Plant Development
Cell and Developmental Biology Part 1B
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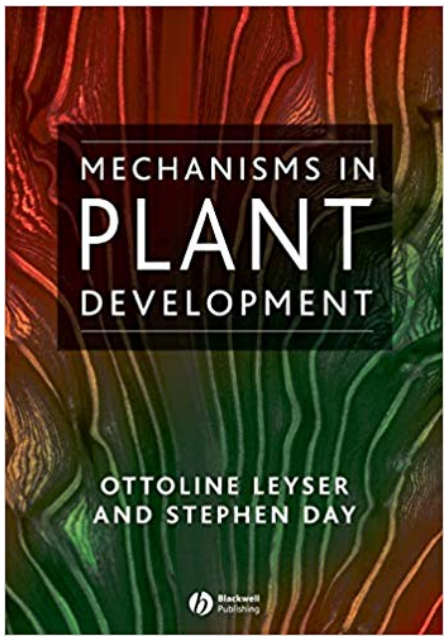
Summary:
In these four lectures we will look at some striking features of biological self-organisation and morphogenesis using examples from the model plant, *Arabidopsis thaliana*. Plant cells are immobile, constrained by a rigid cell wall – yet plant development is plastic and indeterminate. Communication between neighbouring cells controls plant cell fate, and plays a major role in shaping plant growth. We will examine the major role that auxin plays in short-range and long-distance. coordination of growth.

Lecture 1: Plant embryogenesis and establishment of the body plan.
Lecture 2: Polarity, auxin traffic and auxin response.
Lecture 3: Regulation of root initiation and polar growth by auxin.
Lecture 4: Meristems and patterning of plant growth

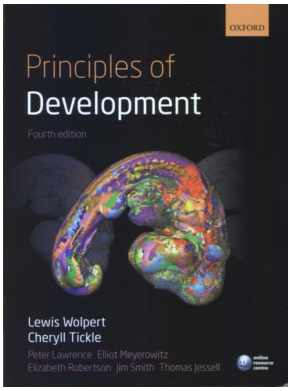
Web resources:
An electronic version of the lecture slides, a colour version of these notes and additional teaching materials can be found on the Moodle site).

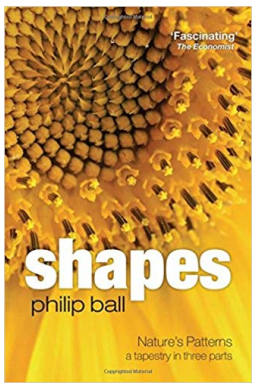


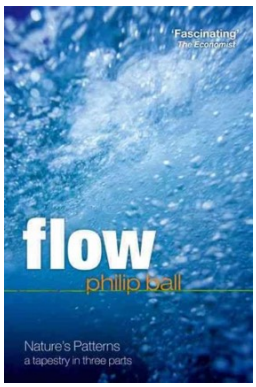
Recommended Text books:
For coverage of plant development see:
***Mechanisms in Plant Development*, Ottoline Leyser & Stephen Day, Blackwell Science, UK, 2002.**

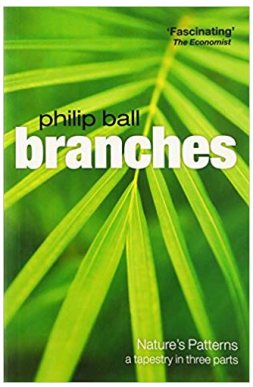
For an integrated overview of animal and plant development see:
***Principles of Development*, Lewis Wolpert and Cheryll Tickle, Oxford University Press, 2011.** Chapter 7 provides a concise overview of content directly relevant to plants.

For a general discussion of self-organisation across physical and biological systems see:
***Nature's patterns: a tapestry in three parts, Shapes, Flow and Branches*, Phillip Ball, Oxford University Press, 2009.**









Lecture 1: Plant embryogenesis and establishment of the body plan.

The genome of Arabidopsis thaliana has been sequenced and its 125 Mb contains over 26,000 genes. Early development of the plant shows regular patterns of cell division and differentiation. Adult growth of the plant is due to the indeterminate activities of meristems which are established during embryogenesis. Arabidopsis is ideally suited for genetic studies, and mutant plants have been screened for defects in development.

