cell, which is being specified (left). During subsequent development, the maximum of auxin activity persists in the proembryo.

Approximately at the 32 cell stage, when the basal embryo pole is being specified, the gradient of auxin accumulation suddenly reverses and forms a new maximum in the uppermost suspensor cells, including the hypophysis (middle).

At later stages of embryogenesis (late globular stage), additional auxin accumulation foci appear in the tips of the developing cotyledons (right).



Bowman et al., *Annu. Rev. Plant. Biol* (2008)

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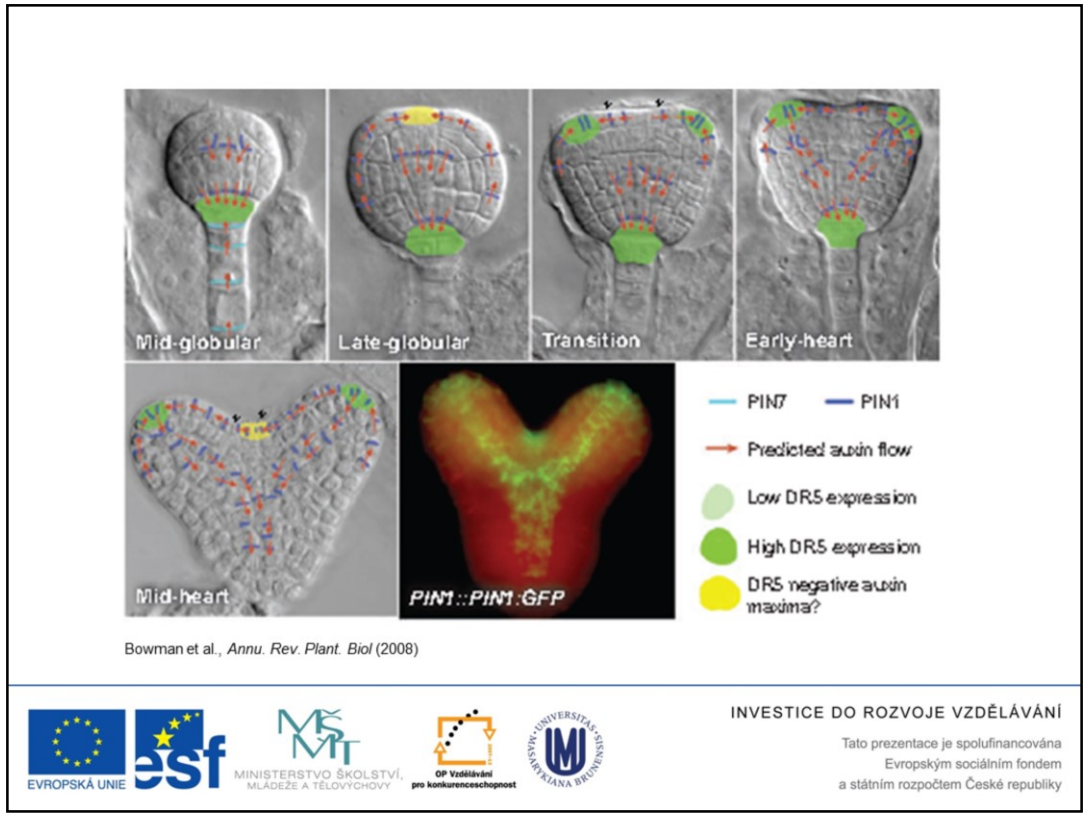
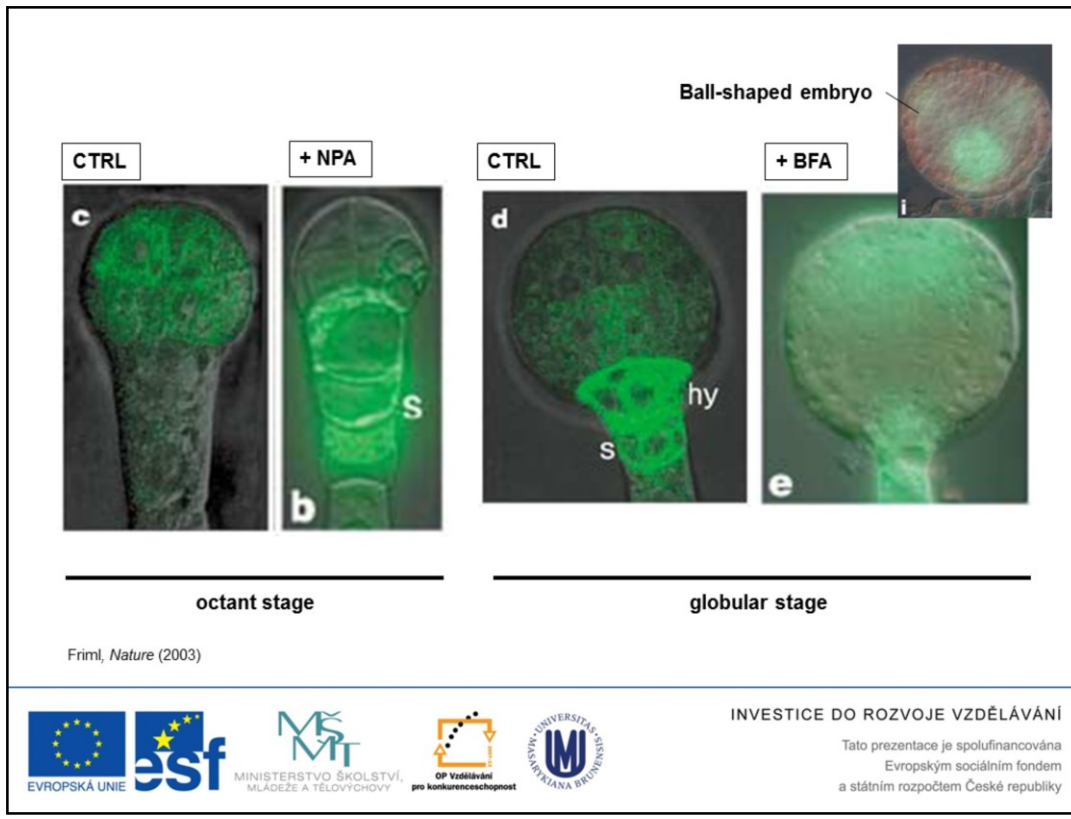
The figures here show the auxin maxima formation during embryogenesis in *Arabidopsis*, as described in the previous slide.



One of the important group of the auxin transporters are so called auxin-efflux carriers from the **PINFORMED (PIN)** family.

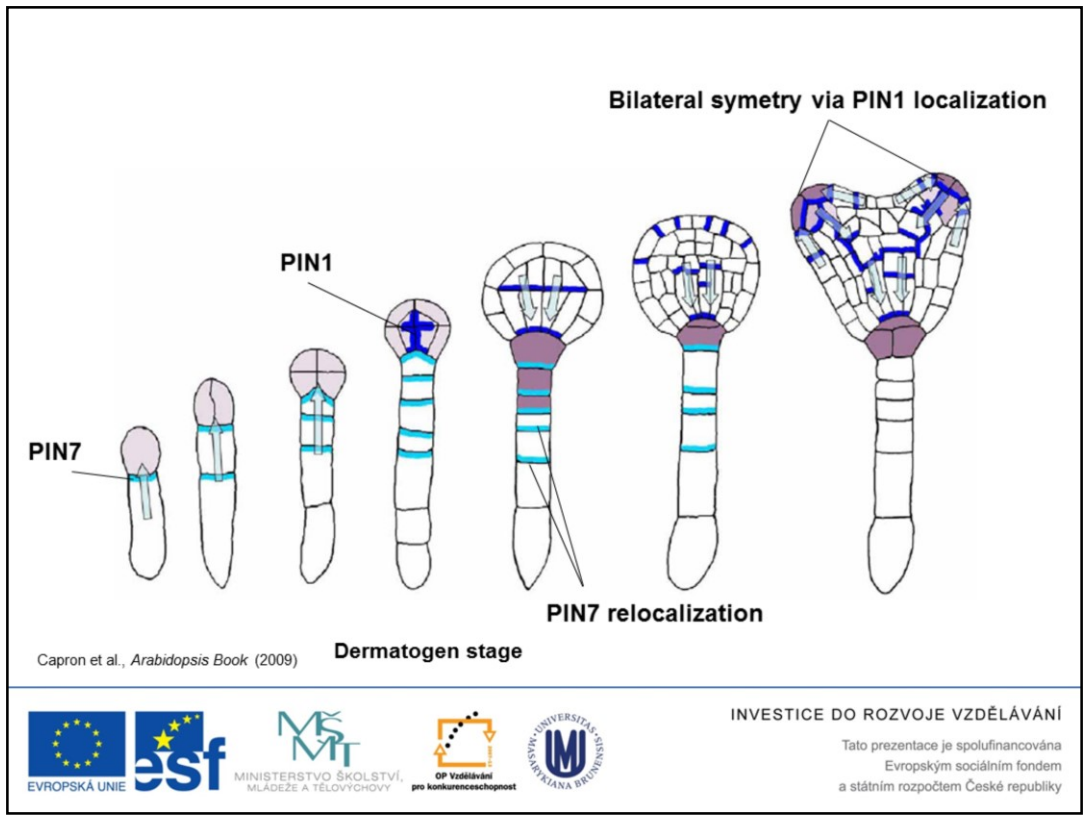
There are eight members of the family and their orchestrated action is important for patterning not only embryos but multiple other plant organs via meristem growth regulation. PIN proteins and importance of polar auxin transport will be in more detail discussed later and is a topic of the lectures of prof. Friml in frame of his advanced course “Bi8930 Developmental and cellular biology of plants”.

It has been found that expression of several *PIN* genes and localization of PIN proteins drives auxin accumulation in specific compartments and cells in the early embryo development.



Manipulation of auxin flux via chemical treatment with auxin transport inhibitors (e.g. 1-naphthylphthalamic acid [NPA], see above) lead to the defects in the auxin accumulation and embryo patterning.

The abnormalities in auxin distribution, which were always accompanied by embryo defects, ranged from cup-shaped embryos with misspecified apical structures and a nonfunctional root pole, to ball-shaped embryos without any discernible apical–basal axis (see the figure, inset).



PIN1 and PIN7 are major players in the process of auxin maxima formation during embryogenesis.

Initially, PIN7 locates at the apical membranes of the suspensor cells and pumps auxin towards apical pole.

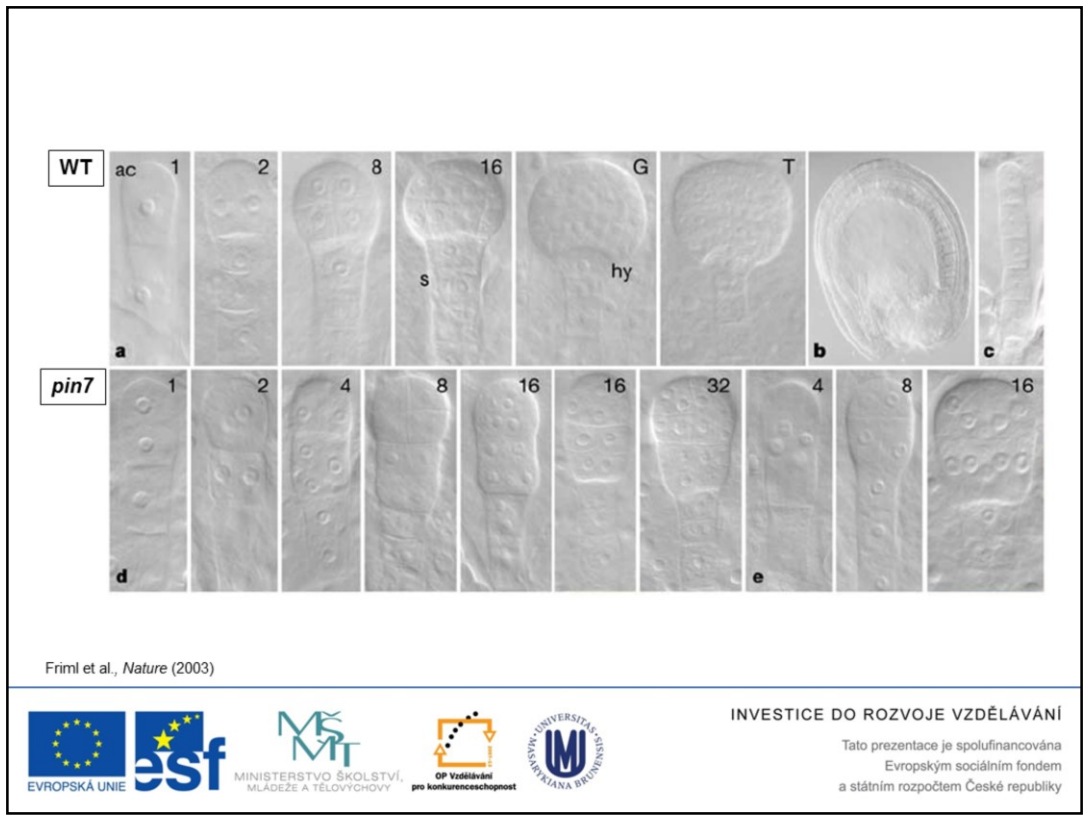
In the dermatogen stage (protoderm formation is apparent, see the figure), PIN1 is expressed in the embryo proper and locates predominantly at the basal membranes. That leads to the switch in the auxin flux and the auxin is preferentially transported rootwards and auxin maximum forms in the hypophysis.

Concurrently, PIN7 relocalizes from the apical to the basal membrane.

Later on, PIN1 localization in the embryo proper allows bilateral organization of the embryo with two auxin maxima in the position of cotyledons formation.

