

C8545 Developmental Biology

Lesson 8

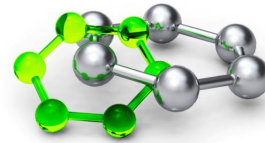
Postembryonic Plant Development

Jan Hejátko

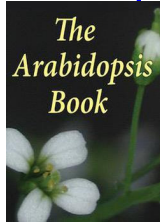
Functional Genomics and Proteomics of Plants
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Literature



- **Fred H. Wilt and Sarah Hake, Principles of Developmental Biology** (W.W. Norton & Company, New York, London, 2004)
- **Capron A, Chatfield S, Provart N, Berleth T 2009.** Embryogenesis: Pattern Formation from a Single Cell. *The Arabidopsis Book*. Rockville, MD: American Society of Plant Biologists, doi: 10.1199/tab.0126, <http://www.aspb.org/publications/arabidopsis/>.
- Essau, K., 1965, Plant Anatomy, 2nd edition, Willey, 978-0471244554
- Selected original papers in scientific journals

Outline of Lesson 8

Postembryonic Plant Development

- The role of plant meristems in the plant postembryonic development
- Shoot apical meristem (SAM)
 - Structure of the SAM
 - SAM establishment and maintenance
- Phyllotaxy
 - Fibonacci series and golden mean in the nature
 - Molecular determinants of phyllotaxy
- Root apical meristem (RAM)
 - RAM structure
 - Positioning of RAM organization centre
 - Radial root patterning
 - RAM size determination
- Lateral root formation
- Vascular tissue formation in shoot and root

Outline of Lesson 8

Postembryonic Plant Development

- The role of plant meristems in the plant postembryonic development



What is the principal difference between plants and animals?



Arabidopsis thaliana, embryo at the torpedo stage

Mus musculus, embryo, longitudinal section

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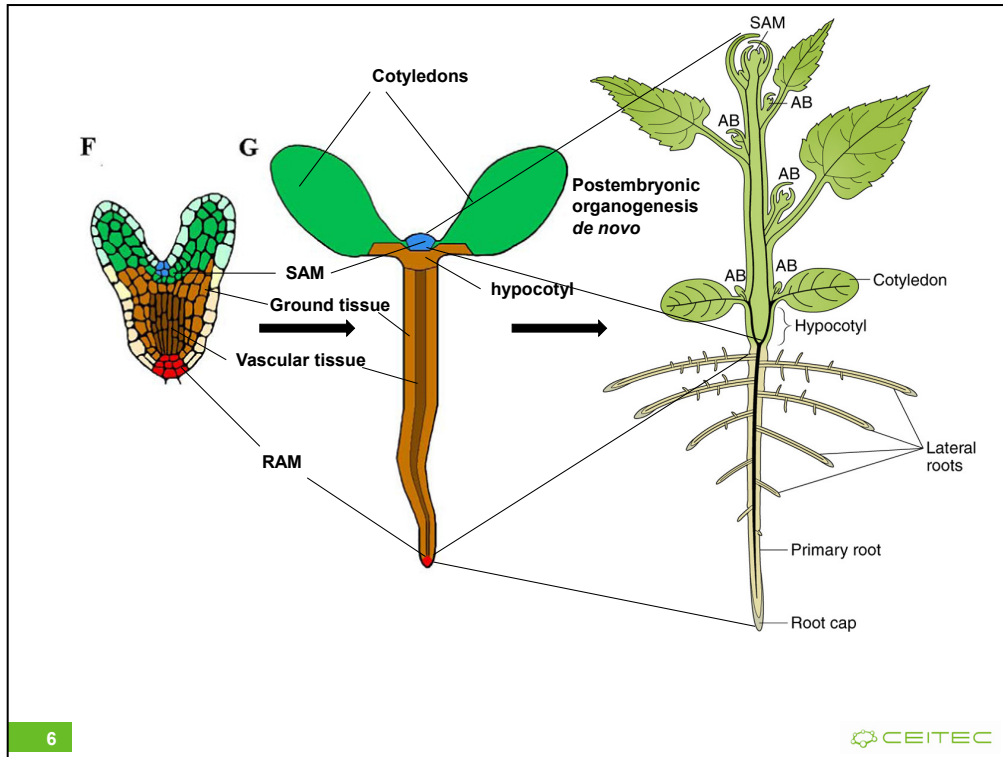
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What is the principal difference between plants and animals?

Plants, in contrast to animals (with few minor exceptions) evolved the ability of **postembryonic *de novo* organogenesis** (i.e. formation of lateral roots, flowers, leaves, etc.).

This developmental strategy is possible thanks to enormous **developmental plasticity** of plants – **totipotency of differentiated plant cells**.

As a result, the **developmental plasticity** allows plants to **adapt to changing environmental conditions**.



The fundamental plant body plan is set up during embryogenesis as discussed in the last lesson.

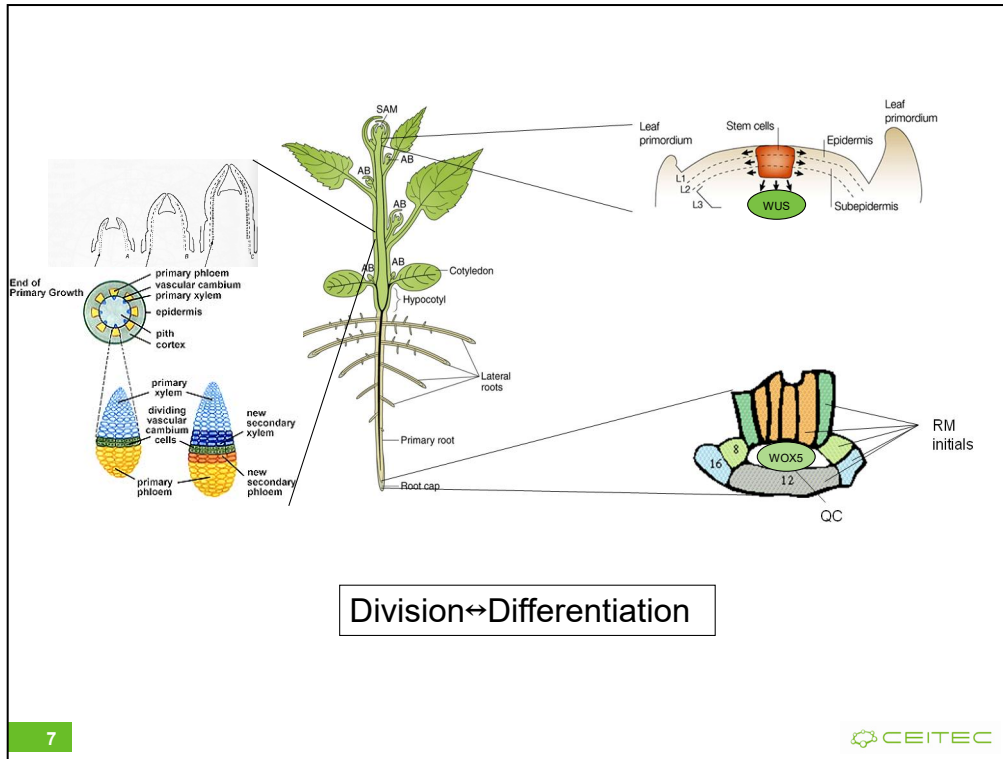
After seed germination, the seedling expands the basic arrangement of tissues and cell types, creating *seedling* with embryonic leaves, *cotyledons*, *hypocotyl*, connecting SAM with *primary root* and RAM.

The further plant development and the final plant architecture is result of interaction between genetically preprogrammed developmental events and surrounding environment, including both biotic and abiotic factors.

The major role in shaping the plant play *meristems*.

Meristems are functionally and anatomically delimited tissues that contain plant stem cells that differentiate into newly formed plant tissues and that thus ensure plant growth and above all, new, postembryonic tissue and organ formation.

This process is called **postembryonic de novo organogenesis** and is characteristic hallmark of the plant development.



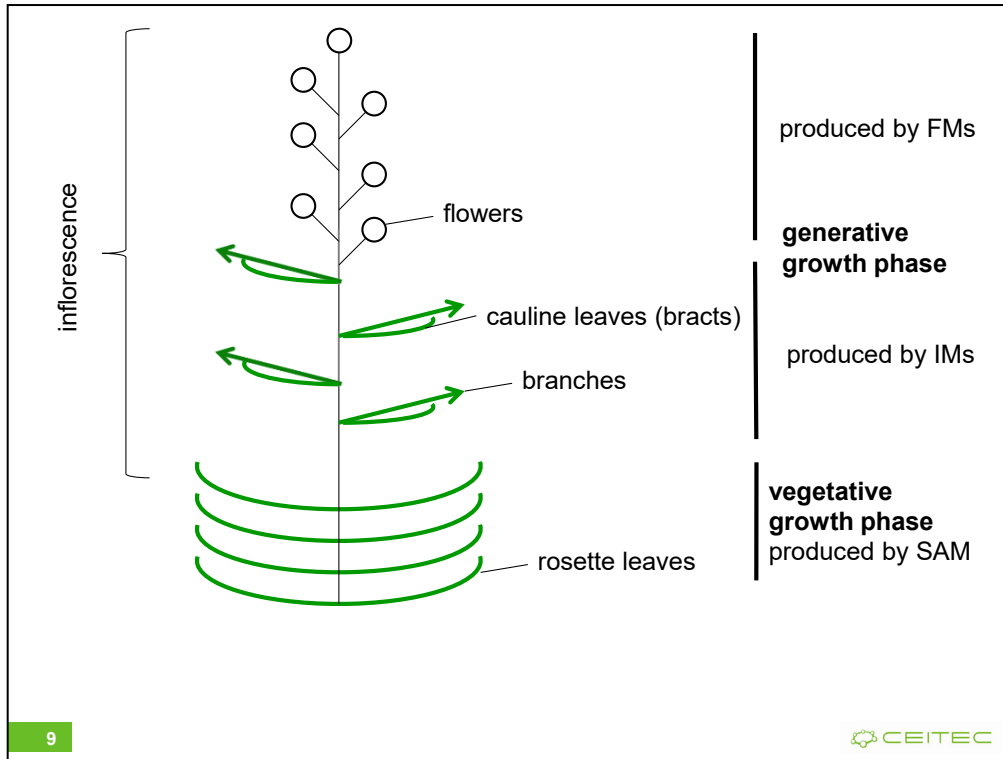
There are two apical meristems in plants, shoot apical meristem (SAM) and root apical meristem (RAM), allowing apical growth of the plant.

Further, there are additional meristematic tissues e.g. (pro)cambium and axillary meristems, allowing lateral plant expansion that will be discussed later.

Outline of Lesson 8

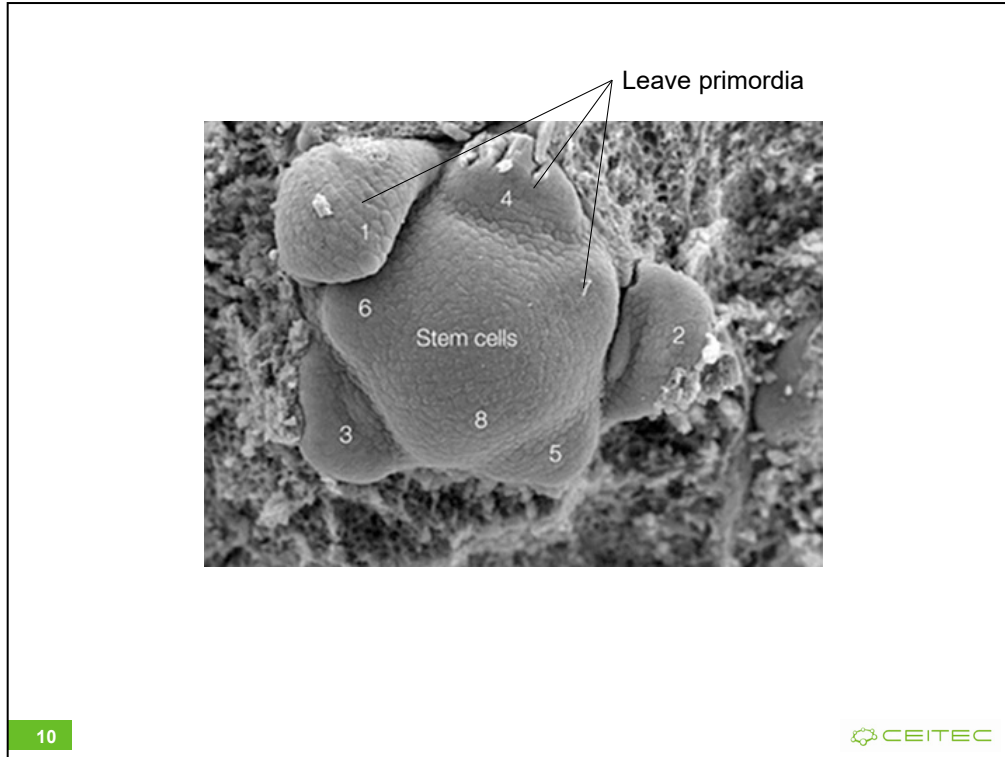
Postembryonic Plant Development

- The role of plant meristems in the plant postembryonic development
- Shoot apical meristem (SAM)
 - Structure of the SAM



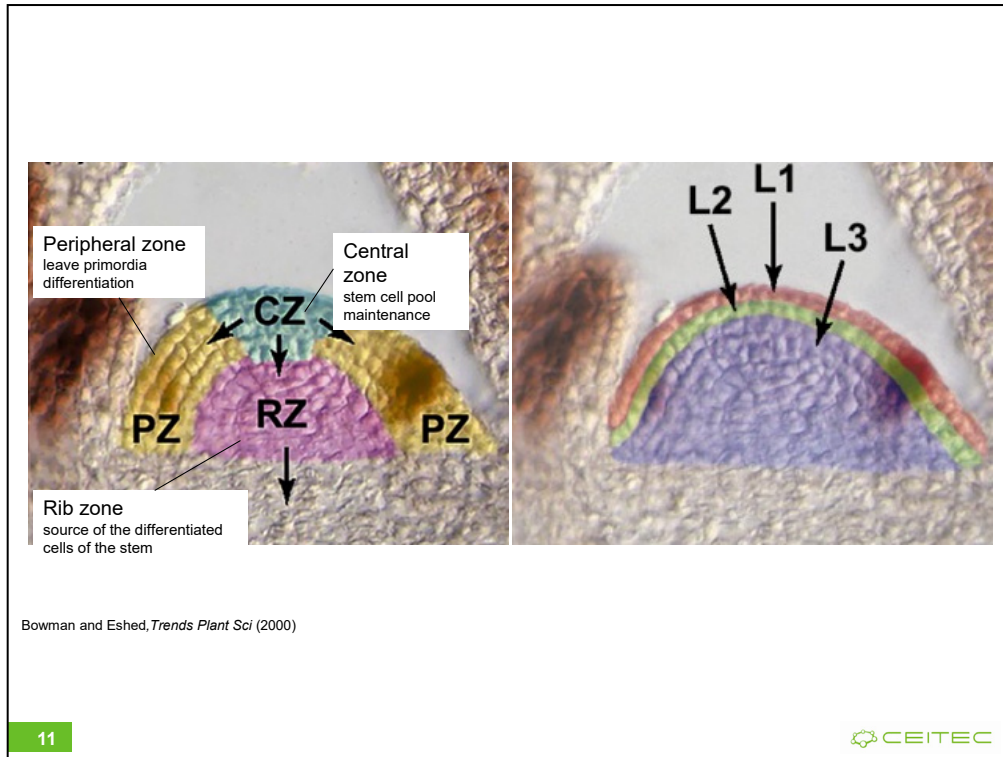
SAM acts during vegetative growth phase, allowing thus production of rosette leaves in *Arabidopsis*.

At the moment of initiation of flowering, the SAM is transformed into inflorescence meristem (IM) that produces inflorescence. Inflorescence meristem differentiate into floral meristems (FMs) that produce flowers.



SAM is an apical structure that allows repeated production of what is called *leaf primordia*, which will further develop into leaves and the connecting tissue, i.e. stem.

Both, leaves and stems are highly vascularized and thus, the differentiation of the vascular tissue occurs in the subapical region.



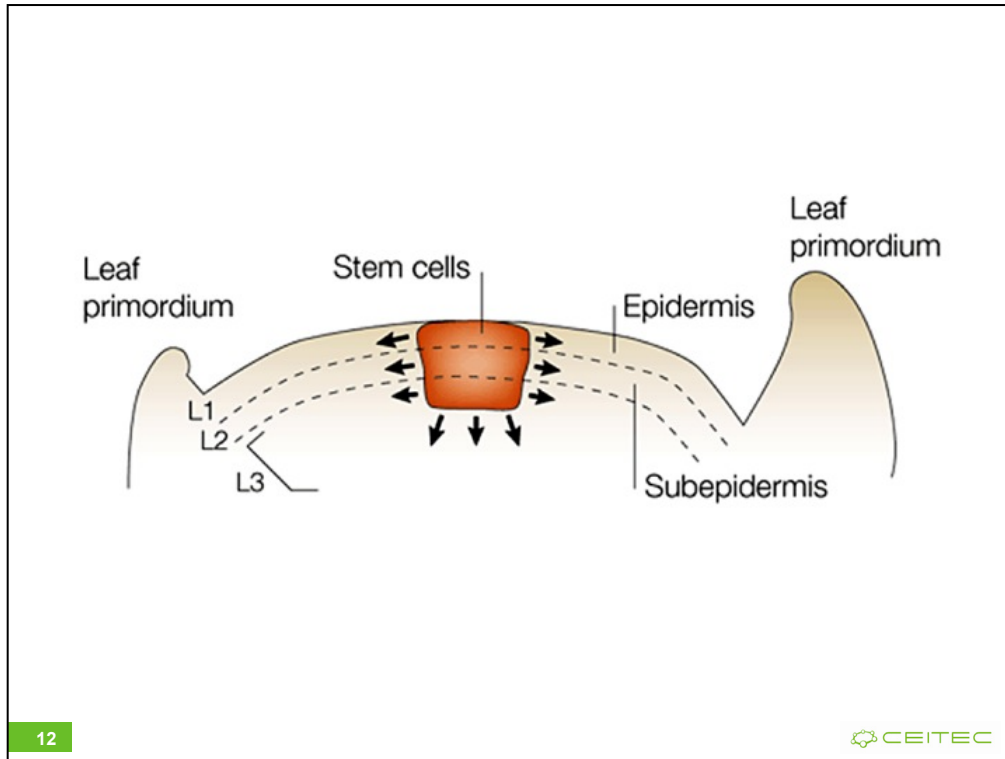
Structure of the SAM involves several functional units.