Extended reading:

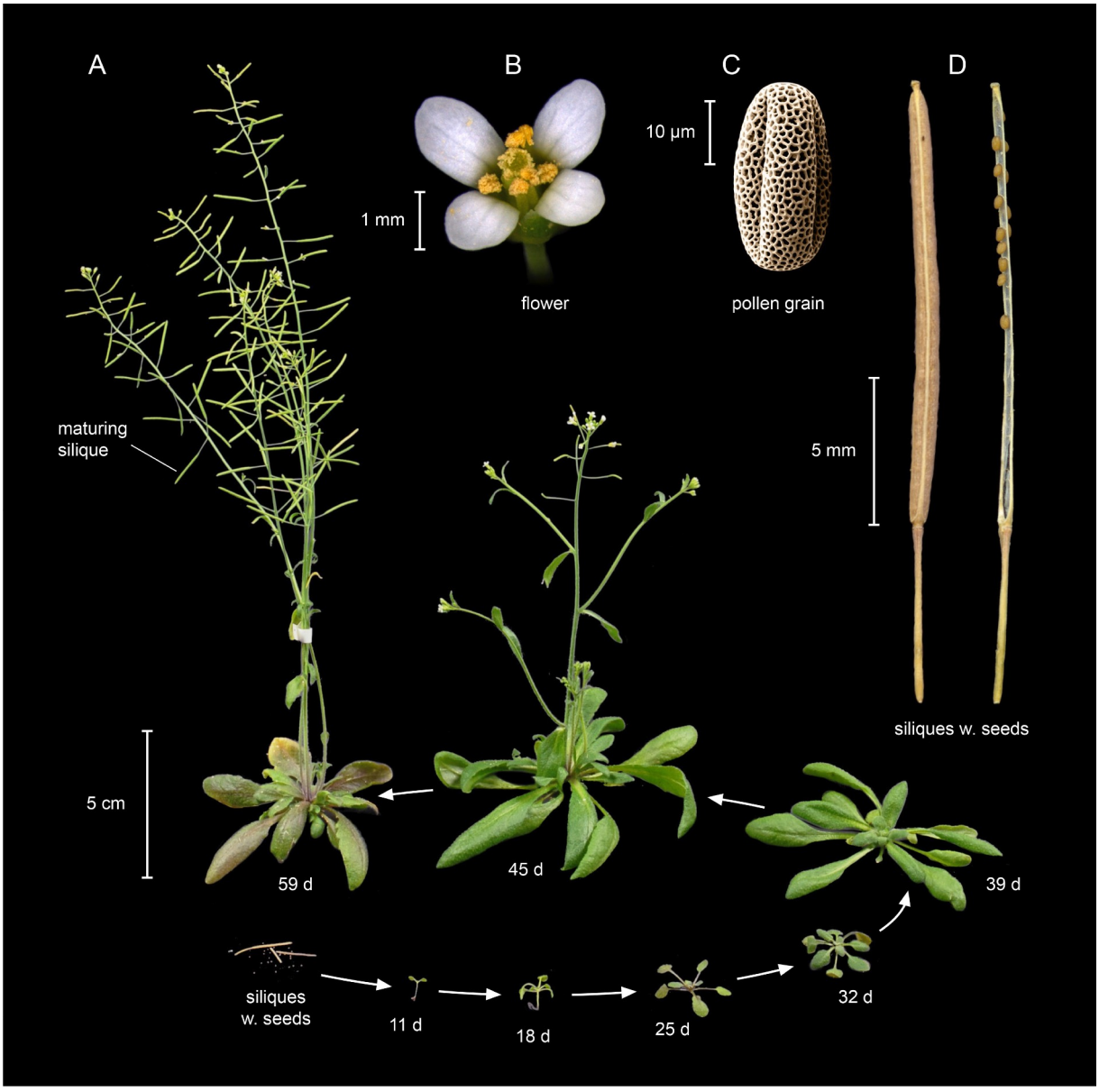
Field guide to plant model systems. Chang, C., Bownam, J.L. and Meyerowitz, E.M. Cell 167:325-339, (2016).

Embryogenesis - the humble beginnings of plant life.

Smet, I. D., Lau, S., Mayer, U., & Jurgens, G. The Plant Journal : For Cell and Molecular Biology, 61:959-70 (2010).

Early plant embryogenesis - dark ages or dark matter?

Bayer, M., Slane, D. and Jürgens, G. Curr. Opinion in Plant Biology, 35:30-36, (2017).