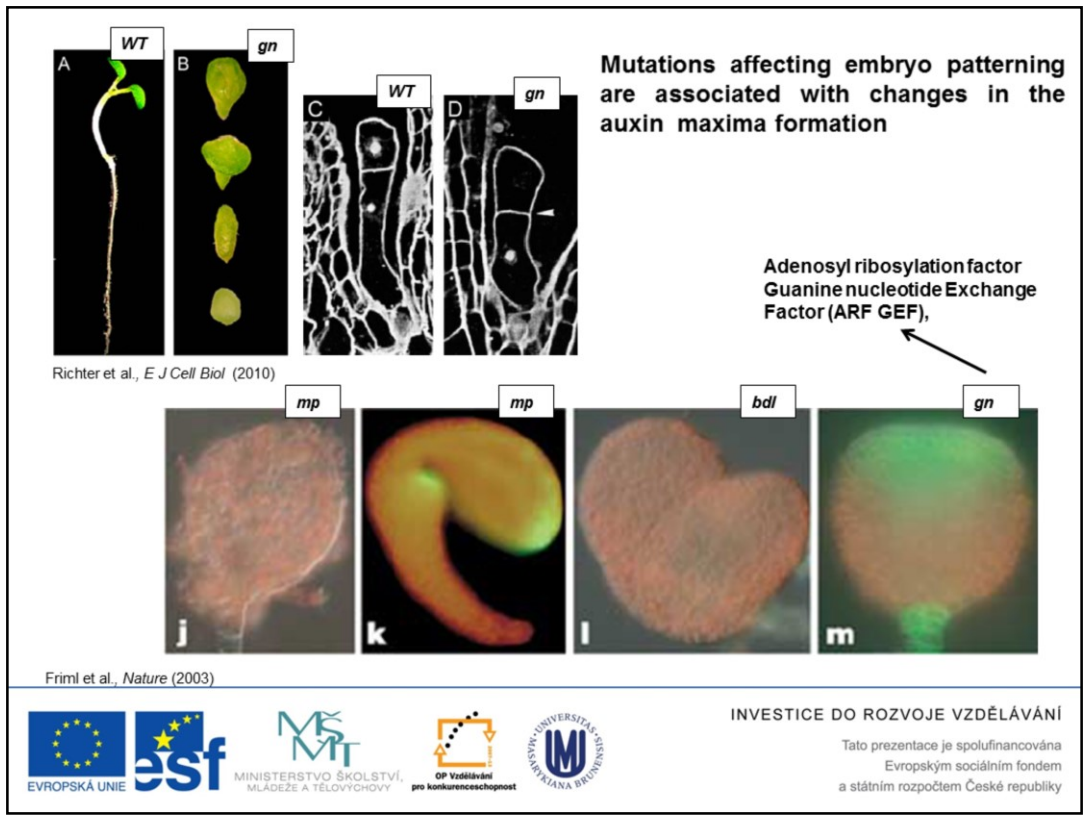
The PIN-mediated auxin flux thus allows not only the initial apical-basal axis formation, but it is also involved in the patterning of the *basal domain*.

As discussed previously, the progeny of LT divides predominantly in parallel to the apical-basal axis, leading thus to the *provascular* (prospective vascular) *tissue* formation. The auxin flux has been shown to be important for the future connectivity of the vascular tissue.



Friml et al., *Nature* (2003)

Mutations in the *PIN* genes encoding the efflux transporters (here, *pin7* mutants) change the developmental pattern of plant embryos.

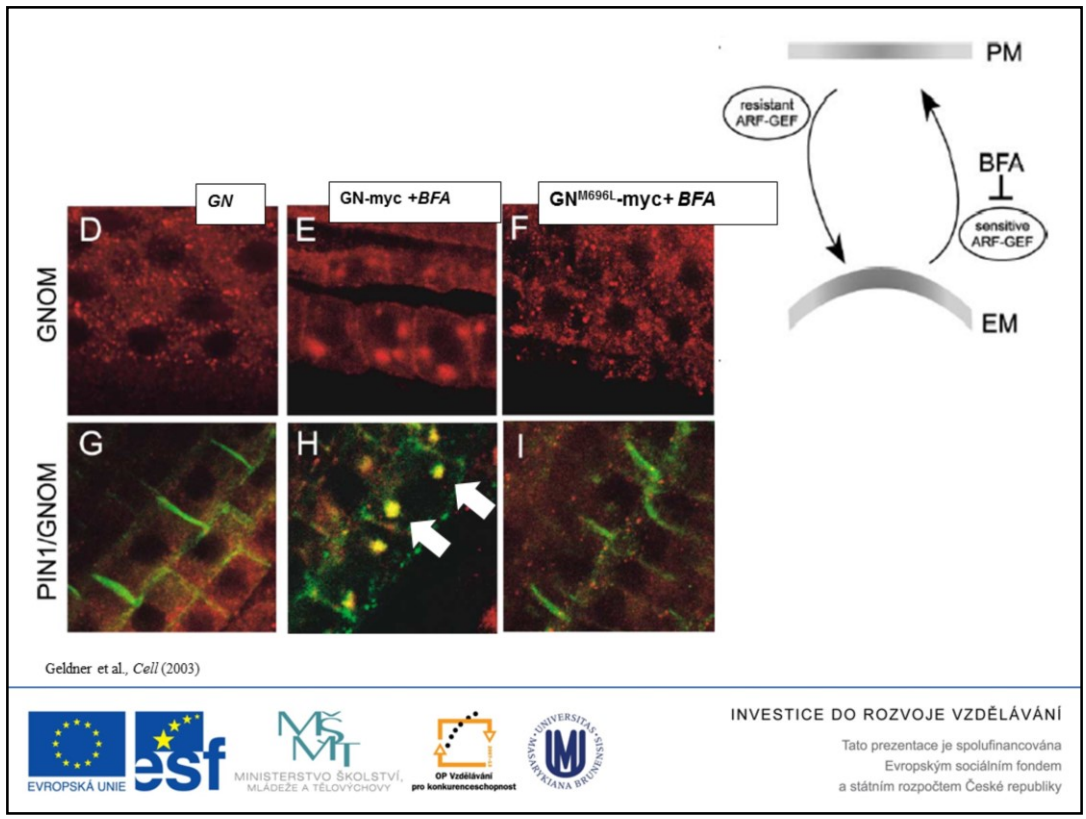


There are plenty of factors regulating polar localization of PIN proteins and thus the auxin flux. In the early embryogenesis, these are e.g. *WOX* genes, discussed before.

EMBRYO DEFECTIVE30/GNOM (EMB30/GN) encodes an Adenosyl ribosylation factor Guanine nucleotide Exchange Factor (ARF GEF), which functions as an endosomal regulator of vesicle budding, part of one of the very important developmental process called vesicle trafficking (see next slides). Importance of vesicle trafficking was already discussed in case of pollen tube growth.

Vesicle transport is critical fro proper PIN localization and thus auxin gradient formation. This topic will be discussed in more detail in lectures of prof. Friml in frame of his advanced course “Bi8930 Developmental and cellular biology of plants”.

Loss of *EMB30/GN* function may produce ball-shaped seedlings with fully differentiated, but randomly oriented, vascular cells at the center, indicative of a selective loss of apical-basal polarity (Mayer et al., 1993), a phenotype that can be mimicked by application of high concentrations of auxin transport inhibitors.



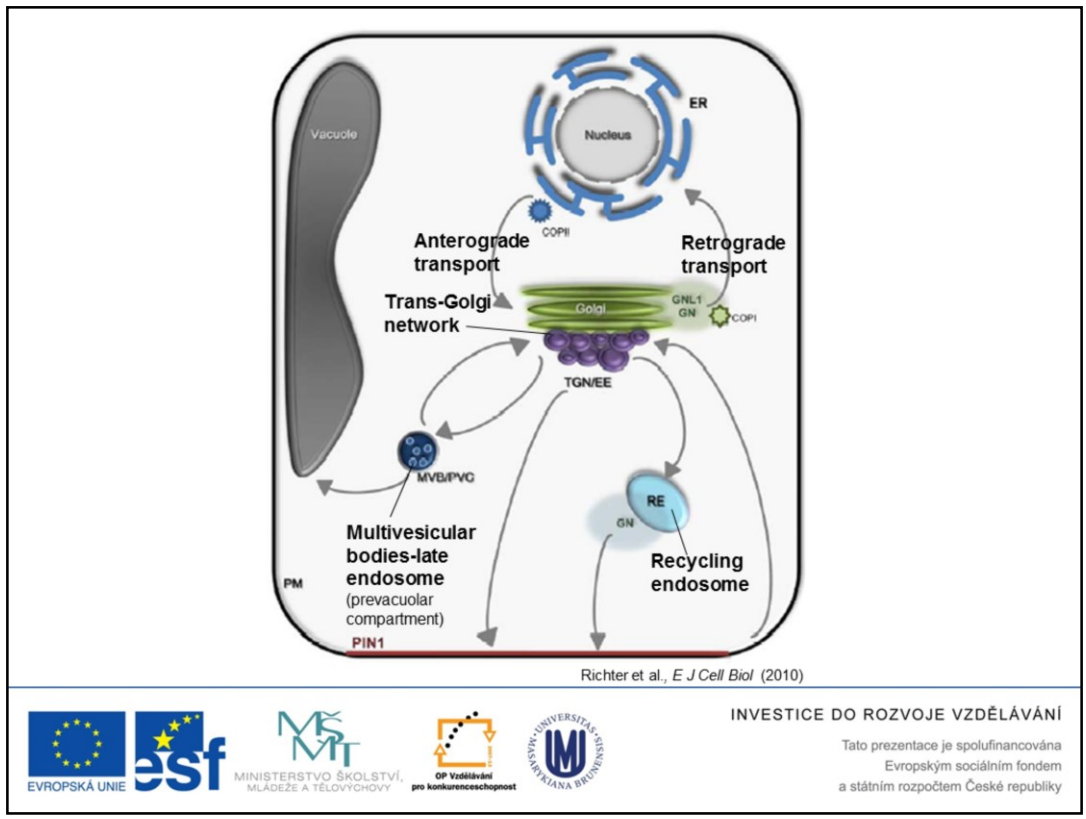
GNOM encodes an exchange factor for ARF GTPase (ARF-GEFs) and regulates vesicular trafficking.

Proteins cycle between endomembrane compartments (EM) and the plasma membrane via a transport mechanisms mediated by small membraneous structures, a process called **vesicle trafficking**.

ARF-GEF GNOM was shown to be important for the vesicle trafficking mediated cycling of PIN proteins.

The exocytosis is sensitive to a lactone antibiotic brefeldin A (BFA). Treatment of cells with BFA leads to the accumulation of PIN1 and associated GN in the endosomes (arrows, see the figure E, H). When the BFA-insensitive mutant was engineered, it allowed BFA insensitivity of the PIN1 cycling (figure F, I).

The cycling of PIN1 protein probably is thought to allow fast changes in the polar localization of PIN1 in a response to diverse stimuli.



In the figure, there is simplified scheme of vesicle trafficking pathways, regulated by GNOM and its closest relative, GNOM-LIKE1 (GNL1).

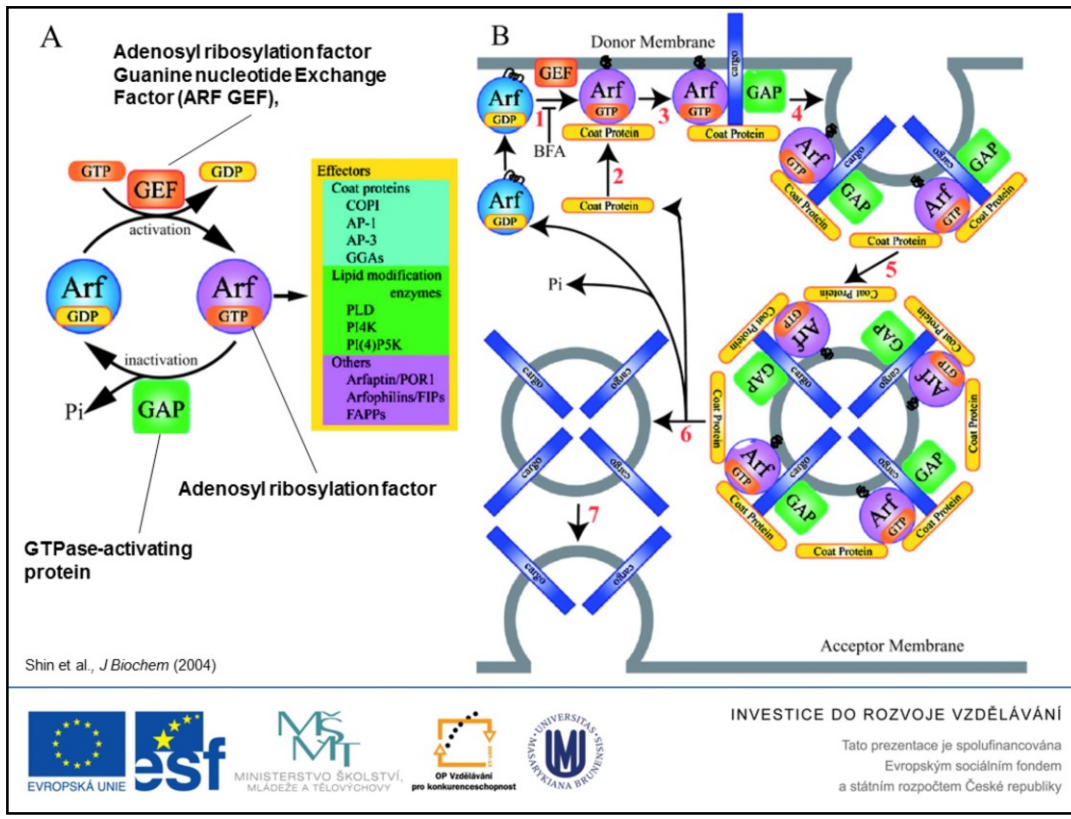
Secretory and membrane proteins are synthesised at the ER (blue) and passed onto the Golgi apparatus (green) by anterograde trafficking in COPII-coated vesicles.

The retrograde route from the Golgi apparatus to the ER is regulated by the ARF-GEFs GNOM (GN) and GNL1, which regulate the recruitment of COPI coats to the Golgi membrane. On the secretory route, proteins are transported to the sorting station, the trans-Golgi network (TGN; lilac).