There is a **central zone**, comprising non-differentiated stem cells.

More laterally, there is what is called **peripheral zone**, where new leave primordia differentiate.

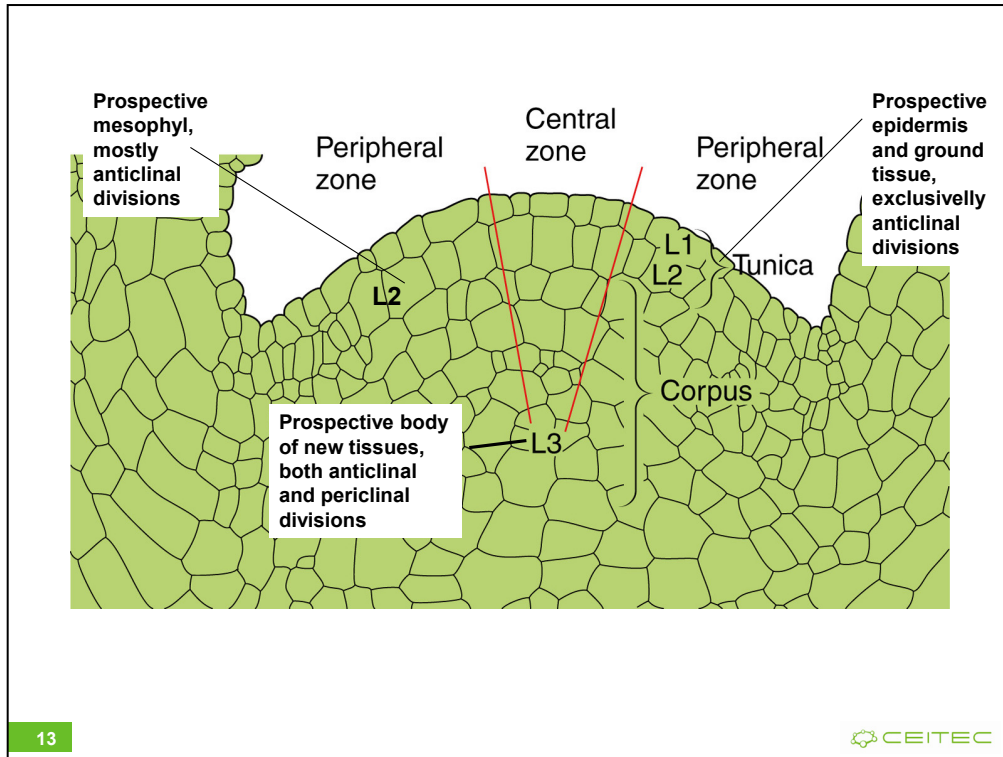
Finally, the mid-proximal **rib zone** gives rise to differentiated cells of the stem.

Further, there could be distinguished three cell layers, going through all of the above described functional units, designated as L1, L2 and L3 (see the figure on the right-hand side).



Stem cell population is being kept in the the central zone and acts as a source of undifferentiated cells for the leave primordia that differentiate in the lateral zones.

As discussed previously in the plant embryogenesis lesson, the equilibrium between stem cell production and stem cell differentiation is critical for the maintenance of the proper size of the meristem.

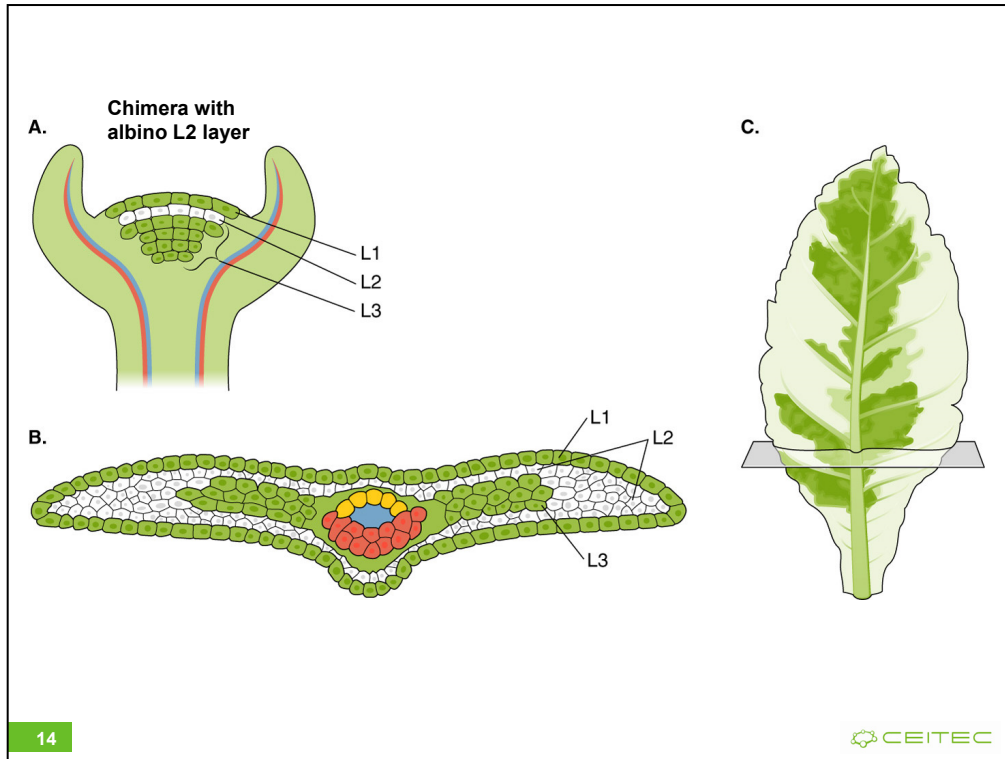


L1 and L2 are single-cell layers, giving rise to *epidermis* and *ground tissue*, respectively.

L1 divides exclusively in anticlinal plane (perpendicular to the surface), expanding thus laterally.

L2 divides also almost exclusively anticlinally and gives rise to *mesophyl cells*.

L3 is multicellular layer, dividing in both anticlinal and periclinal (parallel to surface) planes and forms the *body of new tissues*, including vasculature and germline tissue.



The individual functional units are clonally divergent. The differential contribution of individual meristem cells to the developing lateral organs, e.g. leaves, was investigated using chimeric plants.

Chimeric plants or *chimeras* are plants involving cells of different genotype. These plants can arise via e.g. grafting or mutation.

The plant carrying mutation leading to the albino L2 layer, results in a what is called *variegated leaves*.

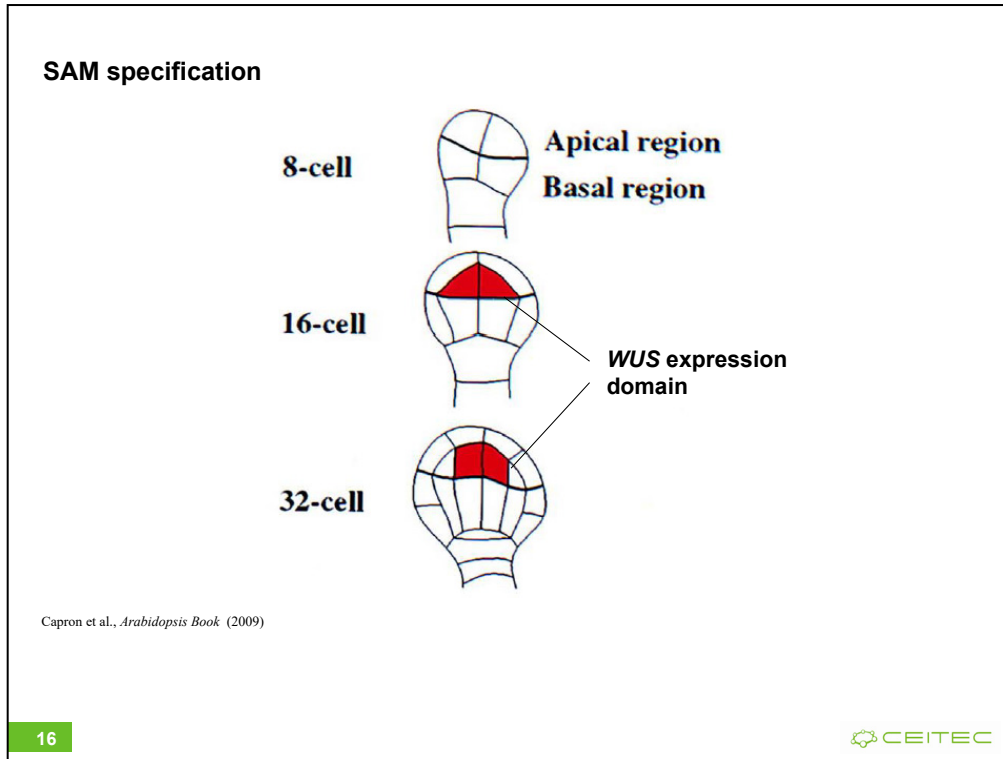
As previously mentioned, the leaf mesophyll cells develop from the L2 layer. The cells mix only rarely in the meristem, however, this might happen in the later leaf development, leading to the jagged boundary of the white and green portions as observed in the leaf on the right (figure C).

However, the cross section through the leaf of the chimeric mutant with albino L2 clearly shows that L2 dominantly contributes to the leaf mesophyll (see figure B).

Outline of Lesson 8

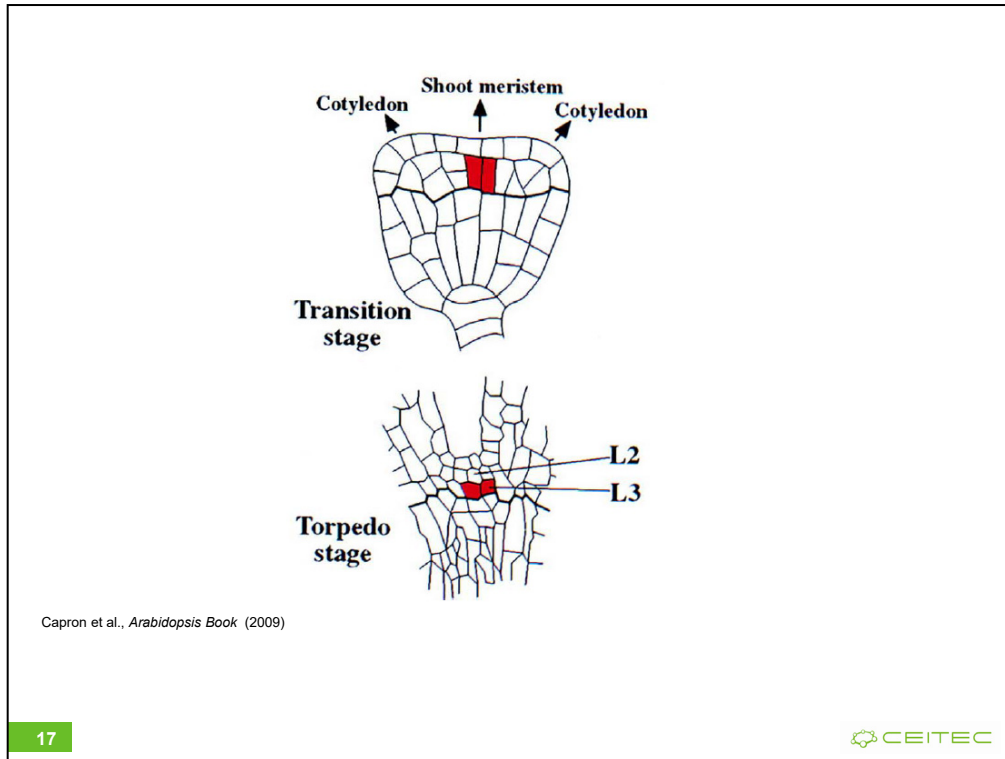
Postembryonic Plant Development

- The role of plant meristems in the plant postembryonic development
- Shoot apical meristem (SAM)
 - Structure of the SAM
 - **SAM establishment and maintenance**

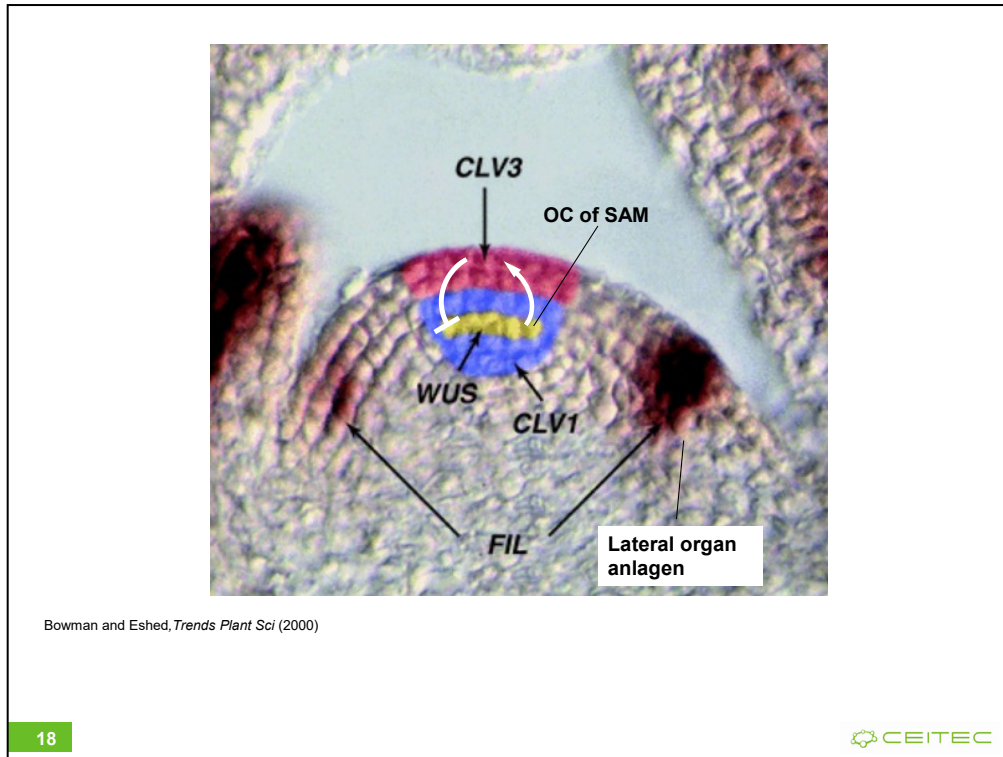


SAM is established in the early embryogenesis (see the Lesson 7).

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



In the later embryonic development, the WUS expression gets delimited to the narrow domain in the L3.



In the postembryonic development, *WUS* expression is detectable just below the upper most layer of L3.

Similarly to previously discussed developmental processes, the spatial-specific gene expression is critical for the equilibrium between cell division allowing maintenance of stem cell pool and their differentiation allowing new primordia formation and growth.

In the SAM, the mutual interactions between *WUSCHEL* (*WUS*) and *CLAVATA* (*CLV*) genes is involved in those processes.

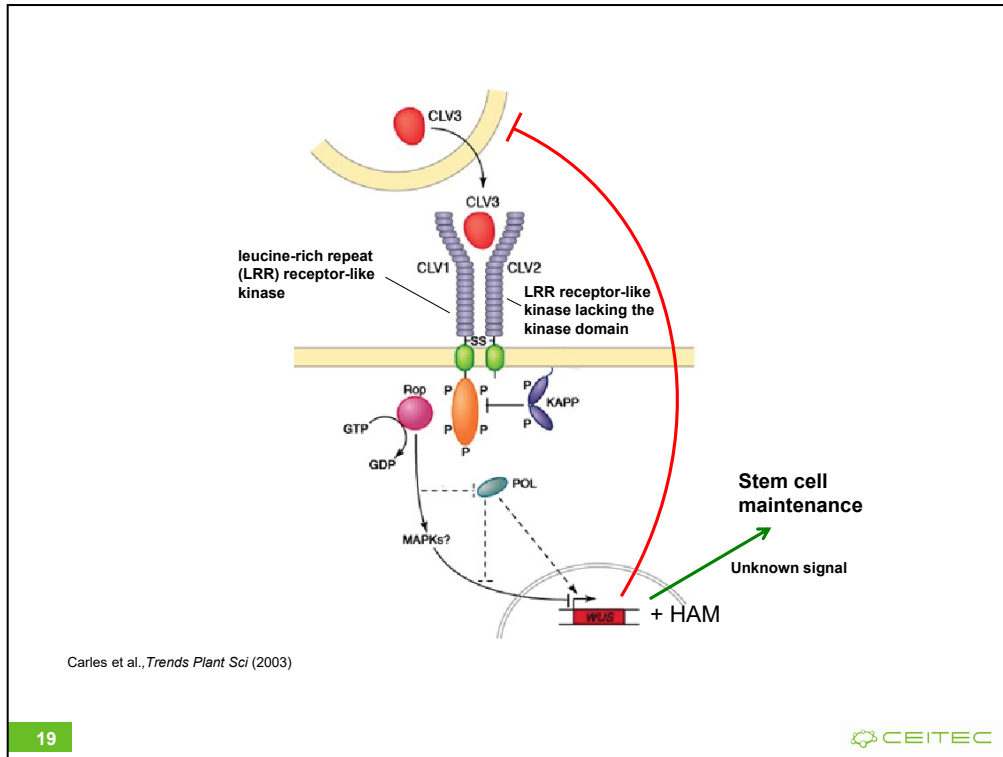
WUS, expressed in the what is called organizing center (OC) induces stem cell fate of the adjacent cells. The nature of the signal mediated by *WUS* (homeodomain-containing TF) is so far unclear.

The expression of *CLV3* is positively regulated by *WUS*. *Vice versa*, *CLV3* downregulates *WUS* and this feed back allows maintenance of the constant size of stem cell niche.

CLV3 encodes a 96 aa peptide that is further processed to give a mature glycopeptide of 12-13 amino acids.

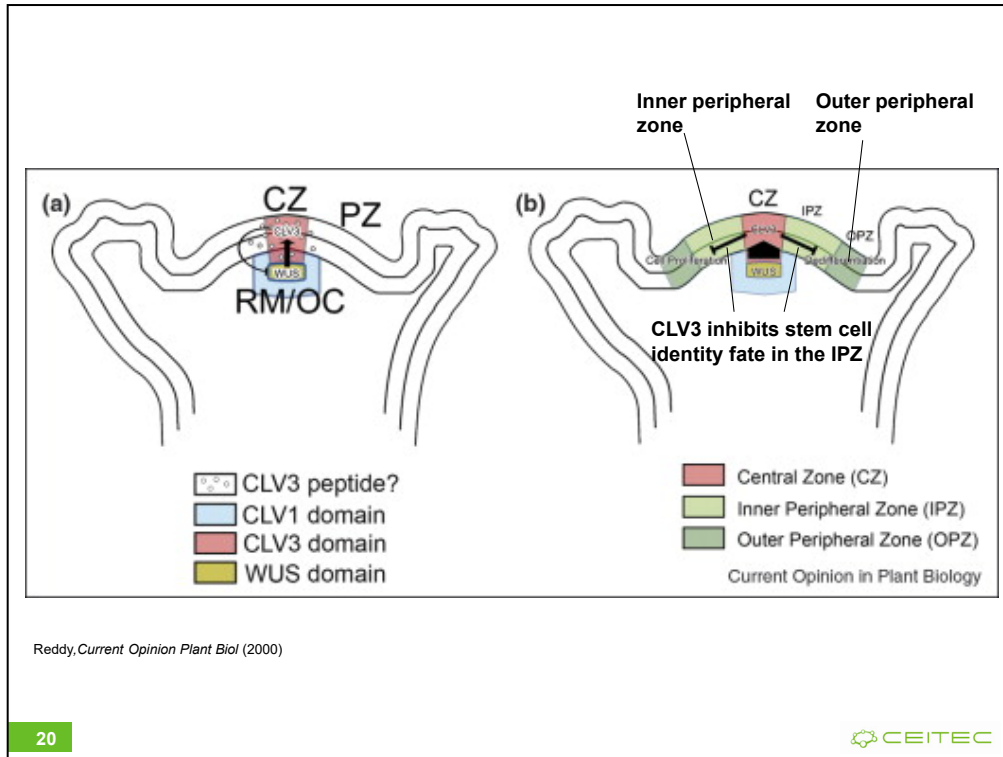
FILAMENTOUS FLOWER (*FIL*) expression occurs in lateral organ anlagen in the peripheral zone.

Anlagen (from German, "Anlage" [pl. Anlagen] corresponds to "conceptus") is used for the morphologically indistinguishable, however, molecularly predetermined tissue where prospective organ primordia will form (compare with expression of *LFY* in floral anlagen, see Lesson 6) .



CLV3 peptide binds to the heterodimer of CLV1/CLV2. CLV1 is a leucine-rich repeat (LRR) receptor-like kinase, and CLV2 encodes an LRR receptor-like protein lacking a kinase domain.

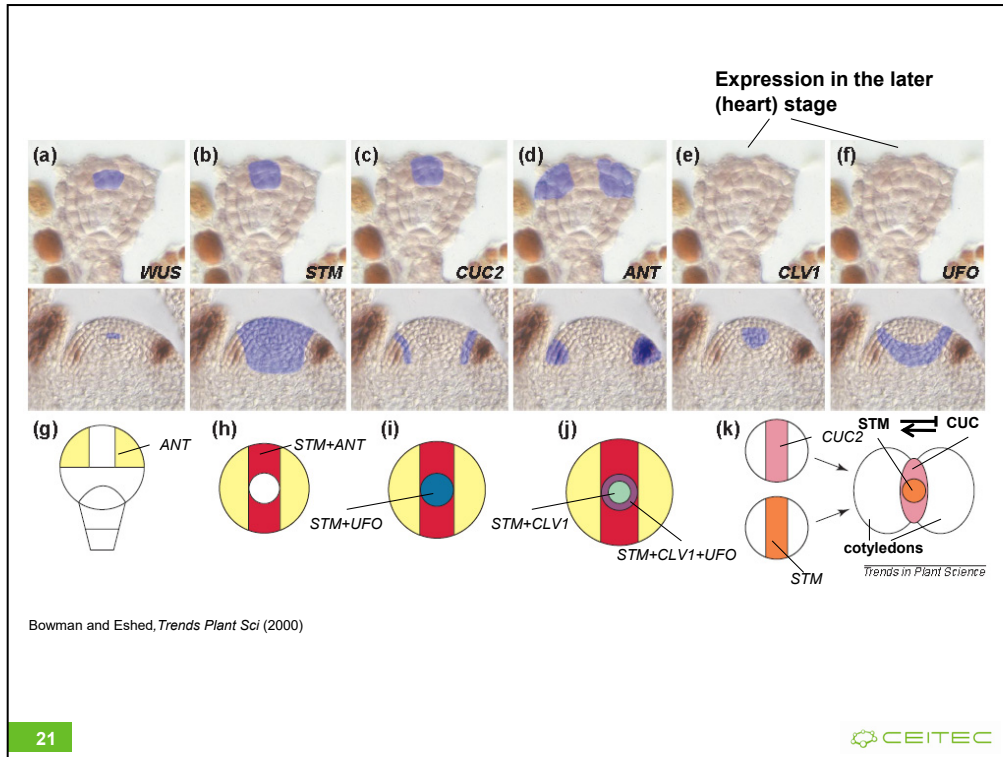
Activation of CLV1 leads to the downstream regulation of mitogen-activated protein kinase (MAPK) pathway, resulting into the *WUS* activation.



Recent experiments with transient downregulation of *CLV3* expression show that the central zone can be further subdivided into outer and inner peripheral zone (OPZ and IPZ, respectively).

Cells located in the IPZ are still responsive to the stem-cell maintaining signal produced by the CZ. However, the cells passing into the OPZ are committed into differentiation pathway and lose their ability to respond to the stem cell signal.

The signal inhibiting the cell proliferation and dedifferentiation in the IPZ is dependent on *CLV3* expression. When *CLV3* is transiently downregulated, the cells in the IPZ invert back to the stem cell developmental status.



There are much more of genes expressed in the SAM, most of them are expressed already in the embryogenesis (see Lesson 7).

Combination of their expression and effects (mostly discussed in the Lesson 7 or above) leads to the establishment and maintenance of the SAM.

On the above figure, there is shown the expression patterns of six genes: the upper panel depicts the expression in a frontal section of a late globular-transition stage of embryogenesis and the lower panel shows expression in a vegetative SAM (see figures a–f).

WUSCHEL (WUS), *SHOOT MERISTEMLESS (STM)*, *CUPSHAPED COTYLEDON2 (CUC2)* and *AINTEGUMENTA (ANT)* are all expressed in the transition stage, whereas expression of *CLAVATA1 (CLV1)* and *UNUSUAL FLORAL ORGANS (UFO)* is not detected until the heart stage.

The expression pattern shown for *CUC2* in the vegetative SAM is an extrapolation of its reported embryonic expression and that observed for *NO APICAL MERISTEM (NAM)*, *PHANTASTICA (PHAN)* and *FILAMENTOUS FLOWER (FIL)* (brown staining in each of the vegetative SAM panels) also mark the lateral organ anlagen in a manner similar to that of *ANT1* (Bowman and Eshed, *Trends Plant Sci*, 2000).

As discussed also in the Lesson 7, there are mutual interactions between genes expressed in the SAM.

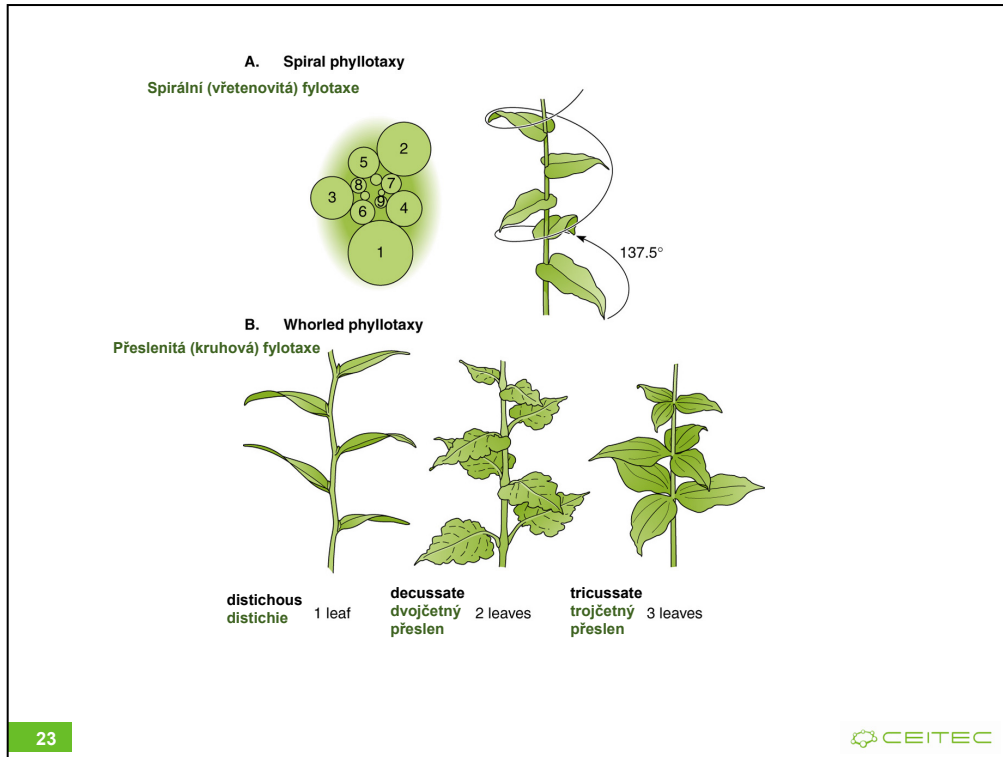
For example, there is spatial interaction between the expression of *CUC* and *STM* genes. *CUC* genes upregulate the expression of *STM* in the central portion, while *STM* negatively regulates *CUC*. That leads to the formation of collar of *CUC* expression around the SAM region (see figure k, view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

There are still being identified new genes revealing distinct expression patterns during SAM (e.g. Yadav et al., *PNAS*, 2008), however, their description is out of the scope of this introductory course.

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- Phyllotaxy
 - Fibonacci series and golden mean in the nature



SAM allows production of vegetative organs, i.e. leaves and the stem.

Leaf primordia are produced by a SAM in a specific pattern, a process called **phyllotaxy**.

There are two phyllotactic patterns in seed plants: Spiral and whorled.

The whorled phyllotaxis could be represented by single leaf per whorl (*distichous*), two leaves per whorl (*decussate*] or three leaves per whorl (*tricussate*, see above).

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0	1	6.2E-01	1 /	1 =	1.0000000000000000
1	1	-3.8E-01	2 /	1 =	2.0000000000000000
2	1	1.2E-01	3 /	2 =	1.5000000000000000
3	1	-4.9E-02	5 /	3 =	1.6666666666666667
4	1	1.8E-02	8 /	5 =	1.6000000000000000
5	1	-7.0E-03	13 /	8 =	1.6250000000000000
6	1	2.6E-03	21 /	13 =	1.615384615384615
7	1	-1.0E-03	34 /	21 =	1.619047619047619
8	1	3.9E-04	55 /	34 =	1.617647058823529
9	1	-1.5E-04	89 /	55 =	1.618181818181818
10	1	5.6E-05	144 /	89 =	1.617977528089888
11	1	-2.2E-05	233 /	144 =	1.618055555555556
12	1	8.2E-06	377 /	233 =	1.618025751072961
13	1	-3.1E-06	610 /	377 =	1.618037135278515
14	1	1.2E-06	987 /	610 =	1.618032786885246
15	1	-4.6E-07	1597 /	987 =	1.618034447821662
16	1	1.8E-07	2584 /	1597 =	1.618033813400125
17	1	-6.7E-08	4181 /	2584 =	1.618034055727554
18	1	2.6E-08	6765 /	4181 =	1.618033963166706
19	1	-9.8E-09	10946 /	6765 =	1.61803398521803
20	1	3.7E-09	17711 /	10946 =	1.618033985017358
21	1	-1.4E-09	28657 /	17711 =	1.618033990175597
22	1	5.4E-10	46368 /	28657 =	1.618033988205325
23	1	-2.1E-10	75025 /	46368 =	1.618033988957902
24	1	7.9E-11	121393 /	75025 =	1.618033988670443
25	1	-3.0E-11	196418 /	121393 =	1.618033988780243
26	1	1.2E-11	317811 /	196418 =	1.618033988738303
27	1	-4.4E-12	514229 /	317811 =	1.618033988754322
28	1	1.7E-12	832040 /	514229 =	1.618033988780204
29	1	-6.5E-13	1346269 /	832040 =	1.618033988750541
30	1	2.5E-13	2178309 /	1346269 =	1.618033988749648



Leonardo Fibonacci (1180-1250)

Fibonacci series: 0, 1, 1, 2, 3, 5, 8, 13, 21...

$$\varphi = \frac{1 + \sqrt{5}}{2} \approx 1,618\ 033\ 988\ 749\ 894\ 848 \dots$$

Wikipedia

Leonardo Fibonacci (1180-1250) discovered the what is called *Fibonacci series*.

It is a series of numbers, where the sum of each two consecutive numbers gives the third in the series.

$a + b / a = a/b = 1.618$
 "golden mean" or "divine ratio"
 "zlatý řez"

1.618= Φ , according to "Fidiios", the creator of Pantheon

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As the numbers in the Fibonacci series increase, the ratio of each two consecutive numbers in the series is getting closer to the value equal approximately **1.618** (see also previous slide).

This is also sometimes called *divine ratio*, *golden mean* or with a greek Φ , according to the name of the Greek architect "Fidiios", the creator of the Pantheon in Roma.

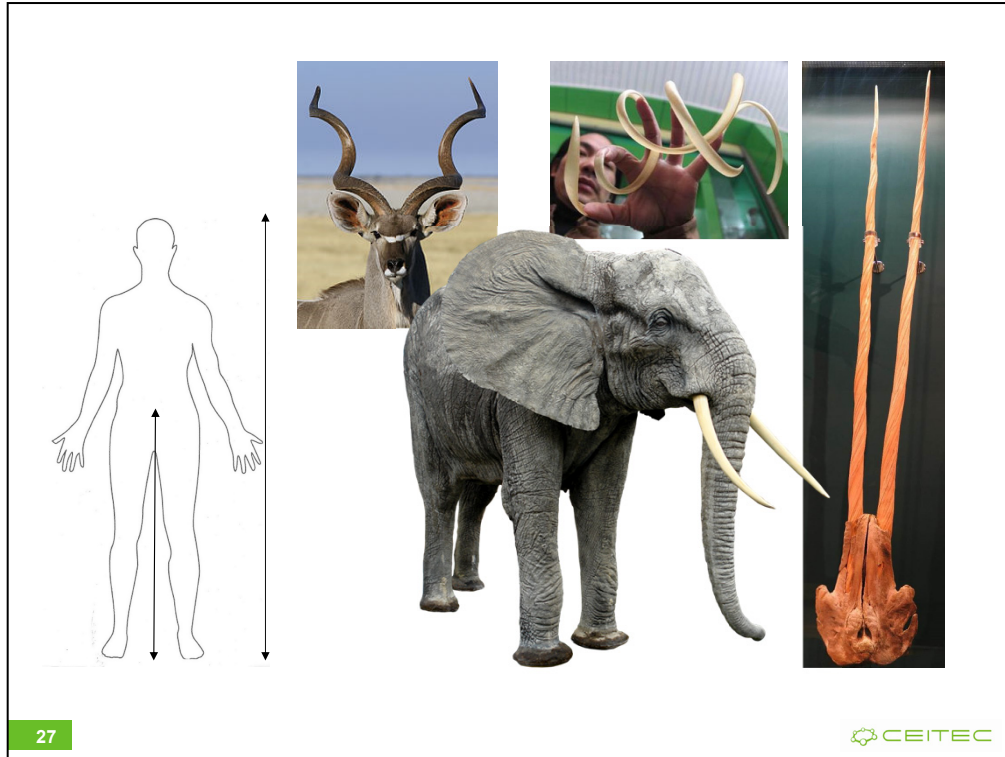
It has been found that the golden ratio occurs in many processes in nature and it is being used by the artists and architects as an ideal and considered to be a measure of beauty.

As an example, a tangencial (Fibonacci) spiral (right, top) is shown and its comparison with a shell of mollusc.

A Fibonacci spiral is created by drawing arcs connecting the opposite corners of squares in the Fibonacci tiling; this one uses squares of sizes 1, 1, 2, 3, 5, 8, 13, 21, and 34.