From there, proteins are either transported to the vacuole (grey) via multivesicular bodies (MVB, also called prevacuolar compartment, PVC, which corresponds to the late endosome; deep blue) or trafficked to the plasma membrane (PM).

Plasma membrane proteins like the auxin efflux carrier PIN1 (red), which accumulates at the basal PM at steady state, are continually internalised and trafficked to the TGN, which resembles the early endosome (EE) in plants.

From the TGN, PIN1 is recycled to the plasma membrane via the recycling endosome (RE; light blue). This pathway is regulated by the ARF-GEF GNOM.



GDP-GTP cycle of Arf and its function in coated vesicle formation.

(A) General regulation of Arf activation and inactivation.

A GDPbound, inactive form of Arf is converted to a GTP-bound, active form through GDP-GTP exchange catalyzed by a GEF. The GTP-bound Arf is able to interact with a wide variety of effectors, such as coat proteins and lipid kinases. The GTP molecule bound to Arf is then hydrolyzed to GDP with the aid of a GAP.

(B) Regulation of coated vesicle formation by Arf.

(1) Through guanine nucleotide exchange catalyzed by a GEF on a donor membrane, a GDP-bound form of Arf in the cytosol or loosely associated with the membrane is converted to a GTP-bound form, which becomes tightly associated with the membrane. The exchange reaction is blocked by BFA.

(2) Arf·GTP then recruits coat proteins from the cytosol.

(3) Arf and the coat together trap transmembrane cargo at the donor membrane. Recognition of a sorting signal within the cytoplasmic domain of the cargo by the coat is critical for this step. A GAP is also recruited to the membrane, but its activity might be inhibited due to the cargo.

(4) The GAP inhibition might shift the equilibrium to drive coat polymerization, and in consequence, the membrane patch deforms into a bud.

(5) The vesicle is then pinched off from the donor membrane.

(6) As the membrane curves, the GAP activity might increase, leading to GTP hydrolysis and dissociation of the resulting Arf•GDP, the coat and the GAP itself.

(7) The uncoated vesicle is now competent to fuse with an appropriate acceptor membrane.

Outline of Lesson 7

Plant Embryogenesis

- Overview of the embryo formation in *Arabidopsis*
- Mechanism of the apical-basal axis formation
  - female gametophyte prespecification, invariant cell division or positional information?
  - differential gene expression
  - auxin gradients formation
  - the role of auxin signalling

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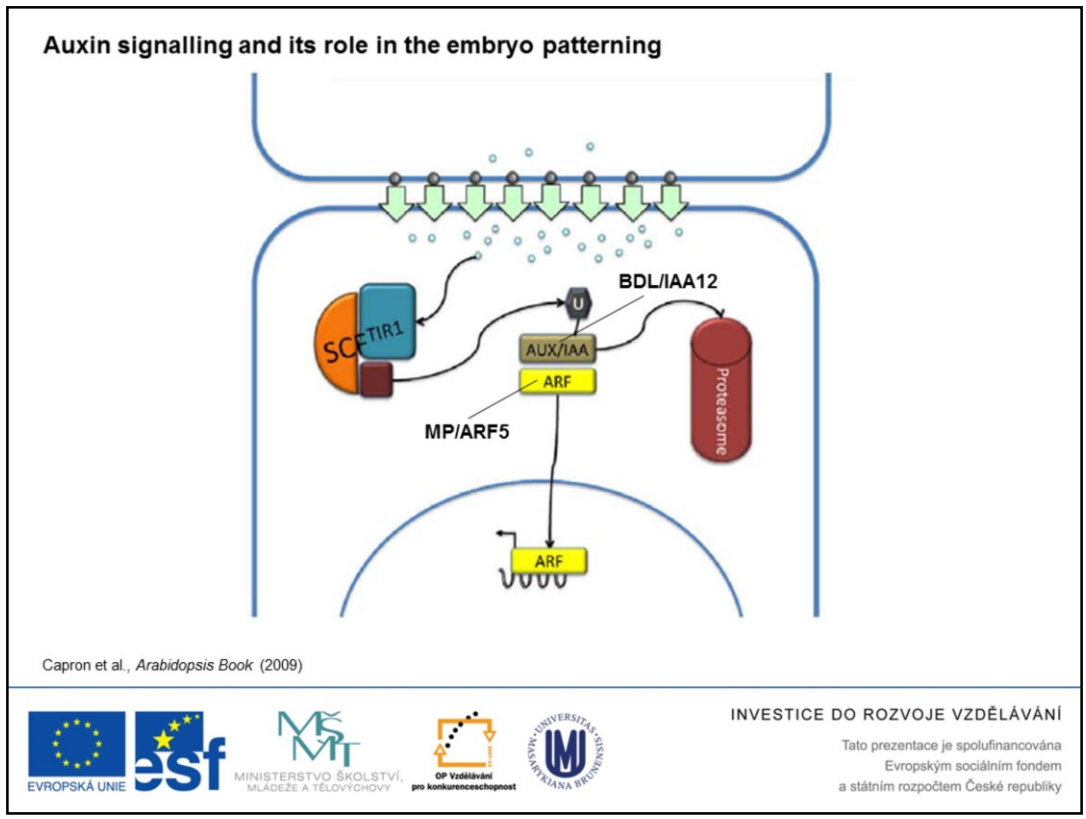
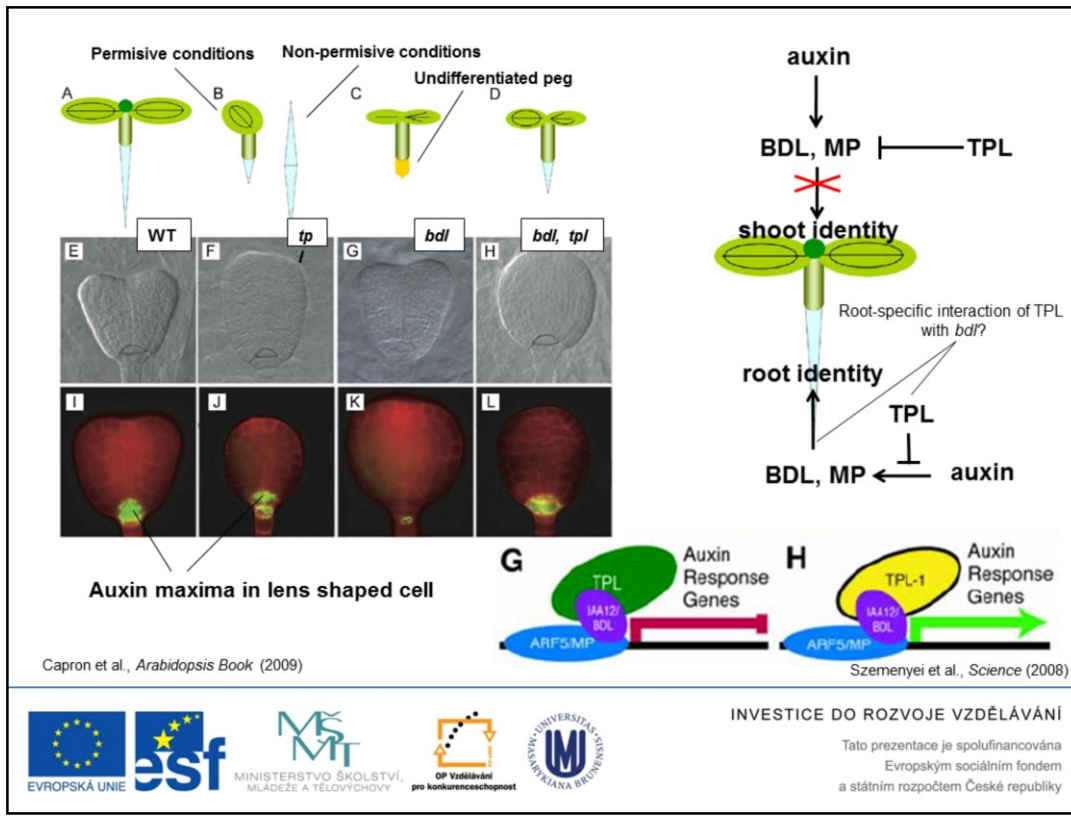


Scheme of the auxin signaling pathway.

Under low intracellular auxin concentrations, the transcription activators of auxin-regulated genes, which are called auxin responsive factors (ARFs), are in a complex with negative regulators of transcription, so called AUX/|IAA proteins. In the complex, Arfs can not activate transcription.

After auxin is imported into the cell, it binds to the TIR1 protein, that allows interaction with AUX/IAA-ARF complex and targets AUX/IAA protein for the degradation via proteasome.

That allows ARFs to enter nucleus and activate transcription of auxin-induced genes.



Accordingly to the previously described role of auxin in the apical-basal axis formation, mutations in the genes involved in the auxin signalling, e.g. *BODENLOS (BDL)* or *MONOPTEROS (MP)*, reveal severe defects in the embryo and seedling patterning.

In figure B, there is the temperature sensitive *topless-1 (tpl-1)* mutant, showing weaker apical defects at permissive temperatures on the left. On the right, at non-permissive temperatures, a complete homeotic transformation of the shoot into a root takes place.

bodenlos embryos lacks the basal (root) pole, and only undifferentiated peg is formed instead of the root (figure C). *bdl* is carrying a gain-of-function mutation, leading to the inability of auxin to target BDL for degradation and thus to release MP (see previous slide).

Both *WT* and *tp* embryos, reveal auxin accumulation in the what is called *lens shaped ce*ll, the derivative of the asymmetric division of the hypophysis (figures I, J). Lens-shaped cell will develop into quiescent centre, the organisational centre of the root meristem (see further).

However, the *bdl* embryos lack the lens-shaped cell and only remnants of the auxin maxima are present (figure K).

TPL protein interacts with BDL and acts to inhibit auxin-induced gene expression.

Auxin-induced gene expression is necessary for the root pole formation. Thus, in WT, TPL allows inhibition of acquiring root identity in the apical portion of the embryo.

In *bdl* background, this TPL-mediated inhibition, however, blocks the auxin-mediated gene expression also in the basal pole, leading thus to the absence of the root structures.

If *tpl* mutation is introduced into the *bdl* mutant, the root formation is rescued (right).

Thus, complex gene interactions regulate the auxin maxima formation (here depicted as a green signal in the bottom row of figures) and plant embryo patterning.

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  - the role of auxin signalling
- Root meristem formation
  - auxin and hypophysis identity

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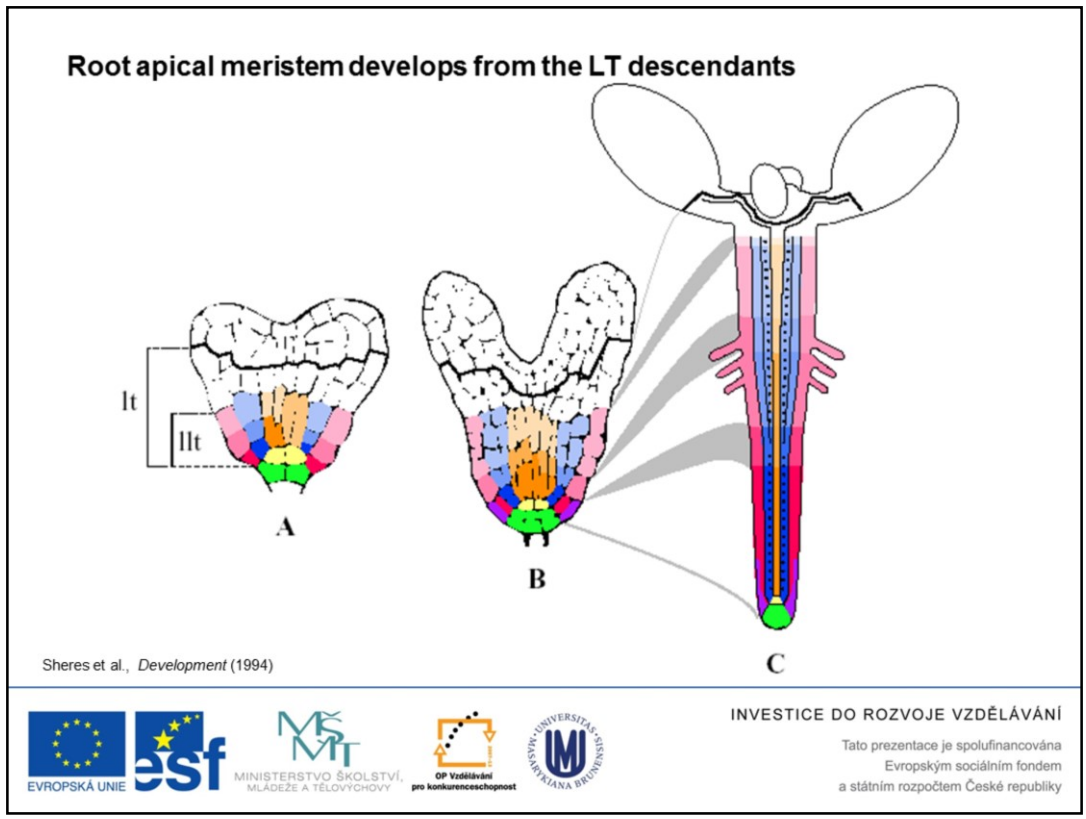
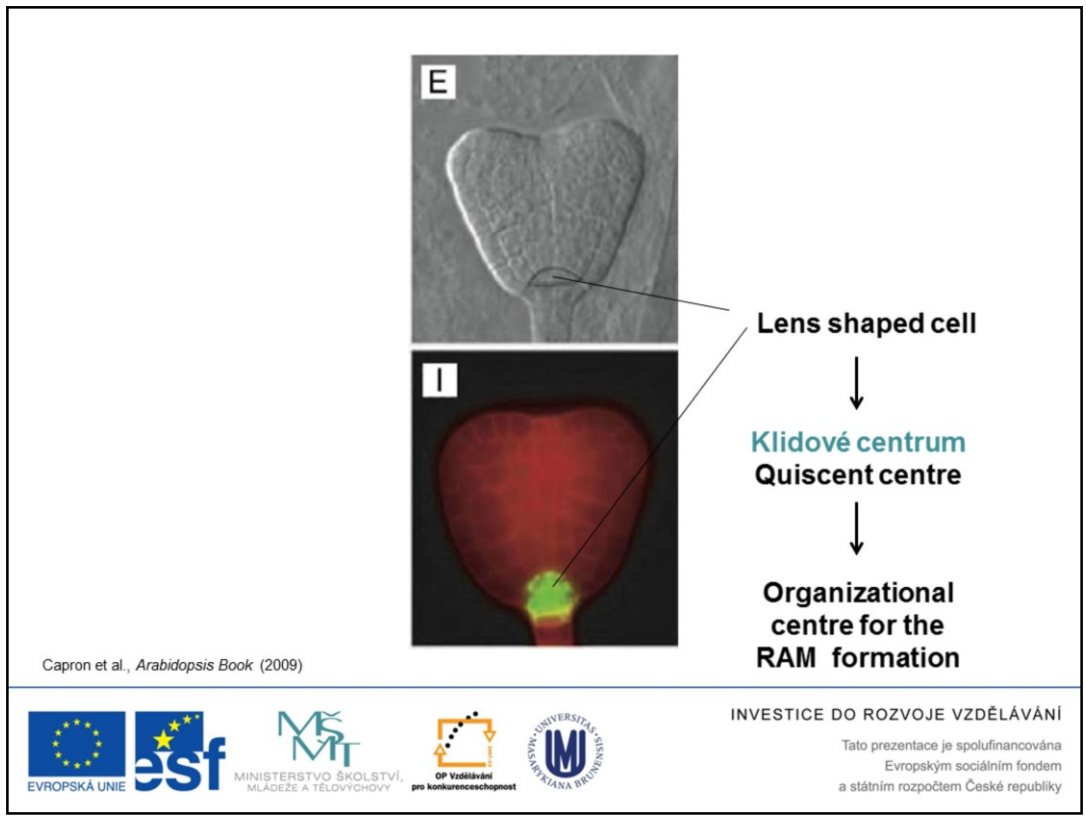