Fibonacci series – the beauty of math
TED lecture by Arthur Benjamin, <https://youtu.be/SjSHVDfXHQ4>)

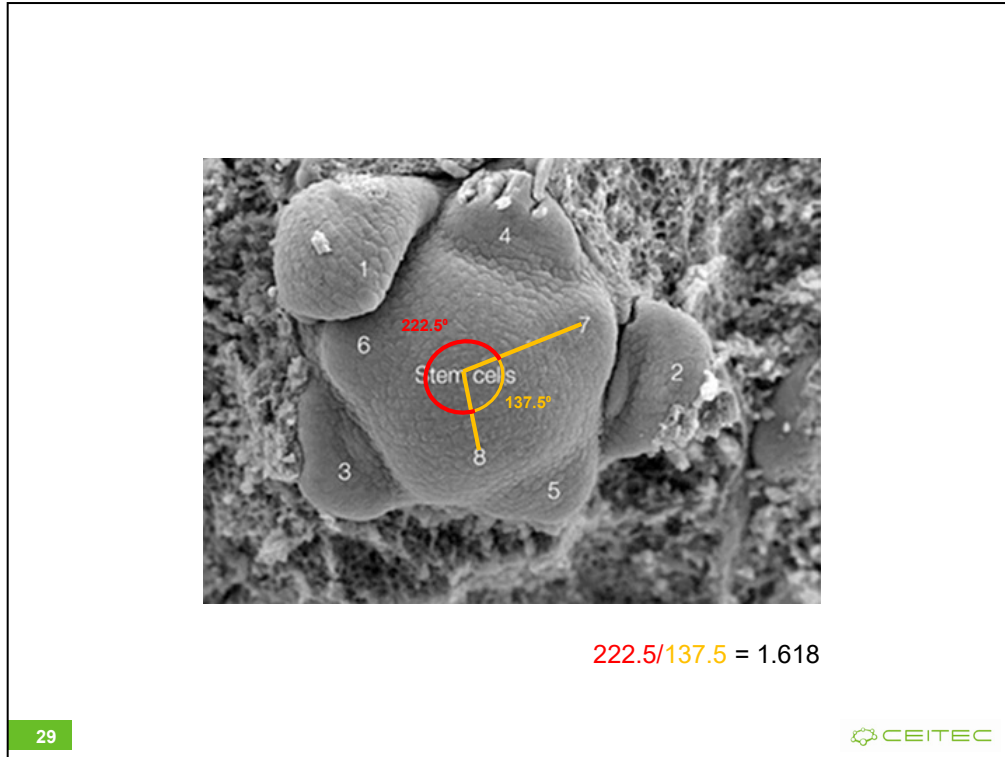




The shape of mollusks' shells is determined by the logarithmic spiral that doesn't change its shape and grows in the same proportion to the width and to the length. In a similar way there could be observed spiral growth of epidermal derivatives, e.g. teeth, nails, hairs, feathers, etc.. This spiral could be observed in case of ivory, the horns of kudu or narwhal teeth. In the human body, the ratio between the golden mean could be also found in humans (the ratio of the height from the top of the body to the height of the umbilicus position).

Golden mean in nature
<https://youtu.be/nt2OIMAJ6o>





In plants with spiral phyllotaxy, the angle between two neighboring primordia is 137.5° .

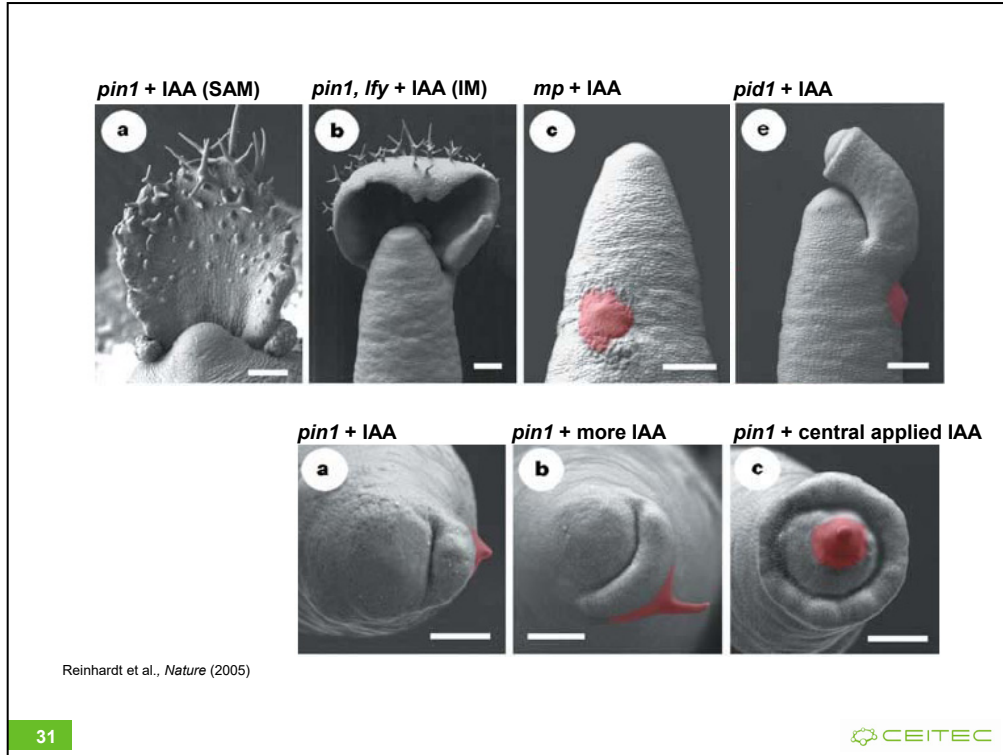
$(360^\circ - 137.5^\circ) / 137.5^\circ = 1.618$. Thus, the Fibonacci series is included in the plant phyllotaxy.

The molecular determinants of that fact have been, however, a mystery for a long time.

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 - **Molecular determinants of phyllotaxy**



Recently, formation of the auxin concentration maxima was found to play a dominant role in the new organ formation by SAM.

In the mutants affected in polar auxin transport or in the plants treated with auxin transport inhibitors, the lateral organs do not form.

However, the auxin application partially rescues the ability of SAM to form lateral organs. In the figure, the red dot corresponds to the auxin application on the mutant in the *PIN-FORMED 1* (*PIN1*), double mutant in *PIN1* and *LEAFY* (*LFY*), *PINOID1* (*PID1*) or *MONOPTEROS* (*MP*).

The *PIN1* gene encodes for a protein involved in the polar auxin transport, *PID1* is protein kinase necessary for the proper localization of *PIN1* and *MP* is *AUX/ARF* TF involved in auxin signalling as discussed in the last lesson (see Lesson 7). *LFY* is floral meristem identity gene (discussed in Lesson 6).

When lanolin paste (coloured red on the figures from the electron scanning microscope) with natural auxin (IAA) is applied laterally on the SAM of *pin1*, it leads to the initiation of the leaf (a).

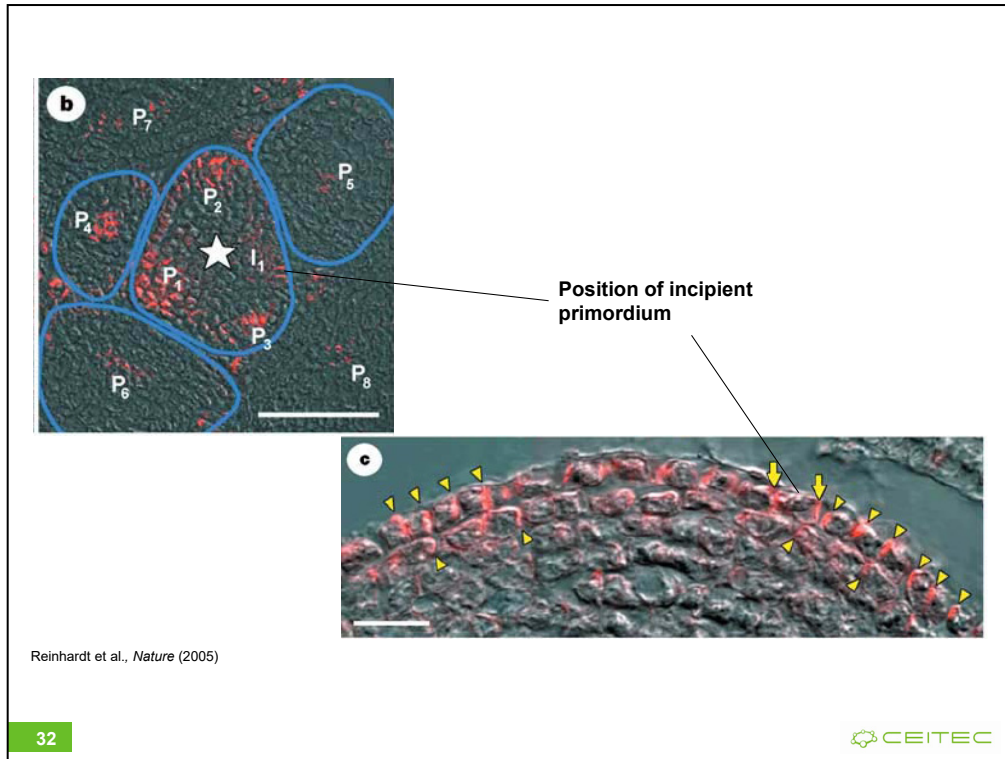
However, when IAA is applied on the inflorescence meristem of double *pin1, lfy* mutant, it leads again to the leaf formation, instead of flower formation. This is due to mutation in the floral meristem identity gene *LFY* (for the role of *LFY* in the flower formation see Lesson 6).

Auxin signalling is necessary for the organ formation as demonstrated by the absence of flower formation upon IAA application on the inflorescence meristem of auxin signalling mutant *mp* (c; for the role of *MP* in the auxin signalling see Lesson 7).

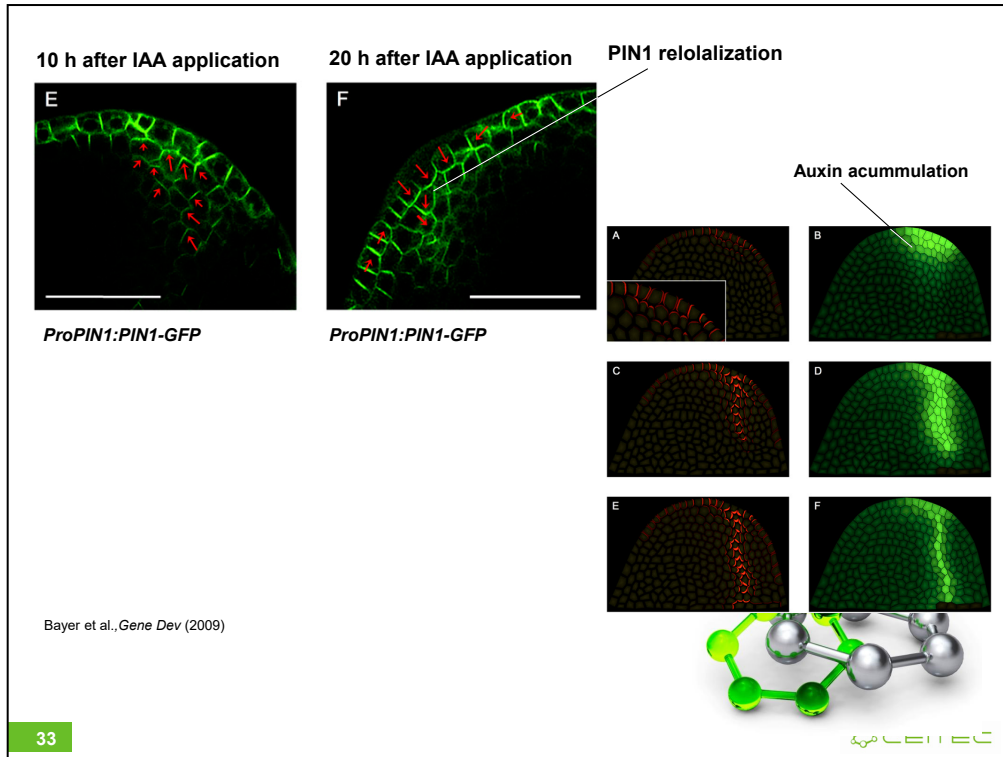
Finally, *pid1* mutation shows flower induction upon IAA application (e).

Importantly, the role of auxin localization on the shape and spacing of the new organ primordia could be demonstrated by the application of different amounts of auxin, as could be seen in the lower row of figures (a, b) and its positioning (c).

The lateral application of higher amount of IAA leads to the wide leaf primordium formation. When IAA is applied to the centre of the SAM, the ring-shaped leaf primordium is induced.



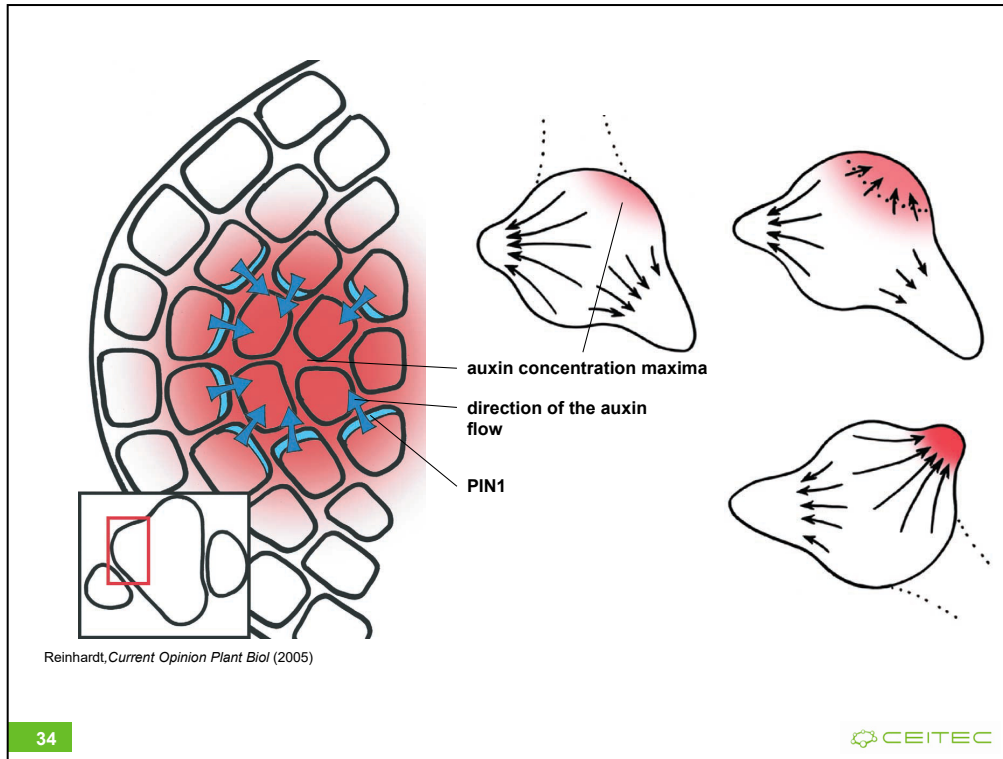
Accordingly, PIN1 was identified to localize in L1 and L2 in that way that it allows auxin accumulation in the position of incipient primordia formation (depicted as I1 in the figure b).



After the new primordium is initiated, PIN1 is induced in the position of prospective vascular tissue (provascular tissue) and its localization is changed from the apical to the basal cell membrane (see the arrows in E and F, respectively).

In figure E, there is PIN1-GFP signal in the SAM 10h (E) and 20H (F) after IAA microapplication.

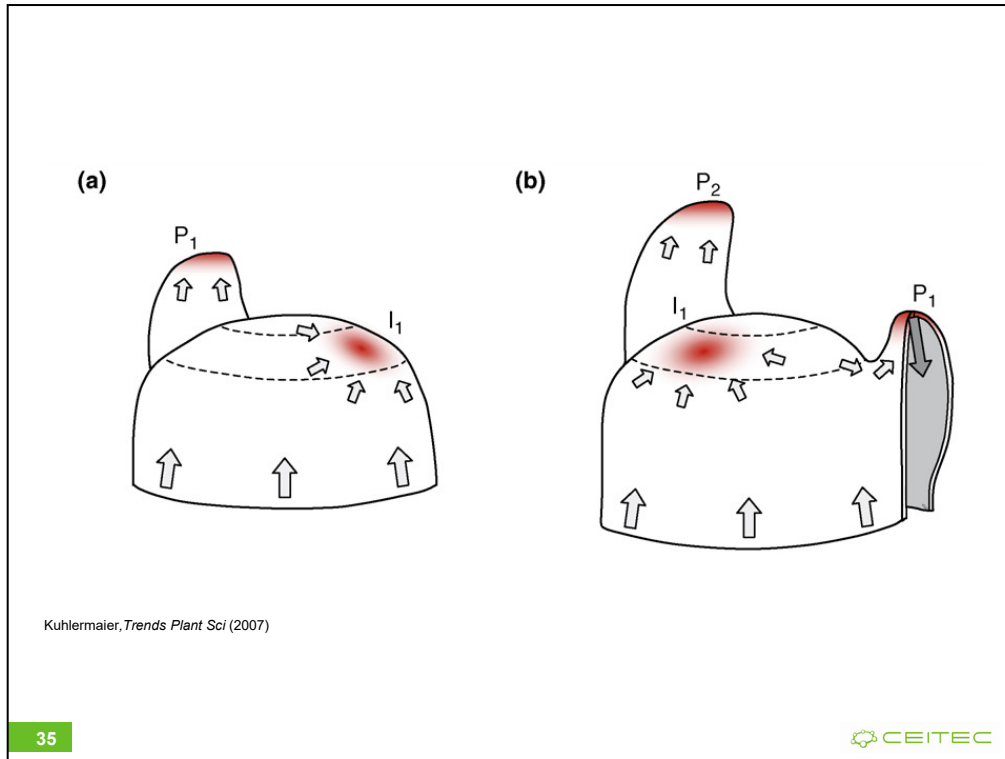
That allows redirectioning of the auxin flow and its “canalization” through the prospective vascular tissue (see the PIN1 localization and model on the right-hand side).



Based on the experimental evidence discussed on the previous slides and several other's, a model describing the role of auxin in the organ formation by shoot apical meristems was proposed.

The model suggests that auxin maxima are created via polar localization of PIN1 in L1.