Another important aspect of embryogenesis in plants is formation of embryonal meristems that give rise to the formation of both apical meristems in plants, the **shoot apical meristem (SAM)** and **root apical meristem (RAM).**

Root apical meristem develops from the descendants of the LT (see the figure). The root apical meristem give rise to formation of all the cells in the primary root (see the figure).

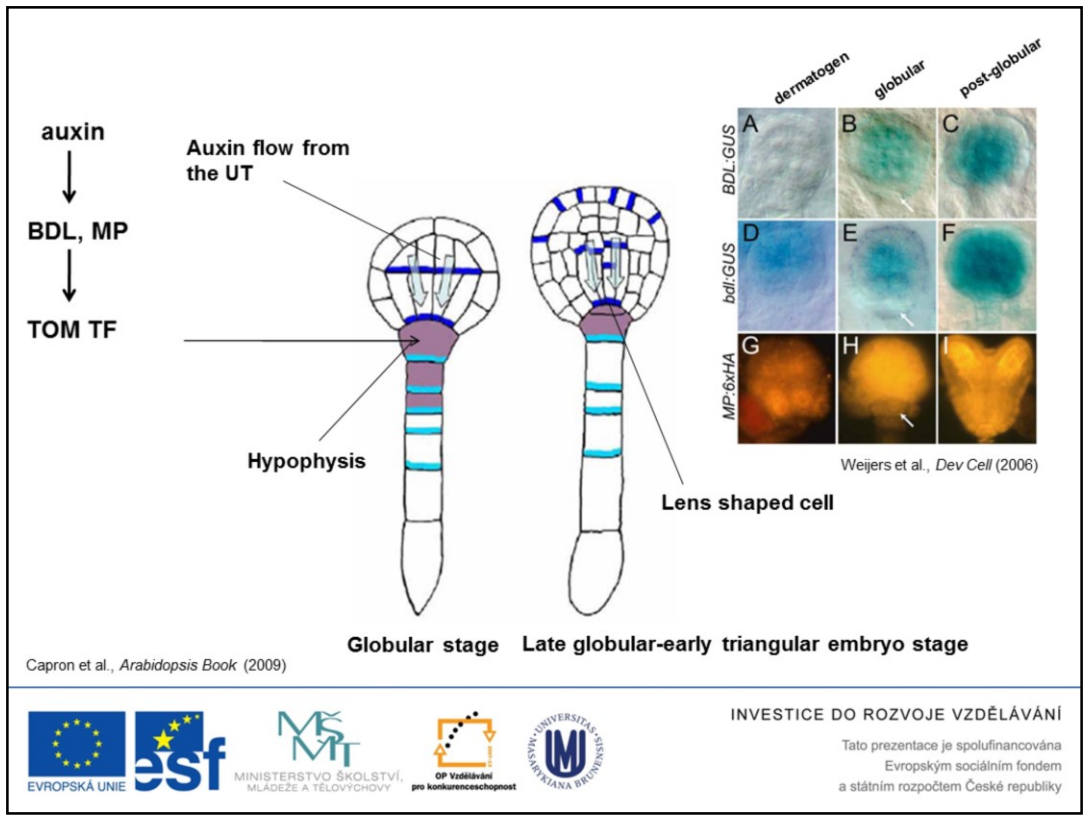


For the formation of root meristem, several signals are important.

First, the signal from the shoot (descendants of the UT) is necessary for acquiring the identity of the **quiescent centre (QC**), an important *organizing centre* of the RAM (compare with another organizing centers mentioned in the animal embryogenesis, e.g. Hansen's node).

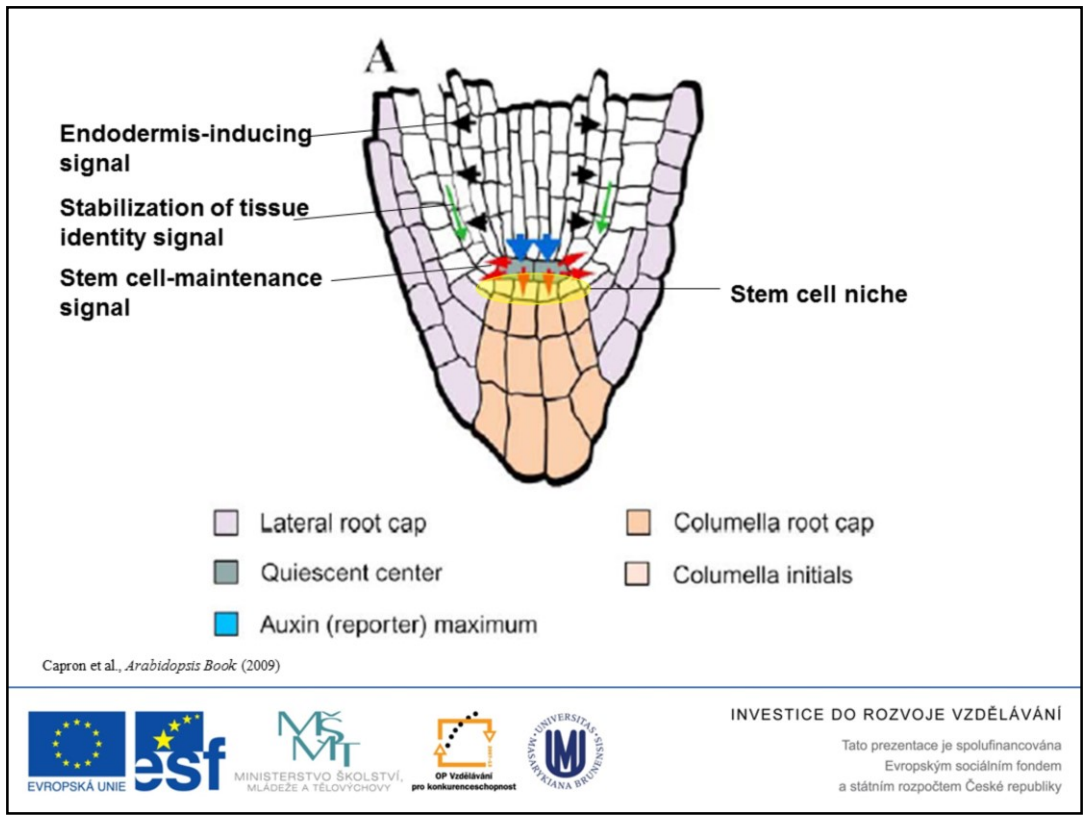
Again, auxin maxima play an important role in those processes.

Auxin maximum defines the QC localization. QC develops from what is called *lens shaped cell* that results from the hypophysis asymmetrical cell division.



In the process of QC specification, the hypophysis is first specified. BD and MP both are necessary for the hypophysis cell identity determination; *bdl* and *mp* embryos are lacking hypophysis.

Interestingly, both *MP* and *BDL* are not expressed in the hypohysis (see right-hand figure), suggesting BDL- and MP-mediated non-cell-autonomous movable signal that allows hypophysis cell determination. Recently, the movable transcription factor TARGET OF MONOPTEROS (TOM) was identified to act downstream of BDL/MP-mediated signalling.



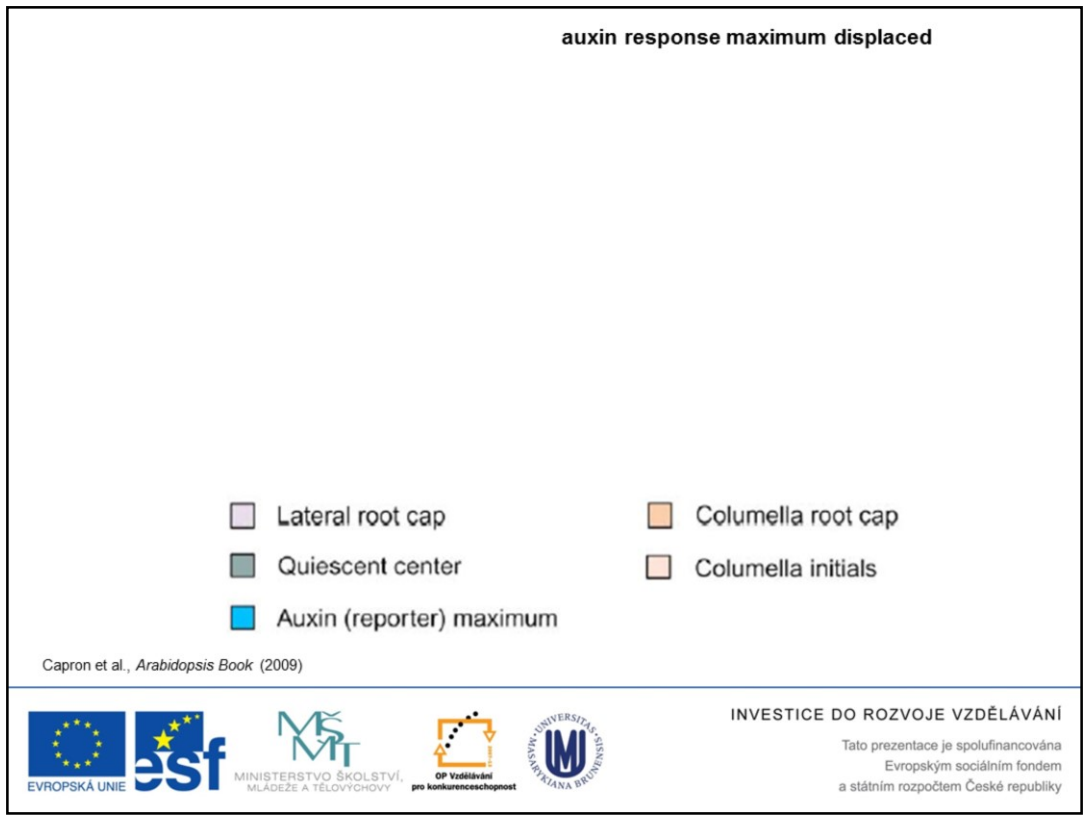
Second, the organizing centre of RAM, the QC emits another signals that are responsible for the maintenance of the *stem cell* population in the RAM (red arrows).

Black arrows represent endodermis inducing signals from the stele (see later) and green arrows the stabilization of tissue identity within each layer.

In general, in every meristem, there are two competing processes taking place. These are *cell proliferation* and *cell differentiation*.

The equilibrium between these two processes allows maintenance of the meristem size and subsequently the size of developing organ (s).

Finally, the differentiating cells emit backward signal that contributes to the identity of less differentiated stem cells. These processes and the underlying molecular mechanisms will be discussed later in more detail.



When the auxin gradient is shifted via e.g. inhibition of the auxin transport, the identity of the cells is shifted, too, allowing thus maintenance of the relative positions of individual cell types.

Compare with additional dorsoventral axis formation induced by extra notochord transplantation in amphibians, Lesson 4.

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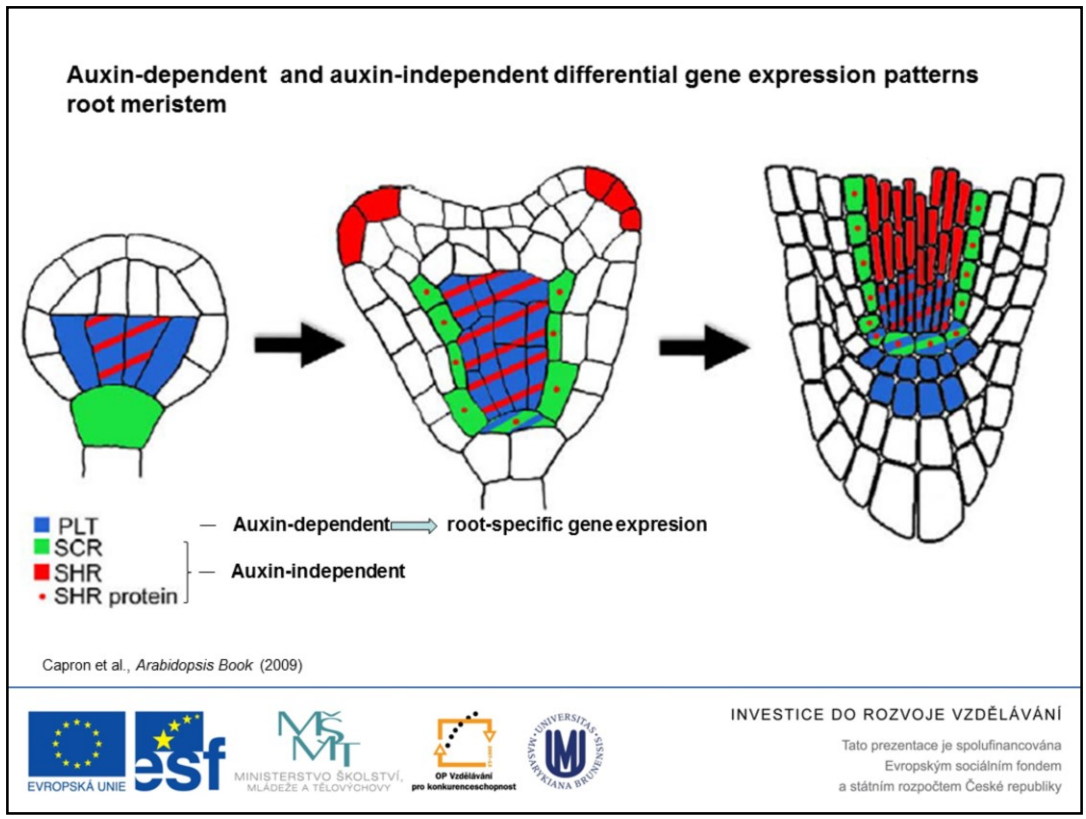

Both auxin-dependent and auxin-independent gene expression regulates proper development of the basal axis and RAM.

Auxin mediates expression of members of *PLETHORA* gene family, PLT1 and PLT2. Changes in the auxin gradients via e.g. *pin* mutations results in changes of the *PLT1* and *PLT2* expression.

Ectopic overexpression of *PLT2* causes homeiotic transformation of the apical domain cells to express root specific genes.

Thus, PLT2 is one of the master regulators (remember the master regulatory genes discussed in the animal eye formation).

Auxin-independent expression of *SCARECROW (SCR)* and *SHORTROOT (SHR)* also plays critical role in the specification of the QC.

SCR is expressed in the hypophysis, while *SHR* partially overlaps *PLT* expression in the globular stage. Later on, *SHR* is transcribed in the provascular tissue, but the SHR protein accumulates in the lens-shaped cell together with SCR. The presence of SHR and SCR in the lens-shaped cell is critical for the QC fate specification.

Member of the previously discusses *WOX* gene family, *WOX5* is also critical for establishing QC. Absence of WOX5 leads to the improper QC specification and subsequent defects in the RAM formation.

Expression of *WOX5* depends on *SHR* and *PL*T activity.

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- Roor meristem formation
  - auxin and hypophysis identity
  - differential gene expression and root meristem patterning
  - auxin-cytokinin interaction and the root meristem organization centre formation

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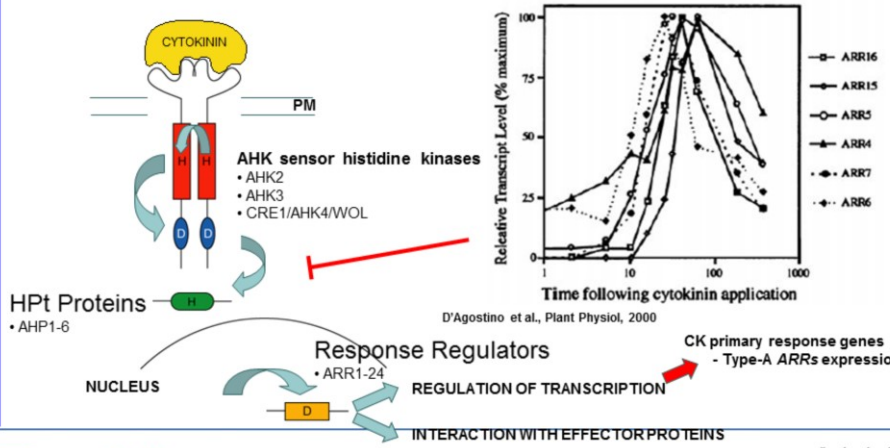
Signal Transduction via TCS

Recent Model of the CK Signaling via TCS Pathway



D'Agostino et al., Plant Physiol, 2000

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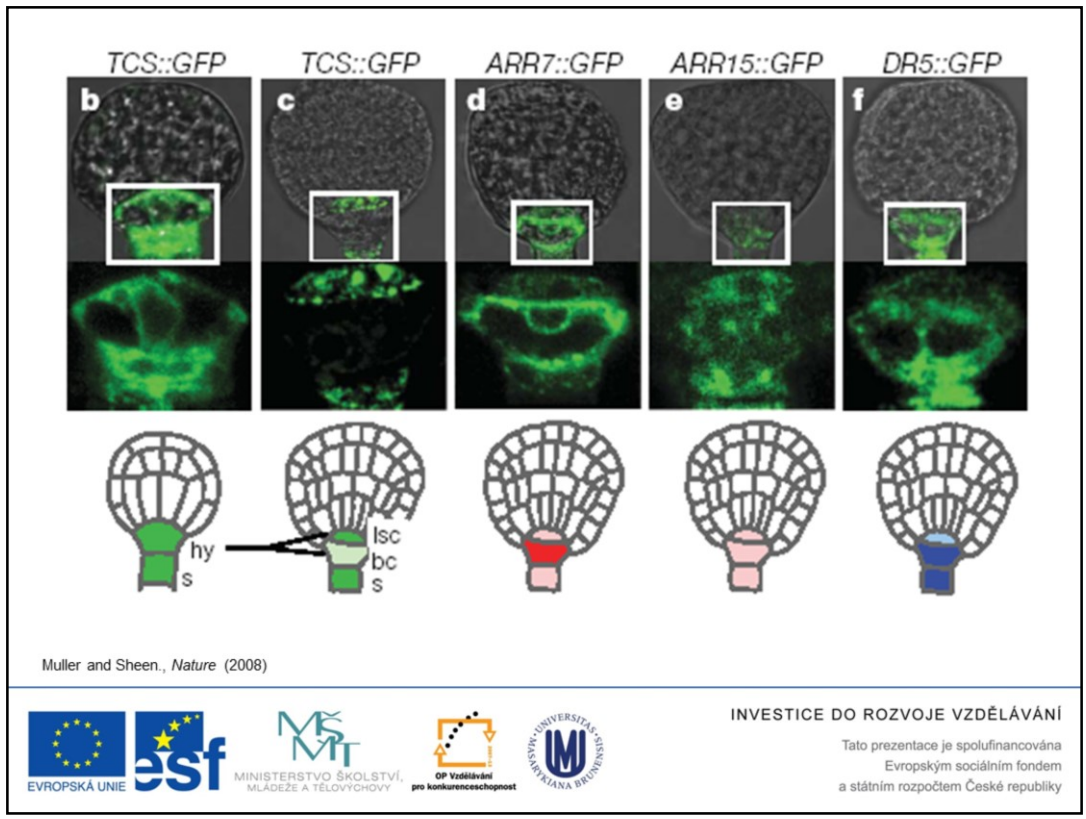
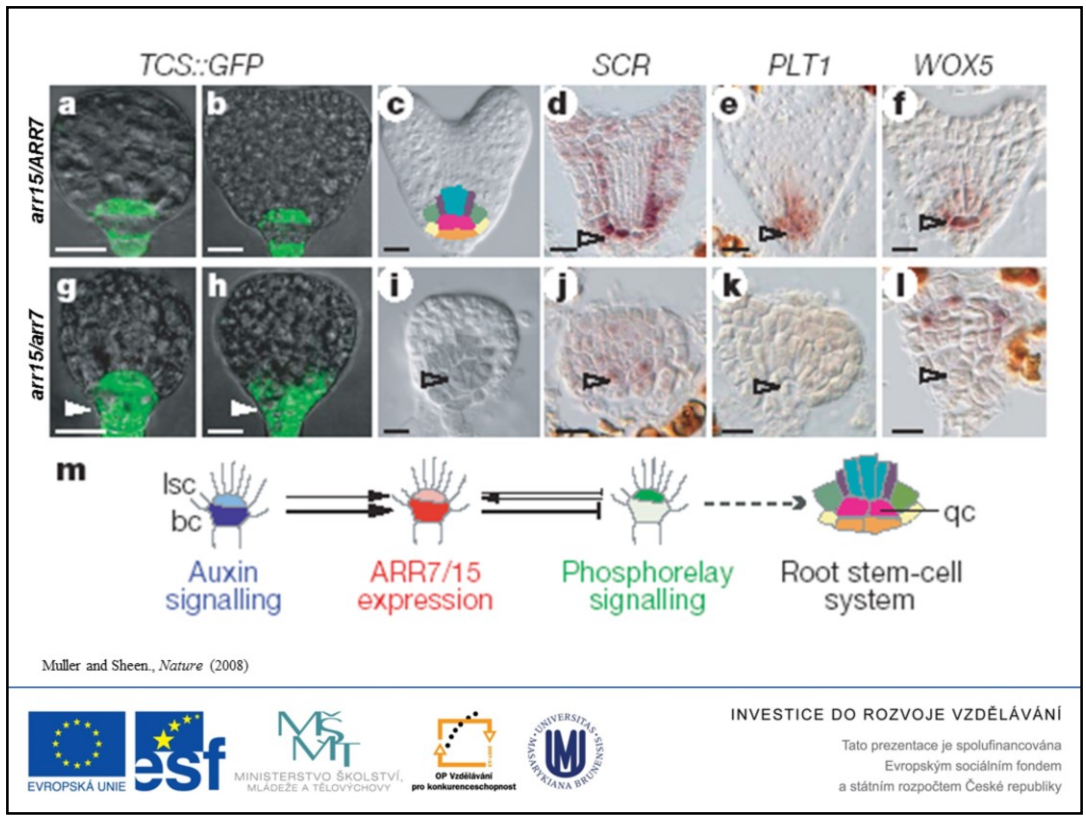


Recently, the role of another important phytohormone, cytokinin (CK) was identified in the specification of QC identity and stem cell niche in the RAM.

Cytokinin signalling could be monitored via artficially constructed recombinant DNA carrying reporter gene (e.g. GFP) under control of CK-responsive element, called TCS.

Using transgenic plants carrying *TCS:GFP* it was found that CK signaling is upregulated in the lens-shape cell. This is achieved via mirrored accumulation of auxin in the lower (basal) cell, formed after hypophysis division.



Recent model suggests that acummulation of auxin inhibits CK signalling in the lens shape cell via induction of negative regulator of CK signaling, the A-type ARRs (will be discussed in more detail in later sections).

That spatiotemporal specific regulation of auxin and cytokinin pathways allows proper expression of regulators of the RAM development (e.g. SCR, PLT1 and WOX5, see previous slides). This results into proper cell identity acquisition and accurate stem-cell niche patterning.

WT is in the upper row, while knock-down mutant via conditional (ethylene induced) *ARR7* RNAi expression is in the lower one.

Outline of Lesson 7

Plant Embryogenesis

- Patterning of the apical pole of the plant embryo
  - generation of cotyledons and shoot apical meristem

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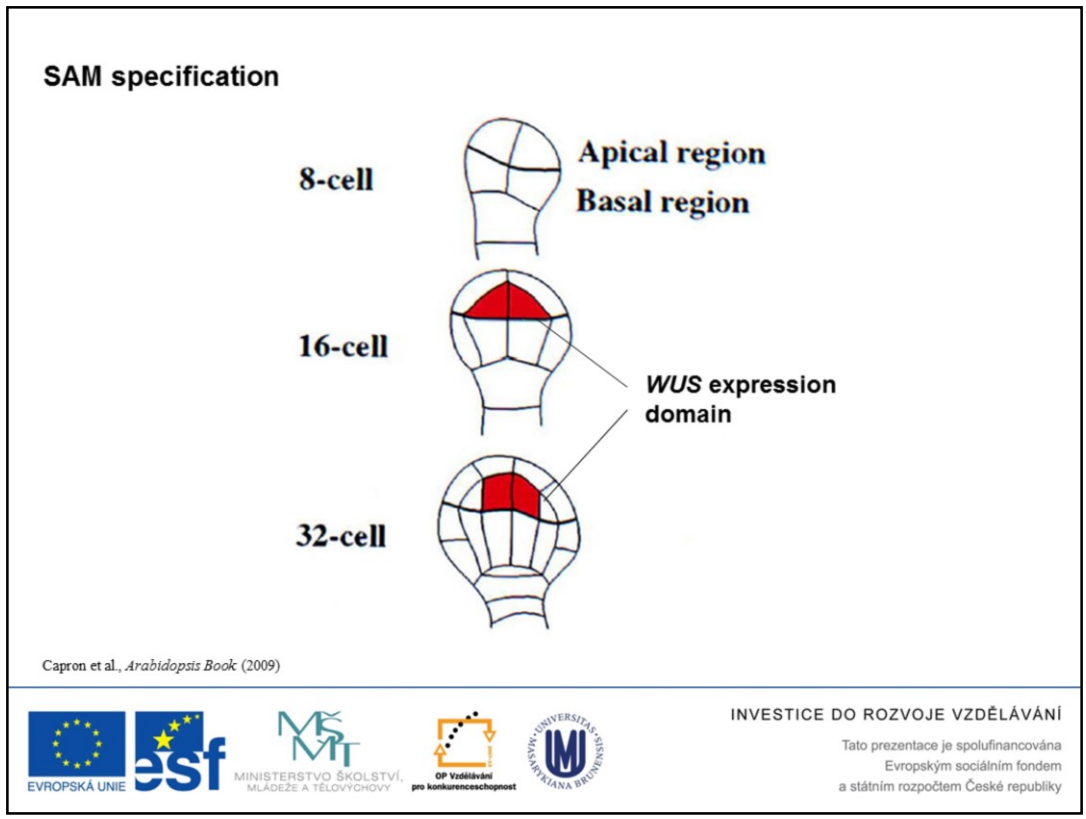


Patterning of the apical part includes several developmental events:

1. Generation of cotyledons and SAM between them
2. Proper spacing of lateral organs
3. Patterning in the adaxial-abaxial axis

Ad1: Generation of cotyledons and SAM between them.