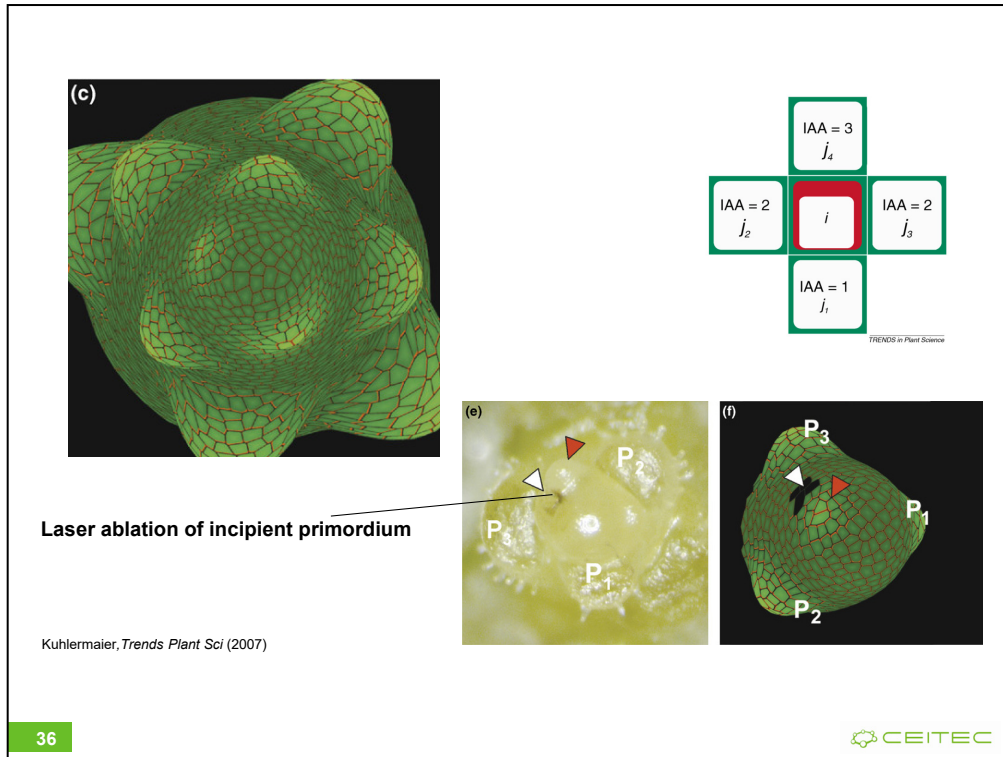
That allows reaching of the threshold auxin concentration necessary for the initiation and growth of the primordium in the position of the auxin maxima (red in the figures above).



The organ primordia act as a sink of auxin, where auxin is pumped away through the provascular tissue.

Consequently, the auxin concentration in the close vicinity of the induced primordia is decreased, leading to the inhibition of new organ primordia initiation.

Thus, auxin maxima seem to provide positional information and by the feed-back the auxin maxima regulate PIN1 localization, accelerating thus auxin concentration.



Quantitative computer models of phyllotaxy were recently introduced.

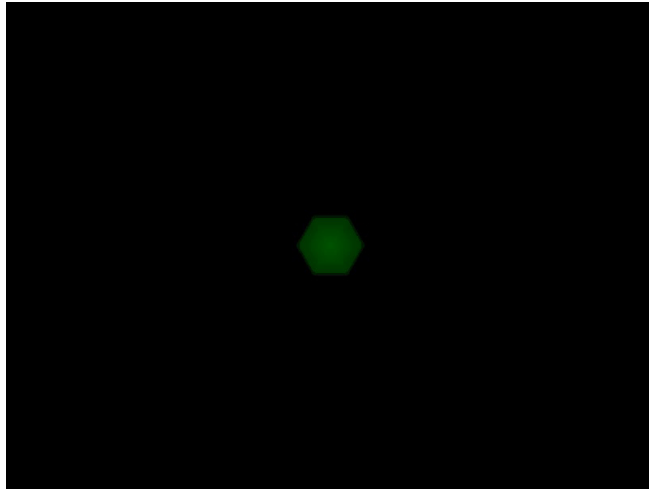
These models are based on several prerequisites:

1. The distribution of the PIN1 in cell i towards the membranes facing each of the four surrounding cells j_1 – j_4 depends on the relative auxin concentrations in these neighbouring cells (see the figure above).
2. The rate of auxin transport depends on auxin concentration.
3. Within incipient primordia, PIN1 polarization is slightly different in the sense that PIN1 orients more strongly towards the centre of the primordium.
4. Cellular growth is modelled by representing the cells as circles with mechanical interactions.

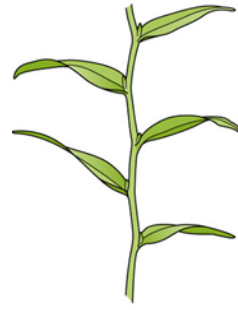
The model published by Smith et al. (PNAS, 2006) is robust enough to calculate formation of both whorled and spiral phyllotactic patterns (see the movies on the later slides, the spiral phyllotaxis is shown in figure c on this slide).

In these simulations, changes to multiple parameters were needed to obtain different phyllotaxis types. The simplest change, from distichous to decussate, involved increasing IAA production, decreasing the width of the peripheral zone, and increasing the size of the central zone. The requirement for such complex changes may explain the almost universal failure to generate mutants that change one stable phyllotactic pattern into another (Smith et al., PNAS, 2006).

The model is also able to predict the effects of surgical modification, i.e. ablation of incipient primordium I1 (white arrowhead in figure e), leading to the initiation of a new primordium in the close vicinity (red arrowhead).

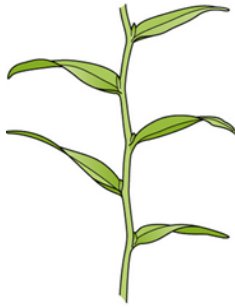


Distichous
Distichie



1 leaf

Distichous
Distichie

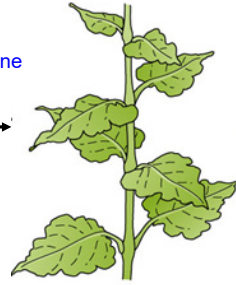


1 leaf

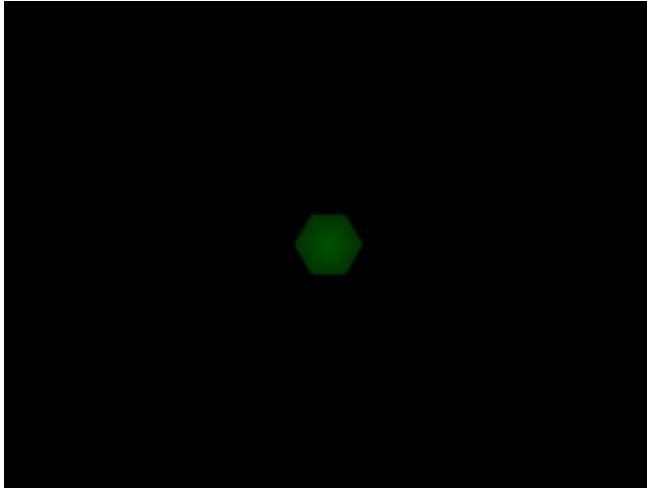
- increasing IAA production
- decreasing the width of the peripheral zone
- increasing the size of the central zone



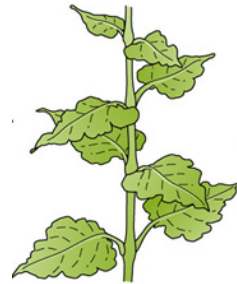
Decussate
Dvojčtý přeslen



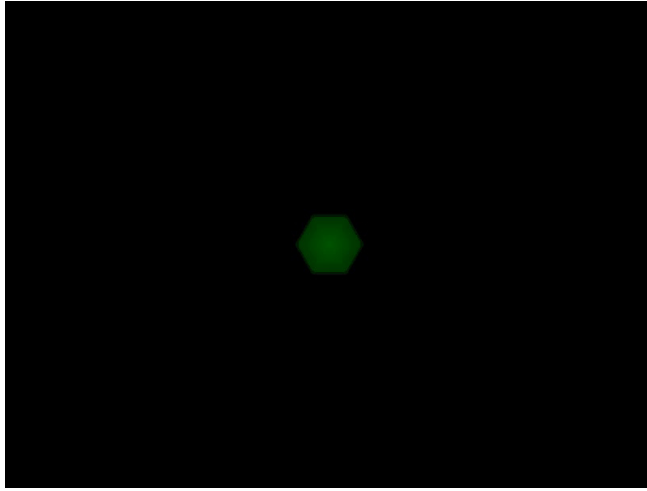
2 leaves



Decussate
Dvojčetný přeslen



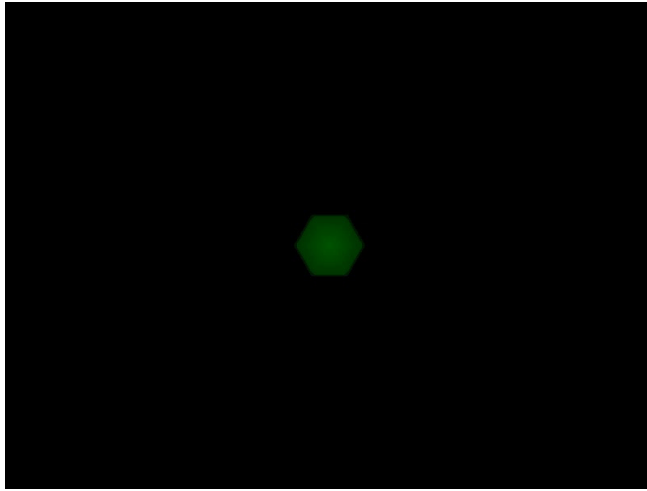
2 leaves



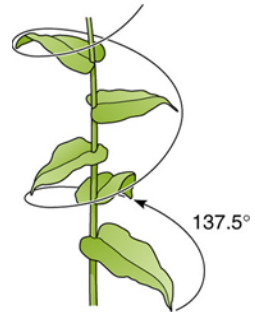
Tricussate
Trojčetný přeslen



3 leaves



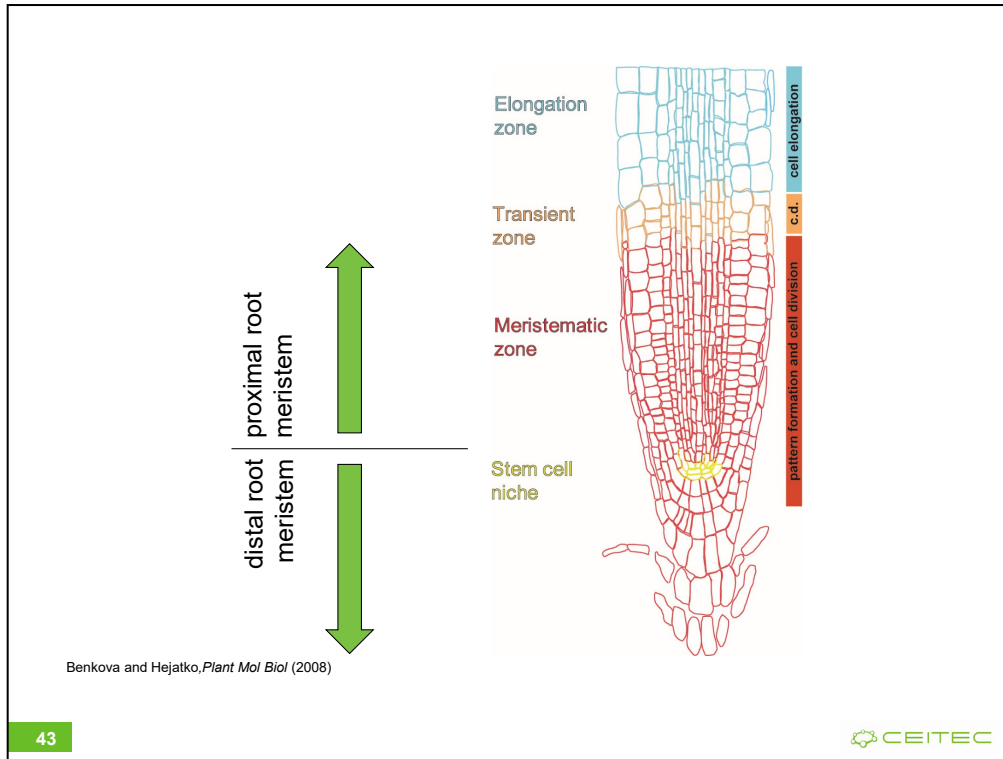
Spiral
Spirálovitá fylotaxe



Outline of Lesson 8

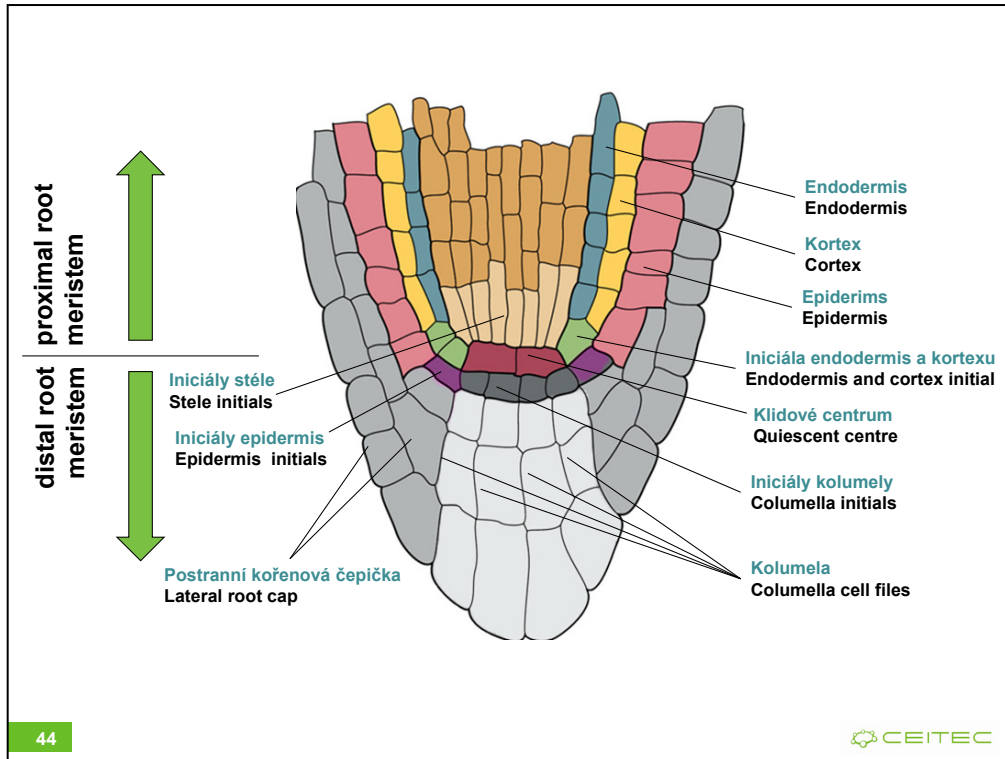
Postembryonic Plant Development

- The role of plant meristems in the plant postembryonic development
- Shoot apical meristem (SAM)
 - Structure of the SAM
 - SAM establishment and maintenance
- Phyllotaxy
 - Fibonacci series and golden mean in the nature
 - Molecular determinants of phyllotaxy
- Root apical meristem (RAM)
 - RAM structure



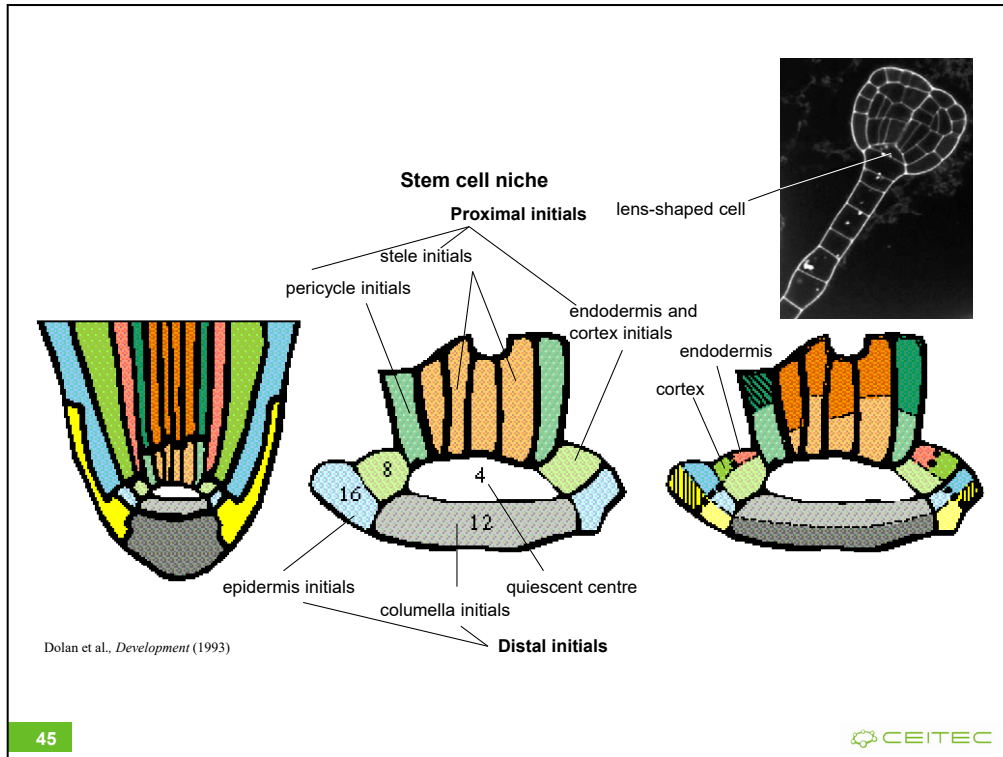
In the root, several functional and anatomical units could be recognized.

Along the longitudinal axis, the root meristem forms a distal root tip, including stem cell niche, columella and lateral root cap, proximal meristem with a population of rapidly dividing cells and elongation zone where cells leaving the root meristem undergo rapid elongation and mature.



The apical growth of the root allows root apical meristem (RAM).

Similarly to SAM, several functional and anatomical domains could be distinguished there.



The “core” of the root meristem is formed by the what is called *stem cell niche*, surrounding quiescent center.