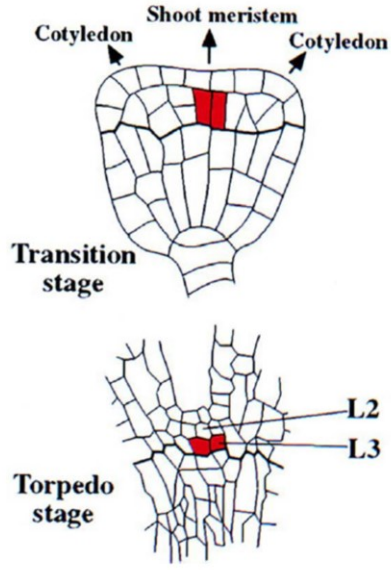
For the specification of the SAM, expression of homeotic gene *WUSCHEL (WUS)* is critical.

WUS activity is necessary for maintenance of the stem cell population in the SAM. Its activity is negatively regulated via CLAVATA genes. For more details, see Lesson 8.

Very briefly, the equilibrium between proliferation of stem cells in the central region of then meristem and differentiation of the "daughter" cells at the margins is critical for the proper size of the meristem and thus the newly formed aerial potion of the plant.

WUS expression starts very early at the stage of 16 cell embryo and is restricted to the region of stem cell population in the later stages (see next slides).

The molecular basis of the *WUS* regulation is unclear.



Capron et al., *Arabidopsis Book* (2009)

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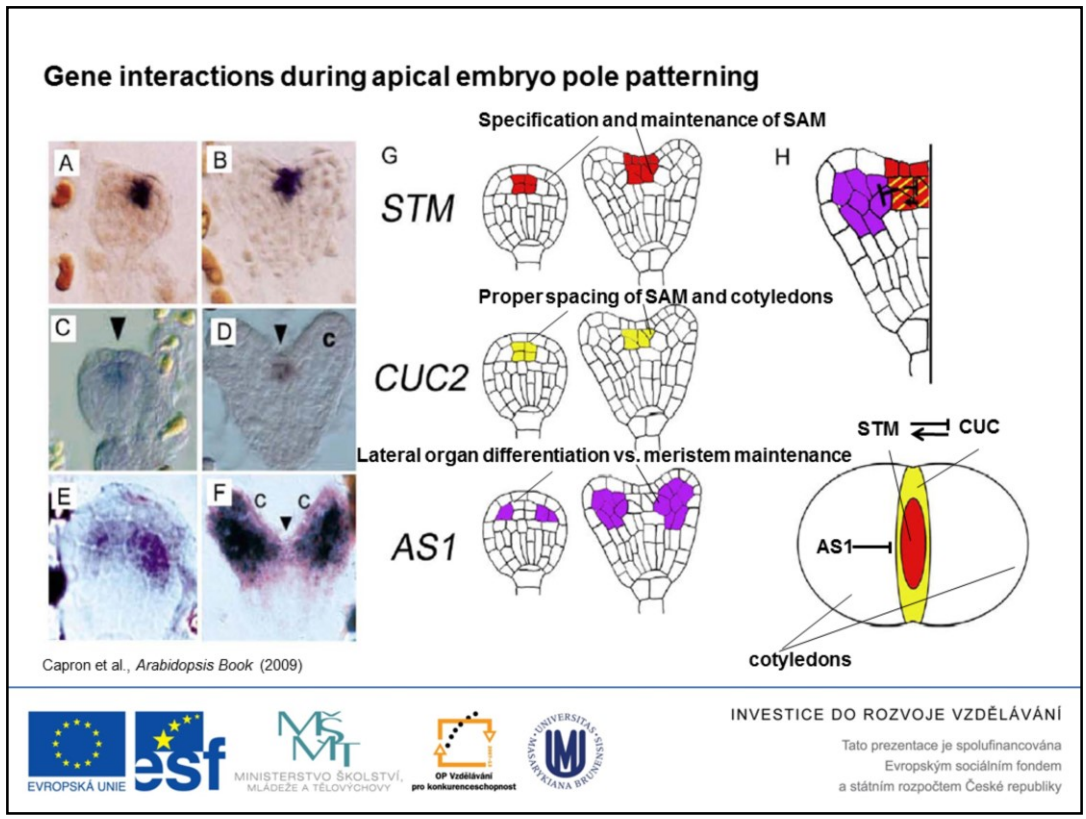

Complex gene interactions are involved in the apical portion patterning of embryo in *Arabidopsis*.

SHOOTMERISTEMLESS (STM) specifies and maintains the activity of SAM.

CUP-SHAPED COTYLEDONS (CUC) genes, some of them regulated by auxin, are necessary for the proper spatial organization of SAM and cotyledons. Double mutants carrying various combinations of *cuc1, cuc 2* or *cuc3* mutant alleles have fused cotyledons. Thus, CU expression determines the boundary between two organs. This situation resembles the role of ENGRAILED, which determines the boundary between diencephalon/mesencephalon during brain development in animals (see Lesson 04).

There is spatial interaction between the expression of *CUC* and *STM* genes. *CUC* genes upregulate the expression of *STM* in the central portion, while, *STM* negatively regulates *CUC*. That leads to the formation of collar of *CUC* expression around the SAM region (see figure I, view from the top).

ASYMETRIC LEAVES (AS) genes counteract the effect of *STM* in the meristem maintenance. Double mutants *as1, as2* restore the meristem formation in *stm* mutants.

Thus, *STM* maintains the cells in the center of the meristem undifferentiated by counteracting the activity of *AS* genes, which are expressed in the cells differentiating to the cotyledons.

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Plant Embryogenesis

- Patterning of the apical pole of the plant embryo
  - generation of cotyledons and shoot apical meristem
  - proper spacing of lateral organs

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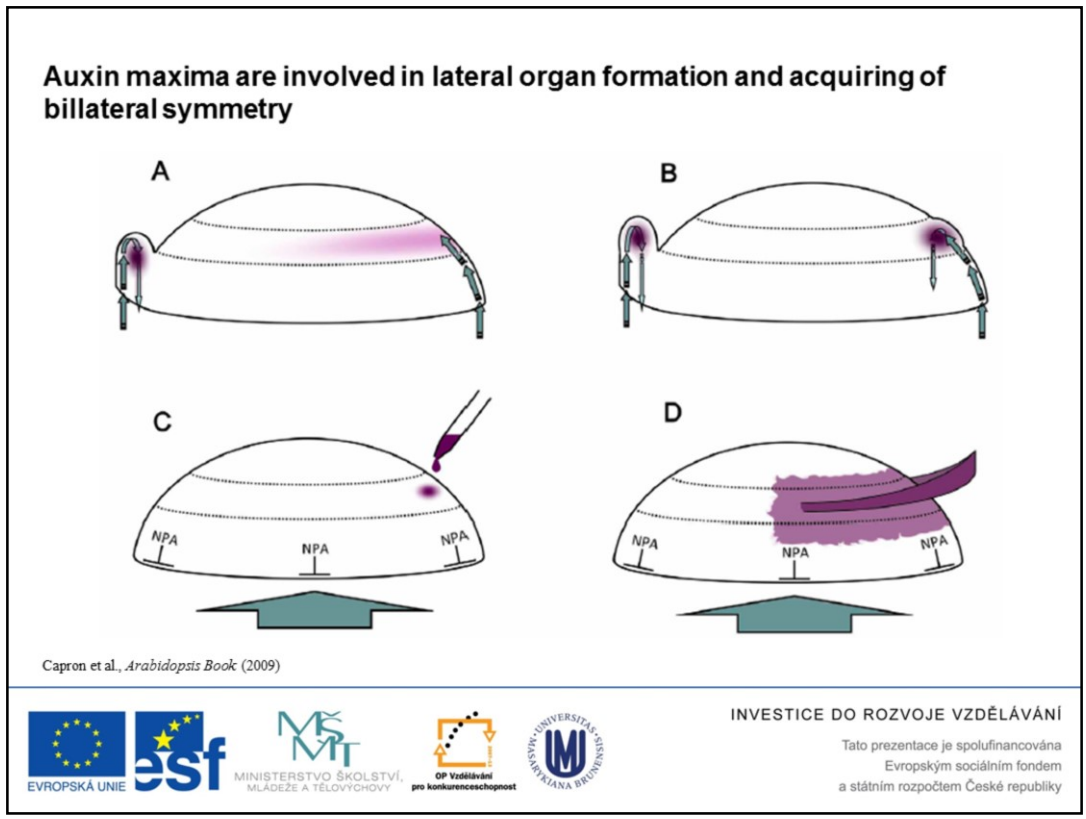


Ad 2: Proper spacing of lateral organs.

Differentiation of cotyledons defines qualitatively novel stage in the embryogenesis-bilateral symmetry formation from the radially organized globular embryo.

Here again, auxin accumulation is critical for the new lateral organ initiation.

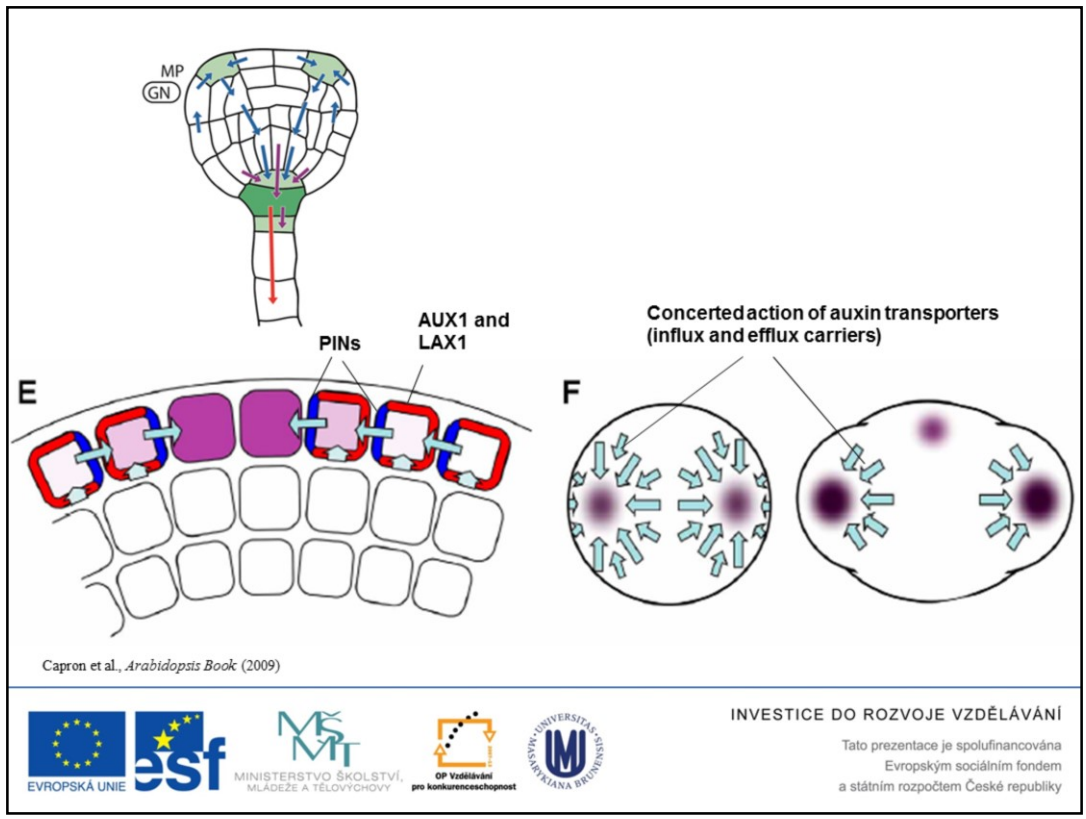
Inhibition of auxin transport using chemical treatment (e.g. with 1-naphthylphthalamic acid [NPA]) leads to the absence of both auxin maxima formation and new lateral organ initiation.

A) Convergent auxin transport within the L1 layer predicts positions of lateral organ initiation. Basipetal auxin transport routes appear to develop within and beneath these new organs. As this removes auxin from the L1 areas near the emerging primordia, the process will most likely reiterated furthest away from the existing primordia.

(B) The localization of PIN efflux proteins in the L1 layer concentrates auxin at sites of subsequent lateral organ initiation. PIN proteins also mediate basipetal transport from the site of initiation.

(C) Auxin transport inhibitors (such as NPA) prevent organ initiation. This block can be overcome and lateral organ initiation can be achieved by localized application of auxin.

(D) If excessive auxin is added to NPA treated meristems, threshold levels of the hormone are achieved over a wide area of the peripheral zone and enlarged organs are produced.



The auxin maxima are formed via coordinated action of auxin transporters, auxin efflux carriers from the PIN family and auxin influx carriers (AUXIN PERMEASE1 [AUX1] and LIKE AUX1, 2 3 [LAX1, 2, 3]).

Subsequently, cotyledons are formed in the position of auxin maxima. The draining of auxin from the sites surrounding the newly formed auxin maxima probably inhibit formation of new auxin maxima and thus new lateral organs in the close proximity of the newly initiated organs. This will be discussed later in more details in case of phyllotaxis.

Localization of PIN auxin efflux facilitators (dark blue) in the L1 layer is consistent with the converging auxin transport and accumulation at sites of incipient lateral organ initiation. Auxin influx associated proteins (red) are thought to help scavenge intracellular auxin and retain it in the cells of the L1 layer.