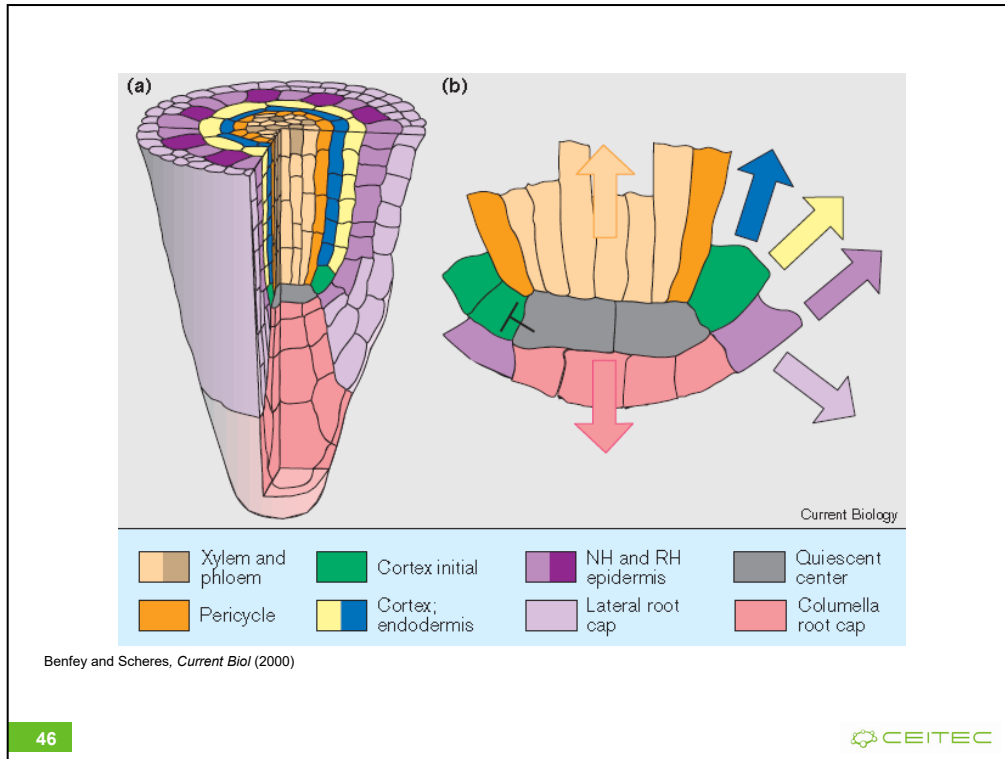
The quiescent center is established soon in the embryogenesis, when the uppermost cell of the hypophysis divides, leading to the lens-shaped cell formation (see inset).

This cell divides only during early embryogenesis, forming thus a group of four cells that later do not divide at all. Because of their mitotic inactivity, these cells are called *quiescent center* (QC).

QC acts as an **organizer of the root meristem** as will be discussed in more details later.

QC is surrounded by the stem cell niche. The stem cells in the root meristem are partially predefined and could be further subdivided in proximal and distal *meristem initials*.

In the proximal meristem, there are initials of *stele and pericycle*, while in the distal portion, there are common initials of *endodermis and cortex* (see the right-hand side figure) and initials of *epidermis and columella*.



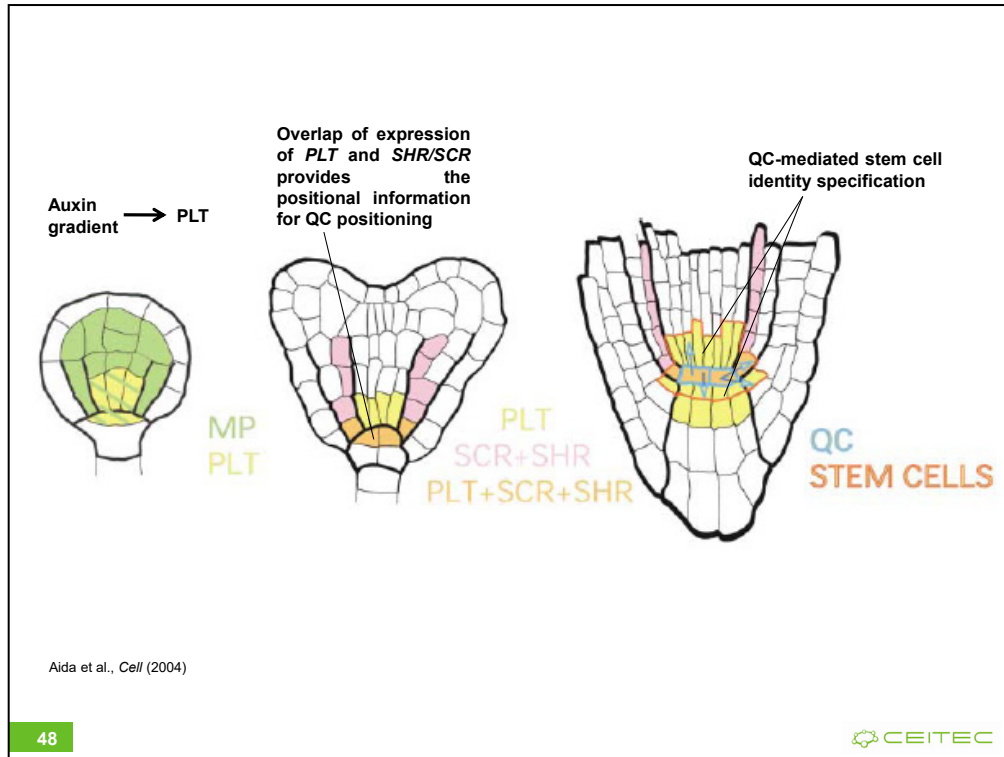
Oriented cell division of the stem cells (initials) and differentiation of their progeny results in the continuous growth of both distal and proximal root portions.

The arrows show the directions of oriented cell divisions of individual initials.

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 - Positioning of RAM organizing centre



Recently, the molecular mechanisms driving the positioning of root meristem organizing centre, the QC and the identity of individual cells were identified. These mechanisms were discussed mostly in the Lesson 7.

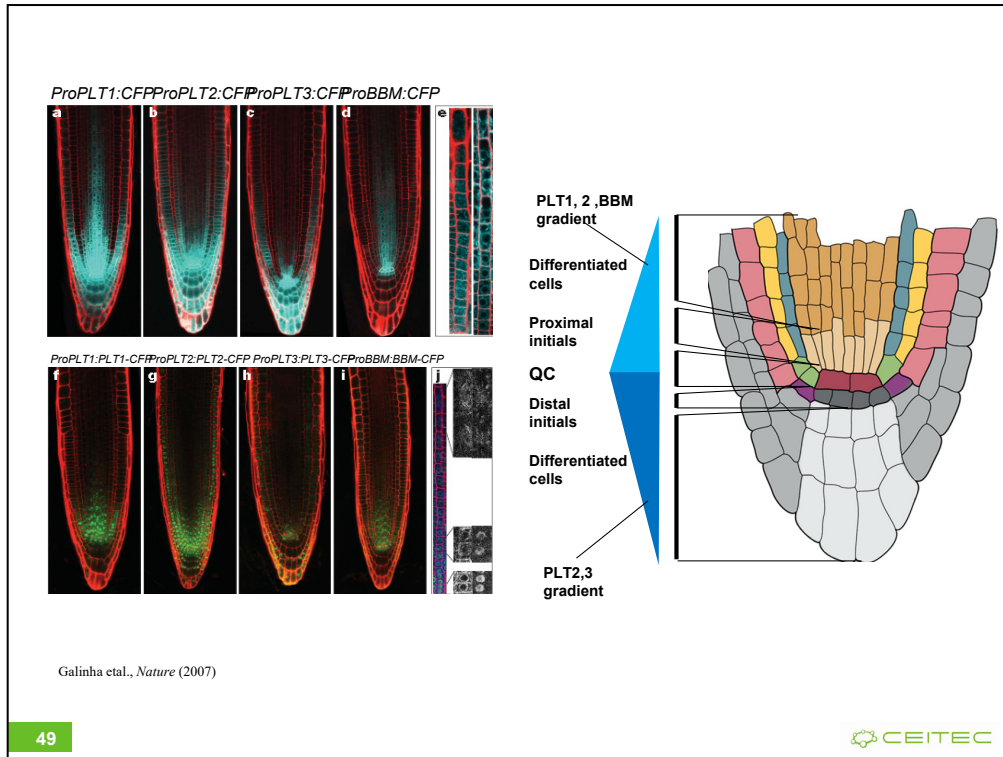
There are several key factors, that are involved in those processes. In brief, the master regulators are auxin gradients and expression of specific transcription factors, some of them being regulated by the auxin gradient.

The role of *SHR* and *SCR*, the putative TF from the GRAS family in the radial embryonal root patterning was discussed before (see the Lesson 7) and will be discussed later, too.

In addition to that, the expression of *SHR* and *SCR* determines the ability of expressing cells (what is called in a *cell autonomous manner*) to become QC.

Auxin gradient is formed via orchestrated action of several auxin transporters, particularly the auxin efflux carriers from the PIN family.

Subsequently, auxin induces expression of genes from the *PLETHORA* family that encode AP2 class of putative transcription factors. The overlap of expression of *PLT*, *SHR* and *SCR* provides the positional clue and specifies the QC cell identity (see the figure).

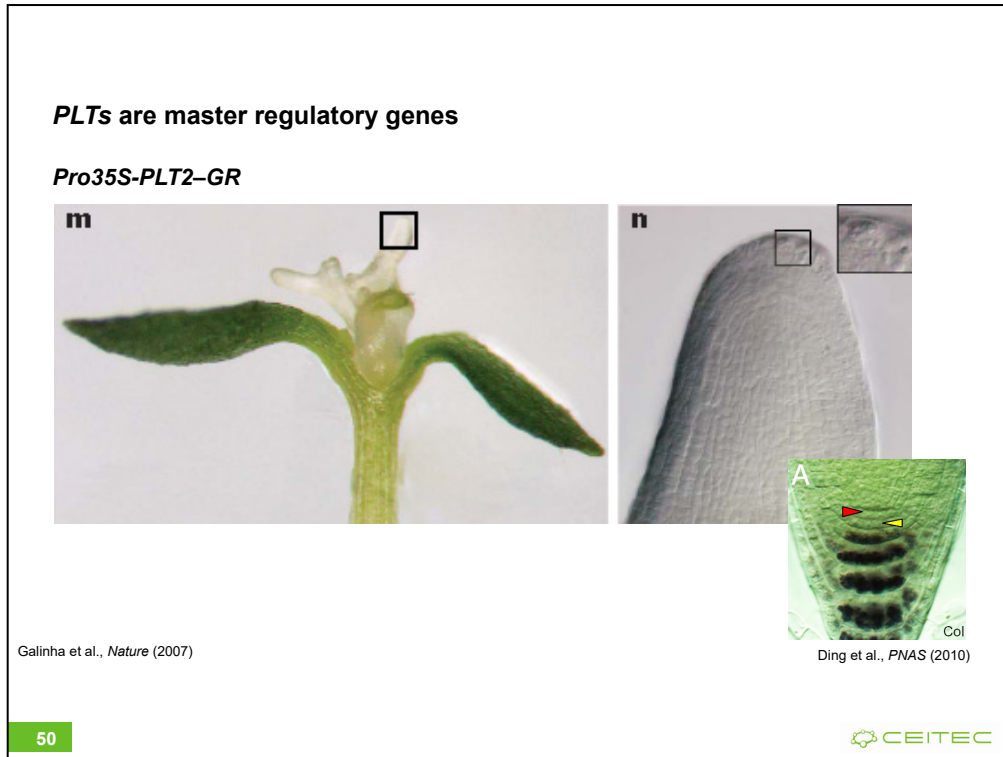


The PLT proteins act in a dose-dependent manner.

Both, transcripts of those genes (upper row) and their protein products (lower row) show the gradient along the longitudinal axis (left-hand figure).

Thus PLT act as a morphogen in plants. High levels of PLT activity promote stem cell identity and maintenance; lower levels promote mitotic activity of stem cell daughters; and further reduction in levels is required for cell differentiation (Galinha et al., *Nature*, 2007).

The gradient of morphogens represents common developmental module in many organisms. Compare gradients of PLT in plants (this Lesson), HUNCHBACK in *Drosophila* (Lesson 2) or SHH in vertebrates (Lesson 4).

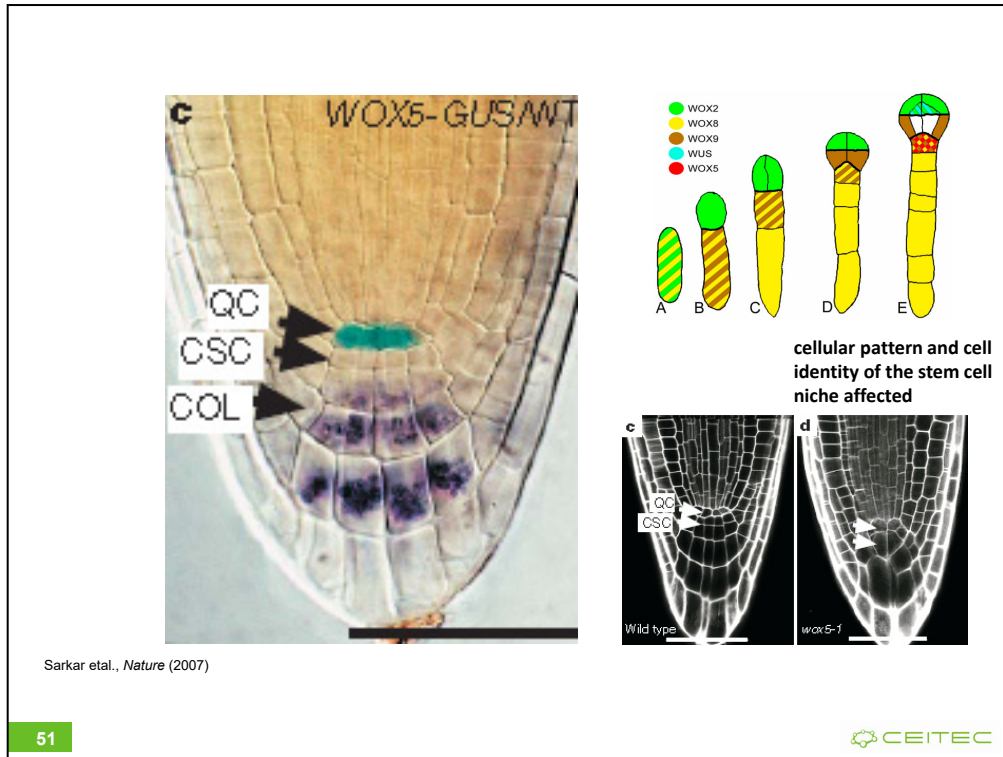


PLT genes act as master regulators of the plant cell identity. That was proven by the ectopic expression of *PLT2*, leading to the ectopic root tissue formation from the SAM.

The tiny purple staining in the inset of the figure **n** (right) proves root tissue identity (lugol staining specific for starch granules normally accumulating in the root columella cells – see the inset, red arrowhead QC, yellow arrowhead columella initials).

In the figure **m**, there is a phenotype of *Arabidopsis* seedlings overexpressing translational fusion of PLT with GR domain after 6 days after application of steroid analogue dexamethasone (DEX). Presence of DEX allows conformational change of GR, allowing nuclear translocation of PLT-GR and its action in the downstream gene regulation.

This represents common mechanism of transgene regulation (for more details on the different mechanisms allowing regulation of transgene expression, see my lecture “Bi7201, Genomics, a basic course”).



Besides the *SHR*, *SCR* and *PLT* genes, the expression of *WUSCHEL-RELATED HOMEODOMAIN 5* (*WOX5*), discussed in the previous lesson (see the inset) was shown to be important for the specification of the root meristem organizing center, the QC.

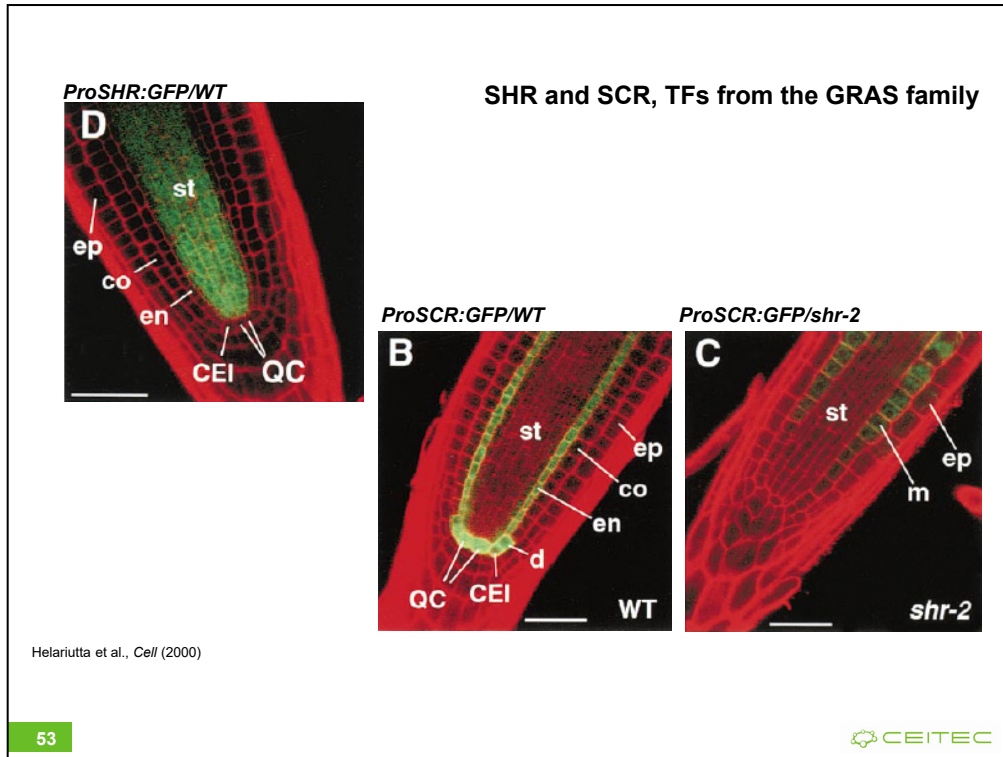
In the *wox5* mutants, the cellular pattern and cell identity of the stem cell niche is affected (see the figures c, d on the right)

This suggests that similar mechanisms act in the shoot and in the root development.

Outline of Lesson 8

Postembryonic Plant Development

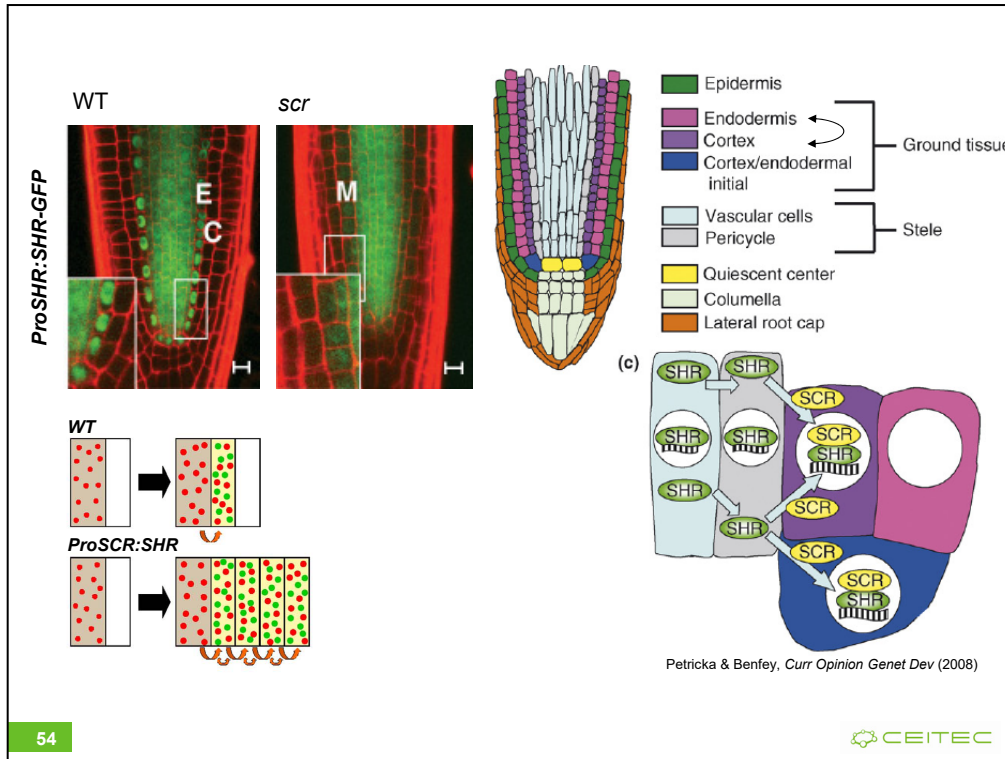
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 - Positioning of RAM organization centre
 - **Radial root patterning**



The radial root patterning is determined by the expression of several genes, among them *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*), the putative TF from the GRAS family.

SHR is expressed in the stele, while *SCR* expression is induced in the adjacent cellular layer, the endodermis. *SHR* protein is transported to the adjacent cellular layer, endodermis, where it induces the expression of *SCR*.

On the figures, there are roots of transgenic lines carrying GFP under control of *SHR* or *SCR* promoters (*ProSHR* and *ProSCR*, respectively). These data show that *SHR* is necessary for the expression of *SCR*, as could be seen by the absence of *SCR* promoter activity in the *shr-2* mutant (*ProSCR:GFP/shr-2*).



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).

This mechanism is necessary for the radial organization (radial patterning or radial symmetry) of the root.

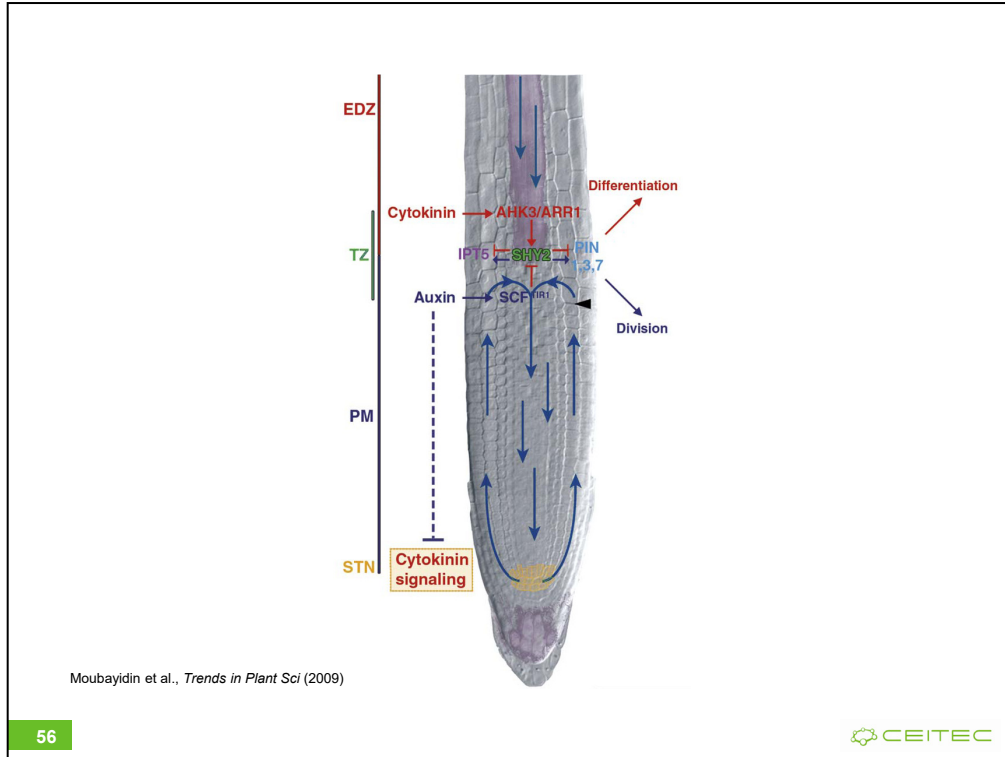
The SHR-induced SCR expression and SCR-mediated sequestration of SHR into the nuclei of single cell layer adjacent to the expression domain of SHR determines the differential developmental fate after asymmetrical division of the common endodermis and cortex initials.

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).

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 - Radial root patterning
 - **RAM size determination**



Similarly to the SAM situation, the size of the root meristem must be kept constant to ensure proper root growth and development.

This is achieved, as previously mentioned in the case of SAM, via equilibrium between cell proliferation and cell differentiation.

Recently, the role of two phytohormones, auxin and cytokinin was identified in those processes.

There is quite complicated network of mutual interactions between auxin transport, biosynthesis and other hormones, including cytokinins.

Briefly, auxin induce cell proliferation, while cytokinins act in the what is called *transition zone (TZ)* to induce cell differentiation. This is observable as a cell elongation (see the figure).

Stem cell niche (STN), the proximal meristem (PM), the elongation differentiation zone (EDZ).

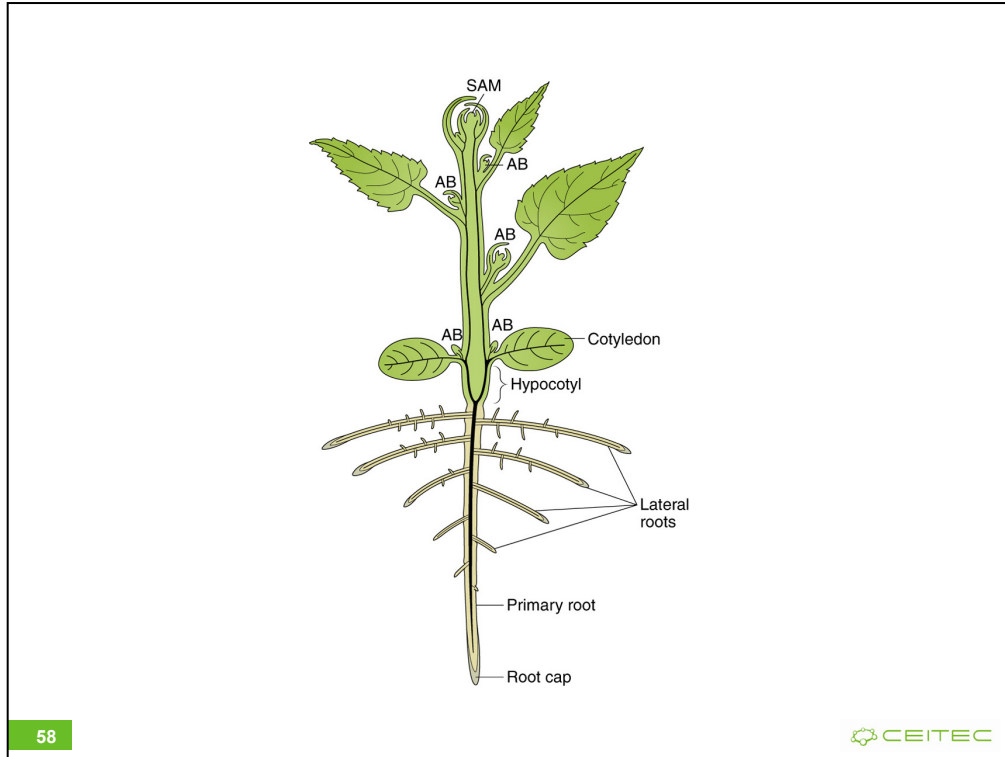
Black arrowhead indicates the cortex tissue file TZ. Blue arrows represent PINs-mediated auxin flux direction, and purple labelling represents the cytokinin biosynthesis gene IPT5 (ATP/ADP-isopentenyltransferase 5) expression.

In the vascular tissue TZ, cytokinin-mediated activation of the SHY2 (SHORTHYPOCOTYL 2) gene, through the AHK3/ARR1 two-component signaling pathway, leads to PIN1, PIN3 and PIN7 downregulation and cell differentiation. By contrast, auxin mediates SHY2 protein degradation through the SCF/TIR1 complex, thus sustaining PINs activity and cell division. The SHY2 negative control on auxin transport on the one hand, and of cytokinin biosynthesis (through IPT5 induction) on the other, confer robustness to the cytokinin-auxin feedback loop regulation [44]. In post-embryonic STN (cells highlighted in yellow), auxin might repress cytokinin signalling activating type-A ARR1s (blue broken arrow), prompting cell division.

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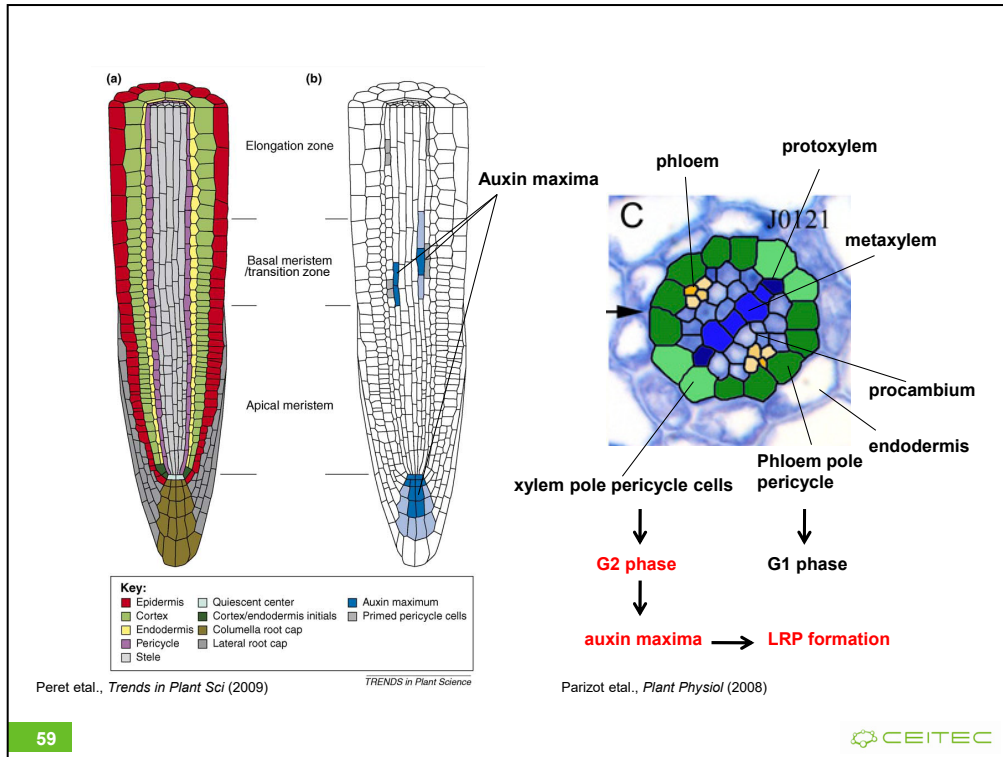
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 - Root radial patterning
 - RAM size determination
- **Lateral root formation**



Besides the main root, there are also lateral organs formed in the root. However, in contrast to the SAM, the lateral roots are not a direct product of the RAM.

Instead, new meristems are established along the main root that allow *lateral root primordia* (LRP) formation.



The problem associated with LRP formation is what determines the cells to acquire the identity of **lateral root founder cell**.

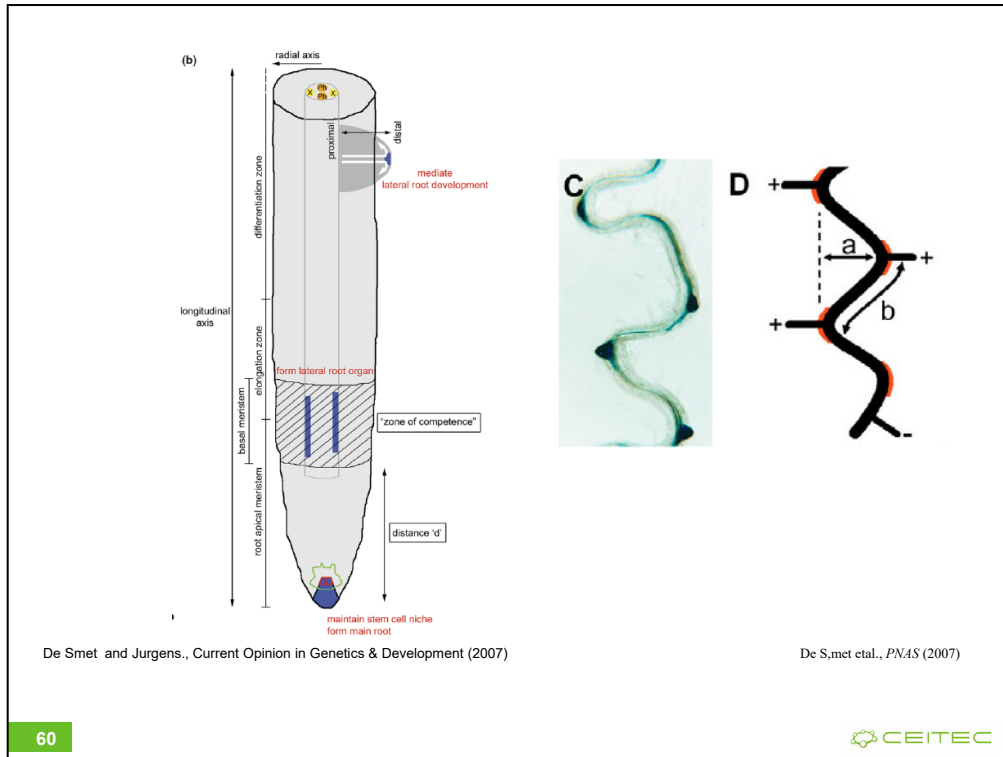
Recently it has been found that the cells are predetermined to develop LRP very early, in the region of the root meristem called basal meristem (see in the figure above, left).

Only specific cells in the pericycle are competent to become the lateral root founder cells. These cells are located at the what is called xylem pole, i.e. in the pericycle cells facing the protoxylem cells (see the figure C).

After leaving the root apical meristem, pericycle cells at the phloem poles remain in the G1 phase, whereas those at the xylem poles advance to the G2 phase of the cell cycle.

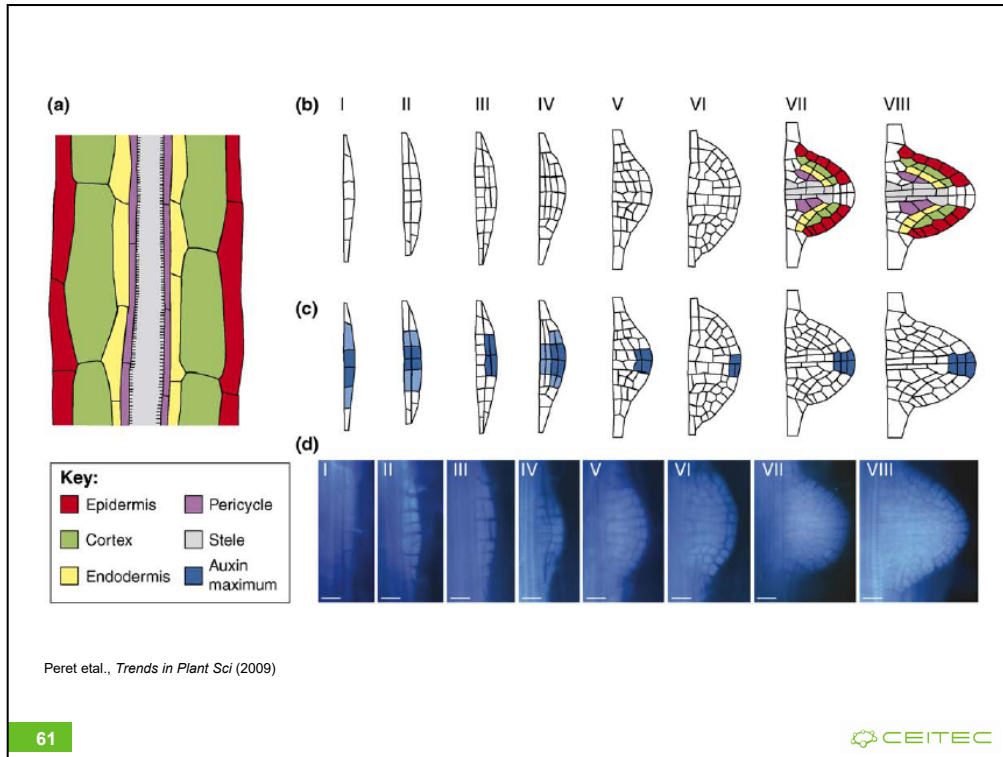
These cells are also considered as an “extended meristem”, as they maintain their ability to divide even after exiting the root apical meristem.

The xylem pole pericycle cells in the basal meristem are primed to divide by auxin signalling.



Priming of pericycle founder cells is correlated with an oscillating auxin response in adjacent xylem cells. It has been demonstrated that these oscillations occur at regular intervals of 15 h and were associated with the induction of new LRP in adjacent pericycle cells.

The spacing of lateral roots along the *Arabidopsis* primary root can also be influenced by tropic responses and mechanical stimuli. For example, it has been reported that a gravitropic stimulus can induce LRP to form on the outer side of bending roots (see Figures C and D). However, the mechanism that would allow changes of auxin distribution in a response to root bending is still not clear.