Outline of Lesson 7

Plant Embryogenesis

- Patterning of the apical pole of the plant embryo
  - generation of cotyledons and shoot apical meristem
  - proper spacing of lateral organs
  - adaxial-abaxial axis formation

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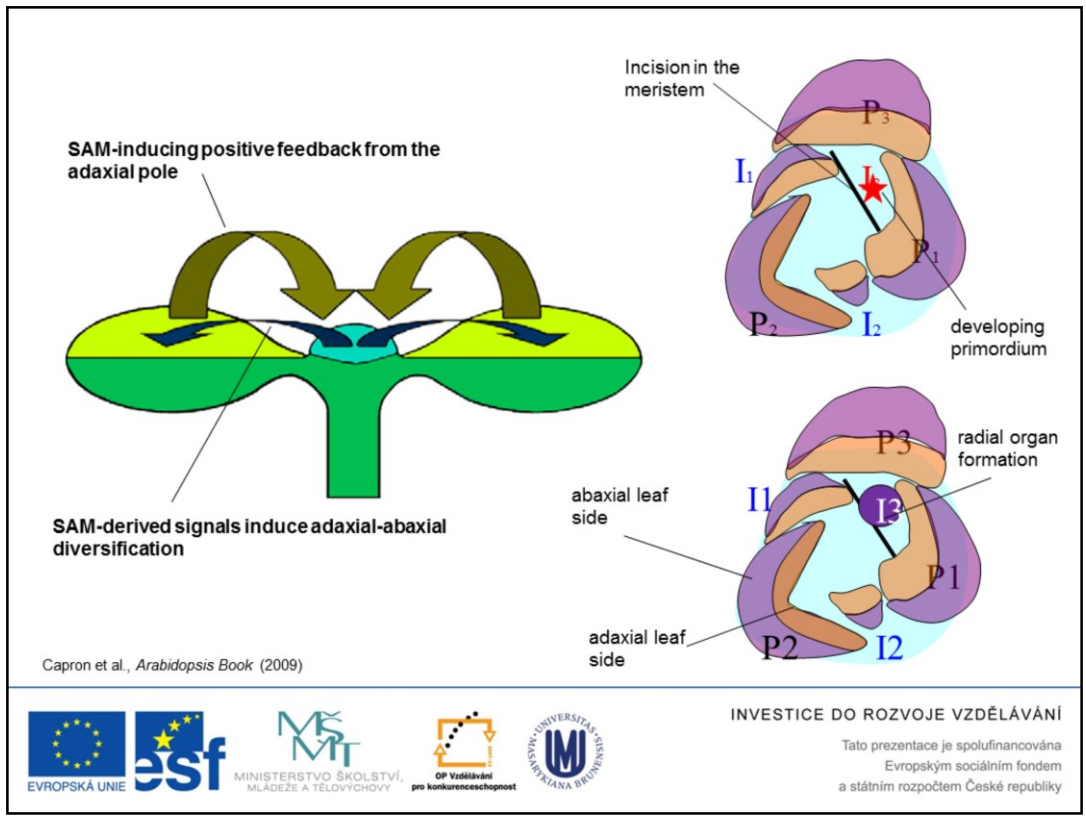
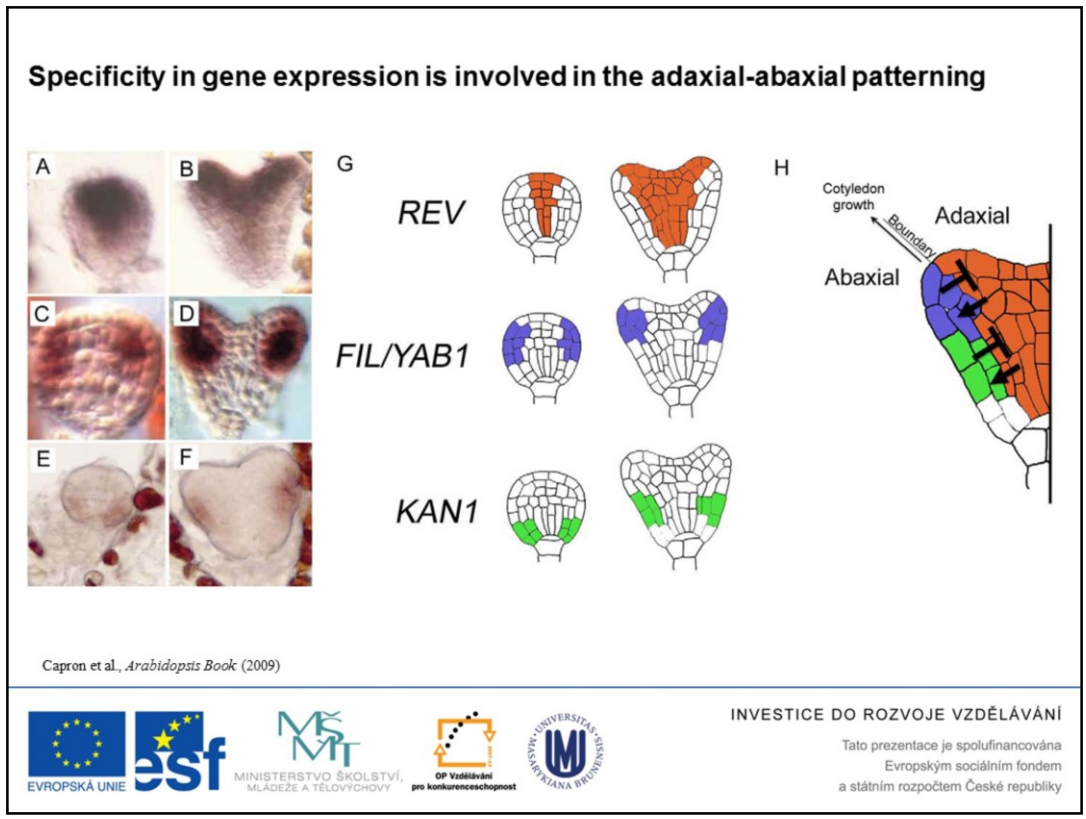


Ad 3: Patterning in the adaxial-abaxial axis.

There is a communication between apical-basal and adaxial-abaxial patterning during embryogenesis in *Arabidopsis*.

The signals coming from the shoot allow the adaxial-abaxial diversification. Incisions in the meristem lead to the loss of the adaxial-abaxial axis formation in the new primordia and lead to the radial organ formation (right-hand panels). That will be discussed in more detail later.

Vice versa, there is a feed-back from the adaxial portion of the lateral organs to the SAM development. The "adaxialized" mutants promote SAM while "abaxialized" mutants show inhibition of SAM development.

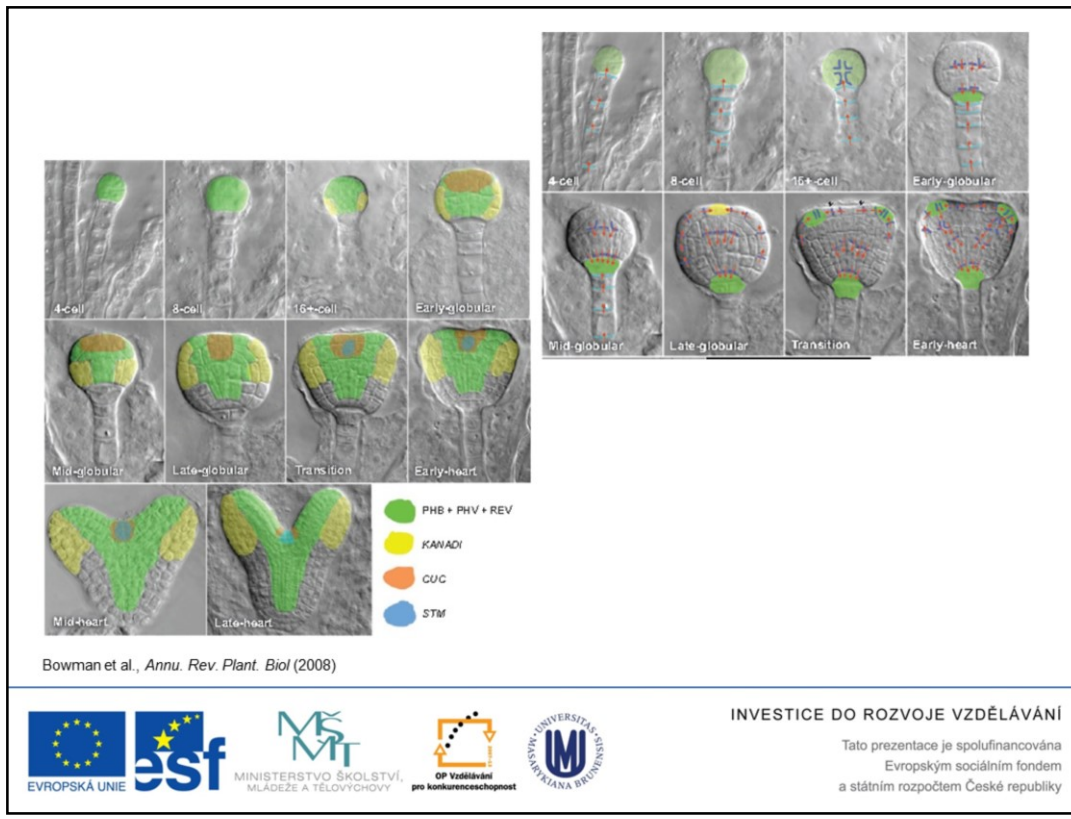


Specification of the adaxial and abaxial axis is determined by the specific gene regulation.

Central and adaxial side is determined by the class III HD-ZIP genes, e.g. *REVOLUTA (REV*).

Abaxial side is determined by the expression of *YABBY* and *KANADI* genes, e.g. *FILAMENTOUS FLOWER (FIL)/YABBY1 (YAB1)* and *KANADI 1 (KAN1),* respectively.

Thus, the equilibrium between anatgonistic activities between adaxial and abaxial genes allows formation of properly specified adaxial-abaxial axis in lateral organ formation.



Taken together, the principal developmental events in the embryo development in angiosperms, e.g.

1. formation of apical-basal axis,
2. meristems formation
3. spacing of lateral organs and
4. acquiring of their bilateral symmetry (formation of adaxial-abaxial axis)

is driven by auxin distribution (upper right figure, green signal) and specific gene expression (lower figure).

The role of other important regulators in the plant embryogenesis, e.g. cytokinins started to emerge. Thus it will be very important to explore in more detail, including interaction of those principal plant growth regulators.

Outline of Lesson 7

Plant Embryogenesis

- Patterning of the apical pole of the plant embryo
  - generation of cotyledons and shoot apical meristem
  - proper spacing of lateral organs
  - adaxial-abaxial axis formation
- Radial embryo patterning
  - epidermal layer specification

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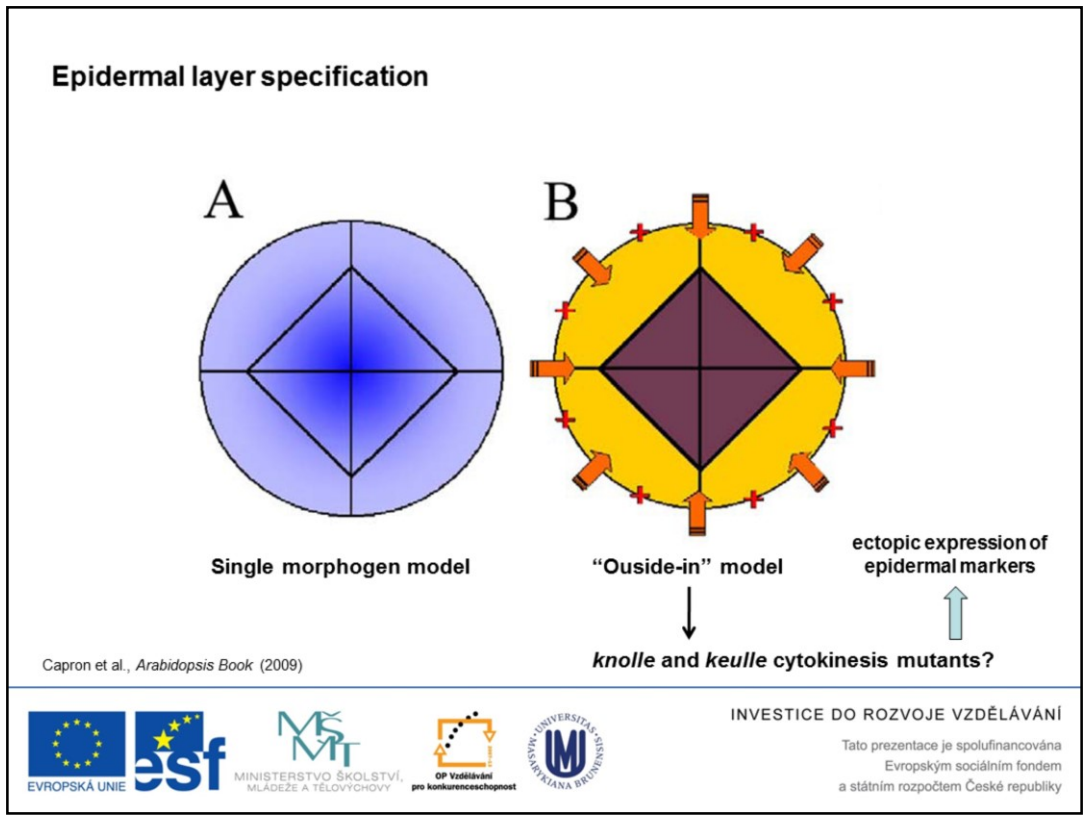
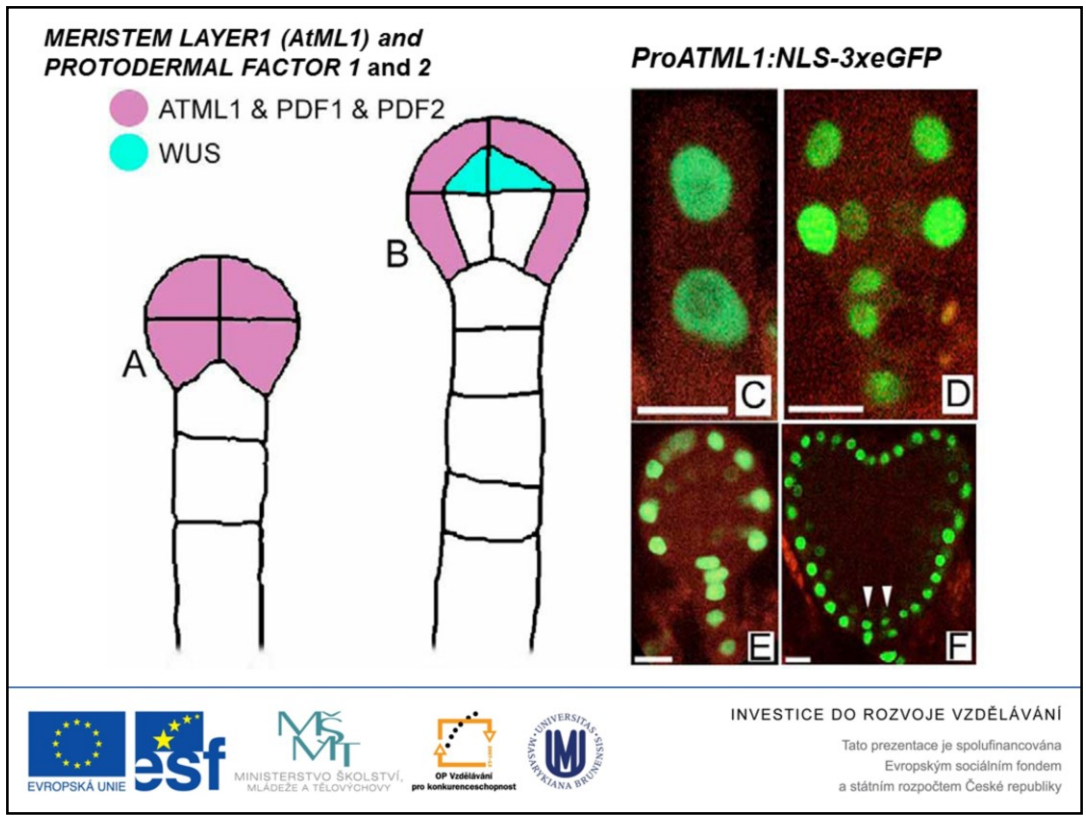


Mechanism of the epidermal layer specification is still not clear in the early plant embryogenesis.