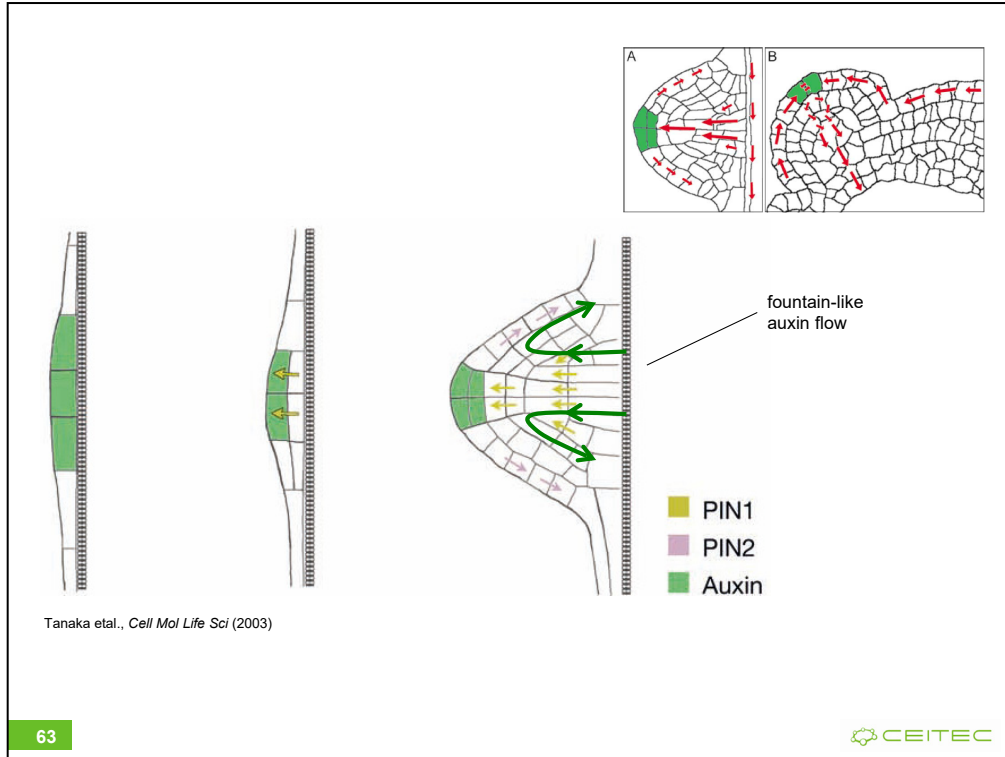


Auxin accumulation triggers the whole developmental pathway leading to the formation of LRP. A complicated gene interactions are regulating the whole process and their description is beyond the scope of this introductory lessons.

However, again, auxin maxima and its interaction with cytokinins are important regulators of the LRP development.



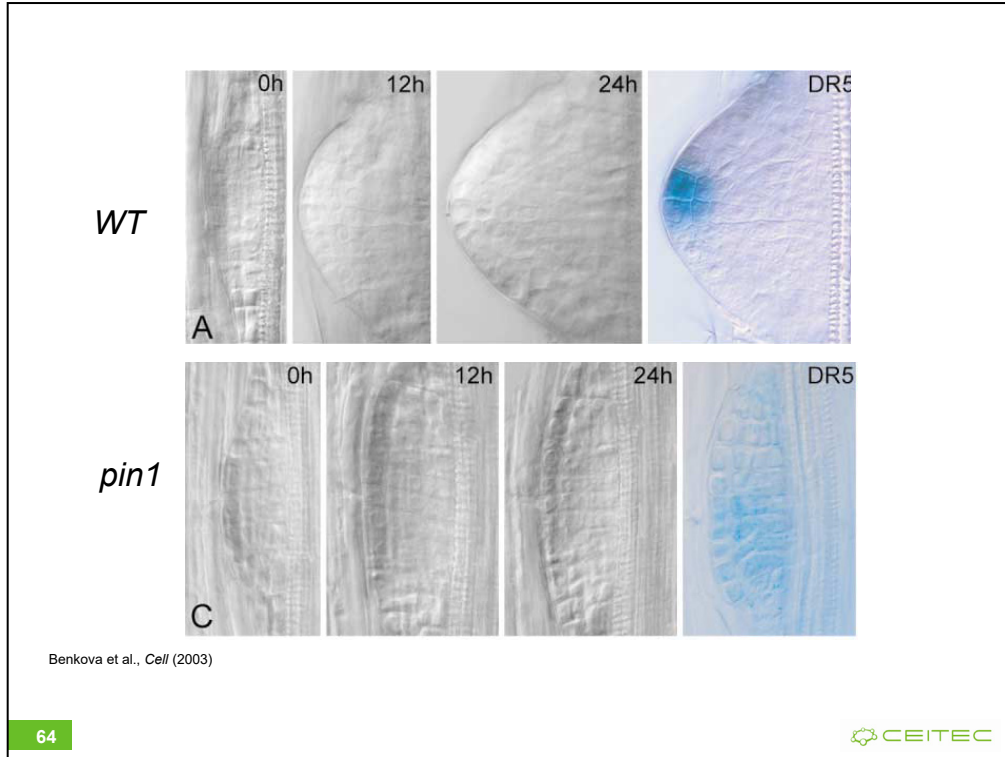
Auxin maxima are kept during the whole process of the LRP development at the tip of the primordia.



As discussed previously, orchestrated action of auxin transporters, particularly from the PIN family, is involved in the oriented auxin maxima formation and maintenance (blue staining in the DIC micrographs above).

The oriented localization of PIN proteins results into “fountain-like” auxin flow (green lines in the figure on the right-hand side).

Interestingly, the auxin flow in the case of roots is reverse in comparison to the auxin flow in the SAM-formed primordia (“fountain” vs. “reverse fountain” respectively, see in previous slides and in the inset).

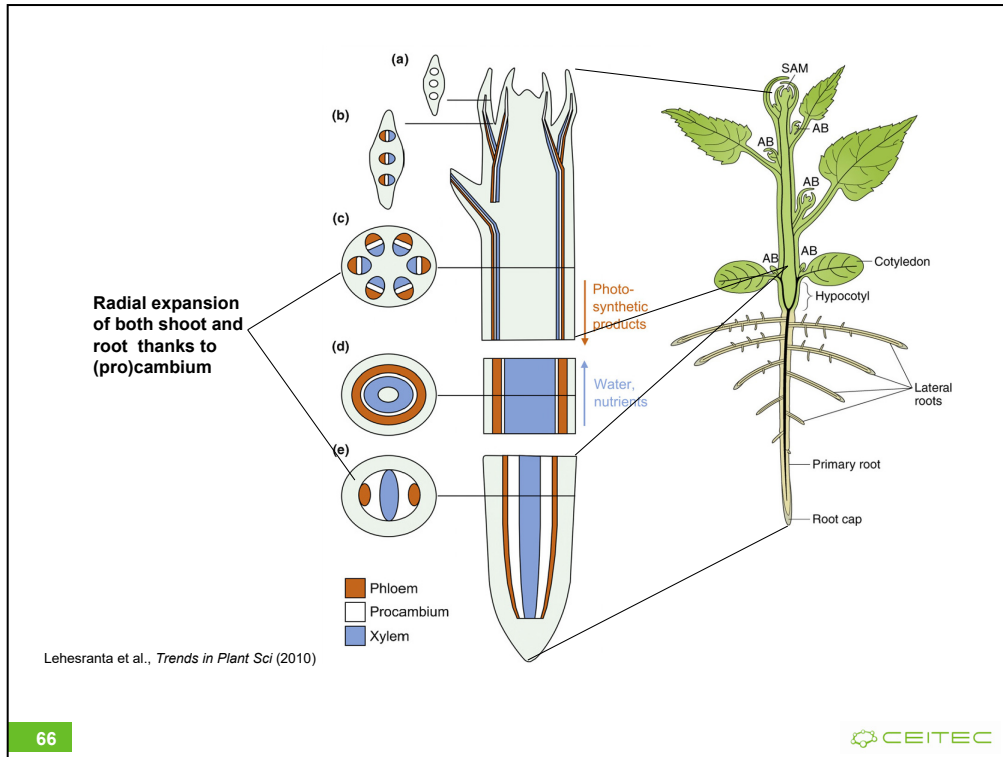


In the *pin* mutants, the auxin maxima formation and LRP development are affected.

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 - RAM size determination
- Lateral root formation
- **Vascular tissue formation in shoot and root**



Besides the apical meristems that allow apical growth, there are also additional meristems in plants, ensuring radial expansion of plants during their lifespan.

These are particularly meristematic tissues involved in the vascular tissue formation.

Plant vascular tissues, similarly to animals, are important for the distribution of nutrients and signals in the plant body.

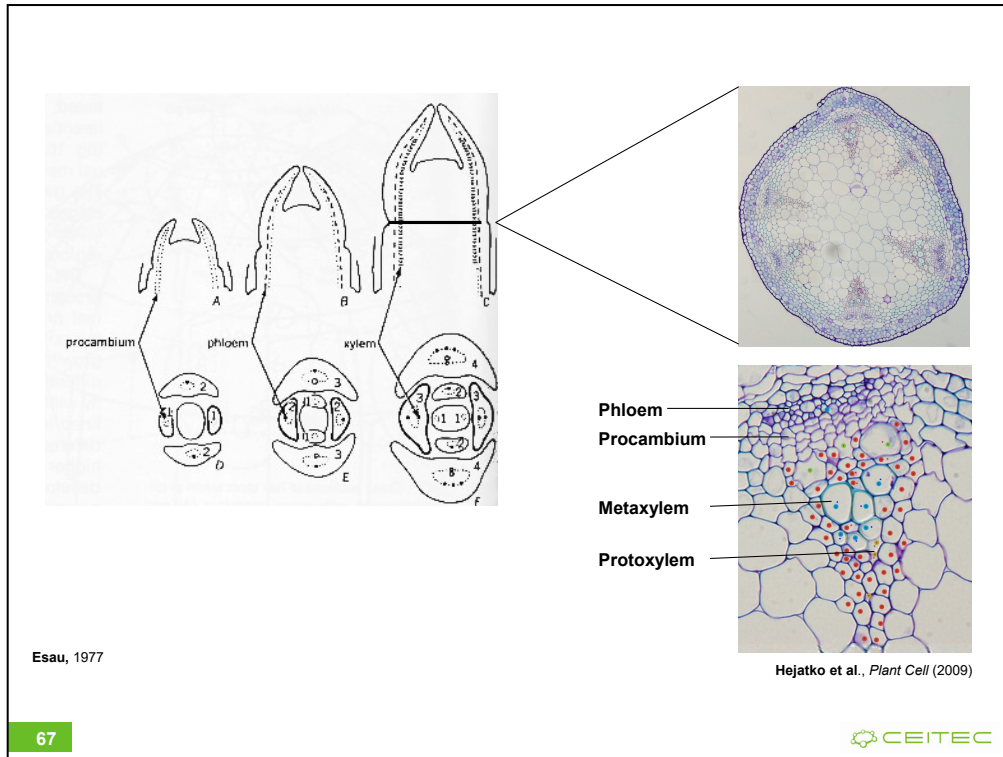
In seed plants, the organization of individual cell types of vascular tissues differ in the shoot and in the root.

As shown in the figure, the vascular tissue in the shoot consist of what is called *vascular bundles* and has radial symmetry.

In the root, the vascular tissue has bilateral symmetry with central xylem axis.

During plant growth, the vascular tissue is formed *de novo* in the tissue adjacent to both apical and root meristem. The meristem allowing formation of primary vascular tissue is called **procambium**.

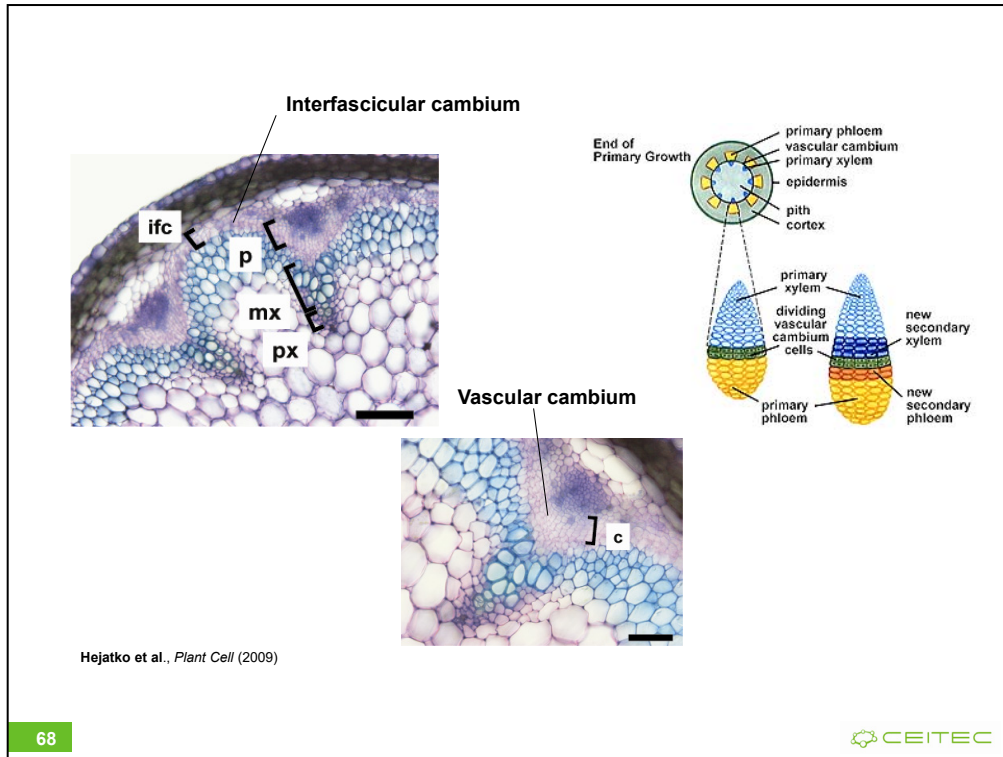
During a process called *secondary thickening*, the new meristem called **cambium** forms and give rise to vascular tissue that continuously surrounds both stem and root.



In the shoot, the vascular tissue development is coordinated with leaf or floral organ primordia formation.

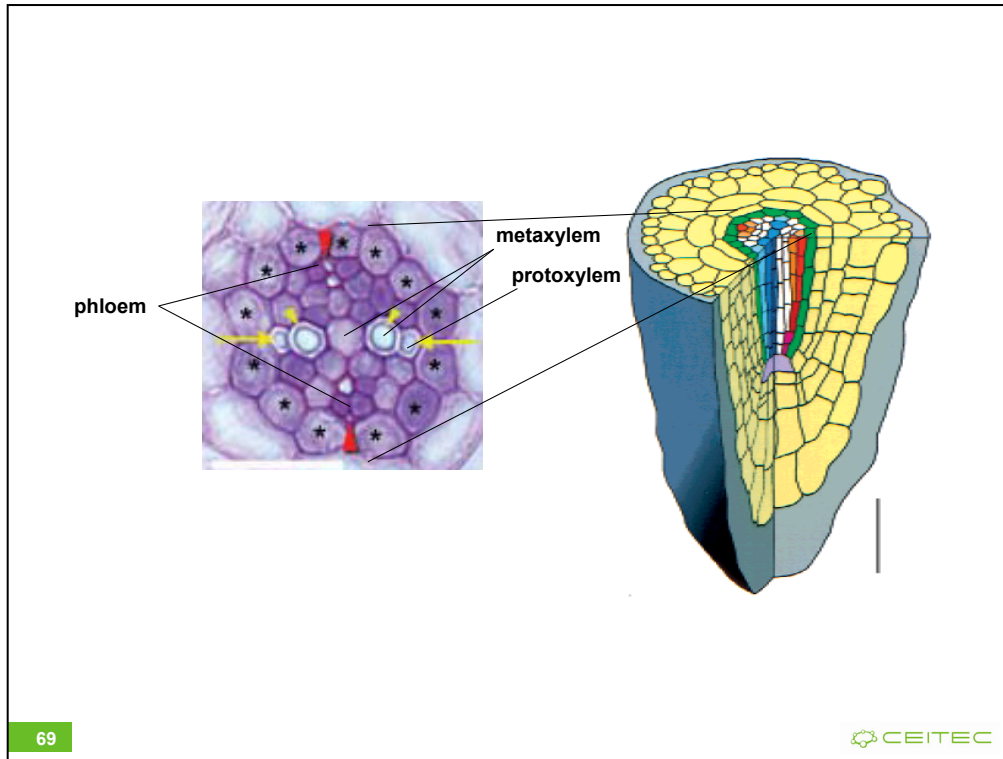
Procambium differentiates first that allows **protoxylem** formation.

In *Arabidopsis*, protoxylem is active only during early stages. Elongation phase of the stem growth leads to its desintegration and his role is overtaken by later developed xylem tissue, the **metaxylem**.



Later on in the development, vascular cambium differentiates from the procambium. In the interfascicular regions, *interfascicular cambium* differentiates from the phloem parenchyma and starch sheath cells.

Both the fascicular and interfascicular cambium give rise to **secondary phloem** and **secondary xylem**.



In the root, the vascular tissue is organized in a central portion and has bilateral symmetry.

The what is called *xylem axis* is formed by the central positioned **metaxylem**, surrounded on both sides by **protoxylem** cells in the root.

Complex gene interactions and hormonal control regulate vascular tissue formation in both shoot and root. The underlying molecular mechanisms will be discussed later.

Key Concepts

Postembryonic Plant Development

- Plants, in contrast to animals, form most of their tissues and organs during **postembryonic development** via **postembryonic de novo organogenesis**.
- Both shoot and root growth occurs via **directed cell proliferation** and **differentiation** in plant **meristems**.
- **Organizing centres** are formed in both shoot and root apical meristems.
- **Auxin gradients** determine **novel organ initiation** and **spacing** in the shoot apical meristem.
- **Auxin-driven morphogen gradient** acts in the **specification of the stem cell niche** and **cell differentiation** in the root.
- **Auxin maxima** specify positions of novel organ formation e.g. **lateral root primordia**.
- **(Pro)cambium** contains **stem cell pool** and allows **vascular tissue formation** and **radial growth** of plants.

Discussion