Two potential models could be considered:

A. Model of the single morphogen gradient. This mechanism would allow for the concentration dependent specification of several cell identities, but only two fates, outer (epidermal) and inner cells, are specified at this stage.

B. "Outside-in" model. This model suggest the presence of the signal that is produced either from the outside milieu or is located to the outer cell walls. There is some experimental evidence that prefers this model. E.g. the mutations in KNOLLE (KN) and KEULE (KEU) are associated with incomplete cell wall formation during cytokinesis that leads to the ectopic expression of epidermal markers in the subepidermal layers.



Several genes were identified with expression pattern specific for the prospective epidermal cell layer.

These include *MERISTEM LAYER1 (AtML1) and PROTODERMAL FACTOR 1* and *2 (PDF1, PDFF2). AtML1* and *PDF1* and *2* are expressed in the outer layers (epidermis|) of the embryo proper and later on in the L1 (upper most layer, see later) of SAM.

Double mutants *atml1, pdf1* form protoderm, but fail to differentiate proper epidermis.

On the right-hand figures, there is *ProATML1:NLS-3xeGFP* expression (translational fusion of three GFP copies with nuclear localization signal [NLS] under control of AtML1 promoter) in the different stages of the embryo formation.

Outline of Lesson 7

Plant Embryogenesis

- Patterning of the apical pole of the plant embryo
  - generation of cotyledons and shoot apical meristem
  - proper spacing of lateral organs
  - adaxial-abaxial axis formation
- Radial embryo patterning
  - epidermal layer specification
  - separating vascular and ground tissue

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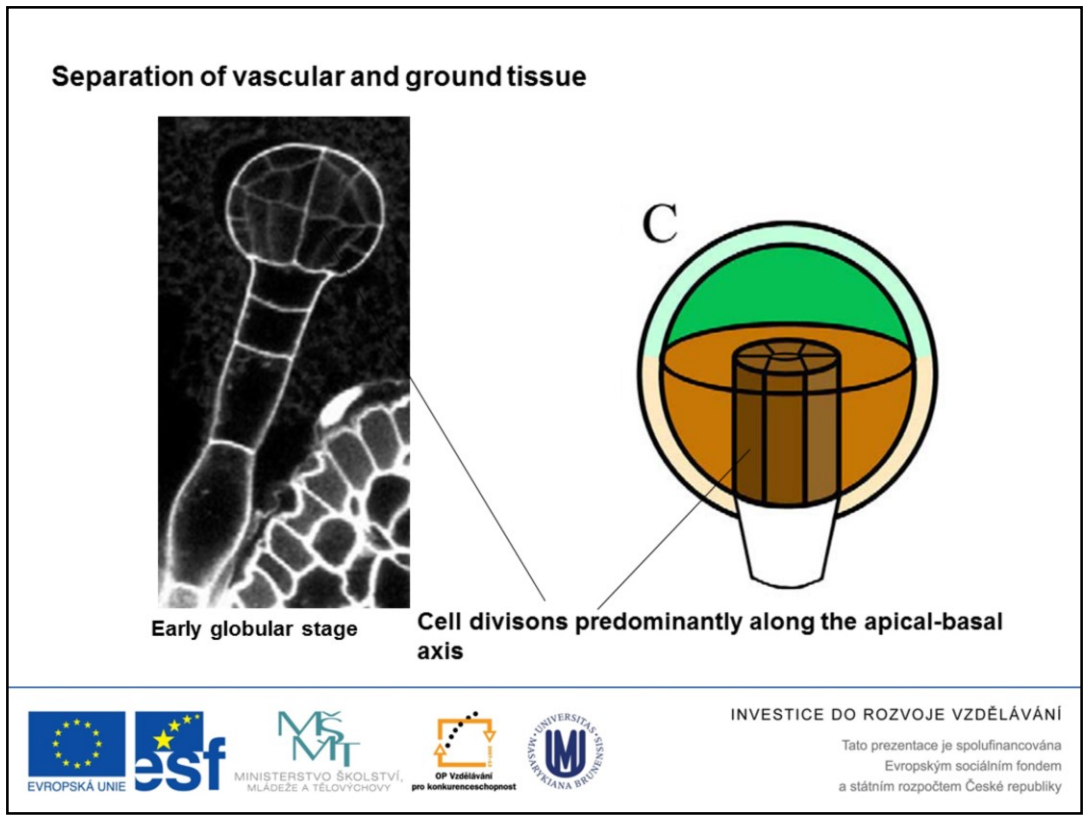
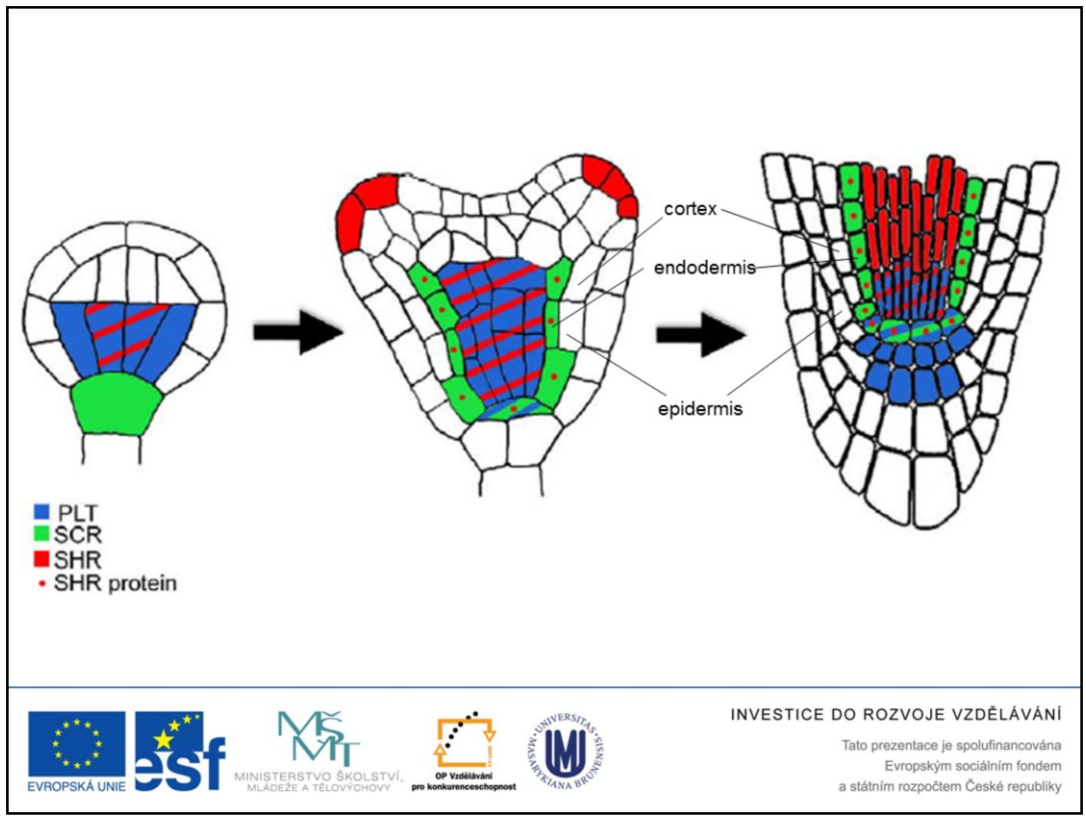


Onwards the dermatogen stage, in the early globular embryo, the LT descendants divide predominantly along the apical-basal axis.

That allows formation of the central cylinder of cell files that will develop into the provascular tissue, the prospective root vascular tissue.

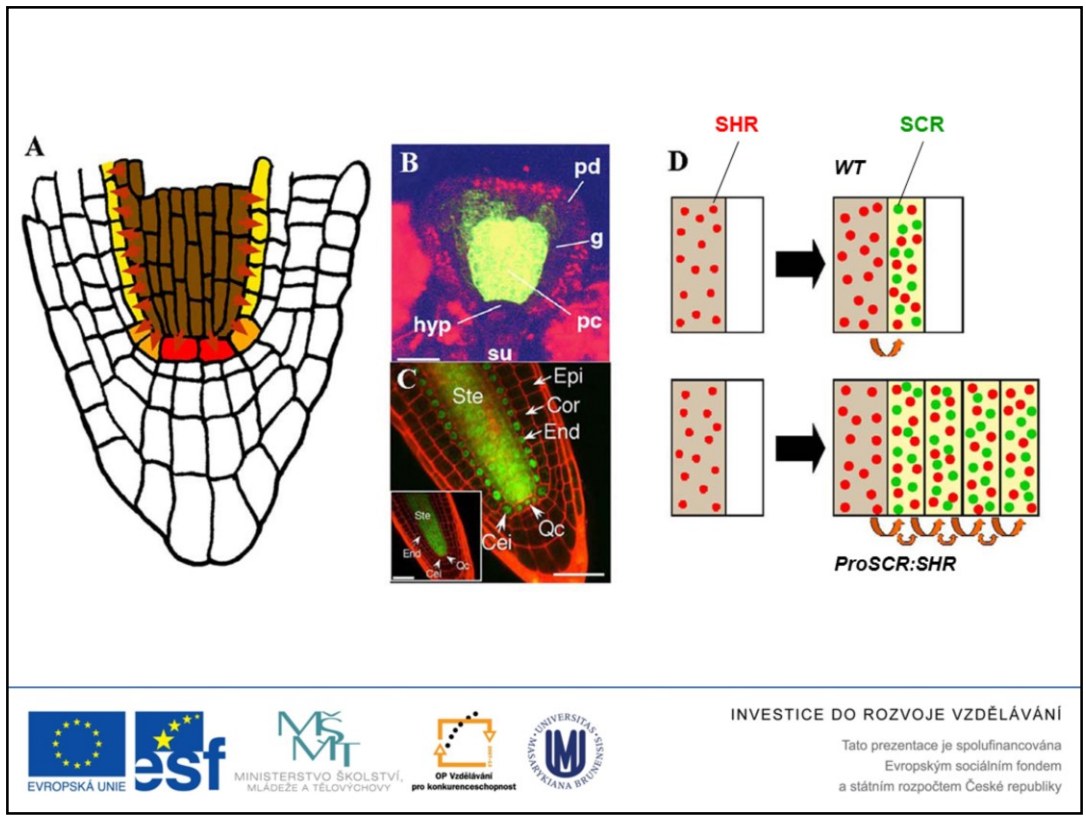


The periclinal divisions allow formation of several layers of cell files that differentiate into individual cell types of the root.

SHR and SCR, previously discussed in the QC fate determination.

In the embryonal root, SHR is expressed in the inner layers of provascular tissue. After additional assymetric cell division of the tissue, the endodermis (inner layer) and adjacent cortex (outer cell layer) are differentiated.

This specification is ongoing also later during root growth, the cortex and endodermis share common progenitor stem cell, the what is called *initial* (see Lesson 8 for more details).



The mechanism of the cell layer specification is based on the SHR protein movement form its expression domain, the vascular cylinder (shown on the figure A) to the adjacent cell layer.

There SHR induces *SCR* expression. SCR induces asymmetrical division of the ground tissue (and/or the progeny of the respective initial), associated with the repression of cortical markers in the inner (endodermal) layer.

SCR also controls expansion of the SHR protein via direct interaction with SHR and its sequestration into the nuclei of the cell layer (see the green GFP signal in the figure C).

Ectopic overexpression of *SHR* under control of *SCR* promoter leads to formation of supernumerary call layers due to repetitive expansion of SHR into the adjacent cell layer and induction of its own expression (because being under control of *SCR* promoter, see figure D).

Key Concepts

Plant Embryogenesis

- Similarly to animals, both **embryonic and extraembryonic tissue** forms during plant embryogenesis
- In plant embryogenesis, **positional information** rather than invariant cell division is decisive for the proper embryo patterning
- **Auxin gradient formation provides positional information** that together with **differential gene expression** directs downstream developmental events during plant embryogenesis
- **Auxin transport machinery** and **auxin signalling** are critical for the proper embryo development
- **Interaction of auxin with other growth regulators**, e.g. **cytokinins** emerges as a crucial regulatory factor for many developmental processes during plant embryo formation
- **Gene** and **protein interactions** allow formation of **distinct cell and tissue spatial patterns** and allow proper organogenesis

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