Plants cannot move, and are developmentally plastic.

A defining feature of plants is that they are sessile (cannot move). To survive they have to alter their development in response to their local environment. This developmental plasticity is conferred by differential growth from meristems at the tips of the shoot (the shoot apical meristem, SAM) and root (the root apical meristem, RAM). These meristems are produced during embryogenesis and contain populations of stem cells that divide to self-renew and generate the rest of the plant body. SAMs produce stems, leaves, flowers and branches and RAMs produce the

branching root system. Plants coordinate the activity of these meristems to generate a huge diversity of shapes and sizes.

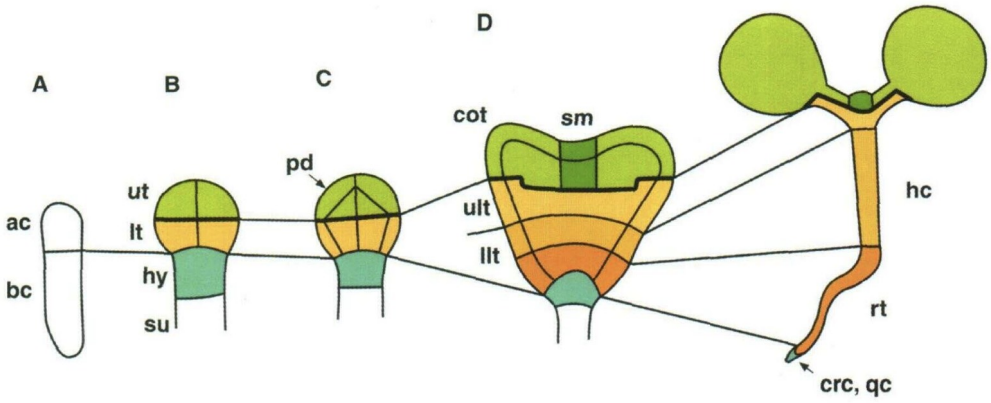
Plant cells are also immobilised, and tissues are formed by coordinated cell division and expansion.
Plant cells are laid down within a rigid cell wall matrix. While animal cells are free to migrate to their final position within a developing tissue, plant cells a laid down almost brick-like, and the final form of a tissue is due to different patterns of cell division and cell expansion. Patterns of cell division can give rise to orderly arrangements of cell files and layers in growing tissues. Such regular layers (or clines) of cells provide references points for classifying the orientation of cell divisions. Planes of division which are orthogonal to an existing layer of cells are said to be **anticlinal**, and result in daughter cells remaining in the same layer, driving proliferation of the layer. Cell divisions that lie parallel to a layer are said to be **periclinal**, creating two daughter cells in the same layer, which can give rise to new layers. The lack of cell migration in plant systems means that cell fate decisions are negotiated in the presence of unchanging neighbours, and their descendants.

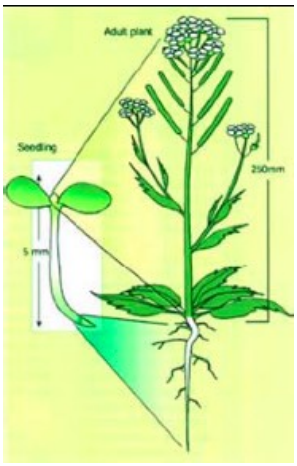
Arabidopsis thaliana is a model plant for studying development.
Arabidopsis thaliana is a common weed in Europe, first described by Johannes Thal in the 16th century. It is a member of the mustard family (Brassicaceae, which includes cabbage, radish, Brussel sprouts), is self fertilising with a short generation time and a single plant can produce 5,000-10,000 seed in as short time as 6 weeks. Arabidopsis is uniquely suited to genetic analysis. The genome has been entirely sequenced (with the exception of some regions around telomeres, centromeres and the ribosomal RNA gene repeat region). The plant has five chromosomes which contain 125 Mb of DNA and 25,498 identified proteins in 11,000 families. About 60% of the plant's genes have a homologous counterpart elsewhere within the genome. 14% of the genome is made up of transposable elements - "selfish DNA". Plastid and mitochondria genomes are small, and encode a further 79 and 58 protein genes, respectively. It has the most thoroughly characterised genome of any plant. Hundreds of mutants in different developmental processes have been identified and these are the basis for our understanding of the molecular control of plant development.

Plant development can be divided into embryonic and post-embryonic stages.
Plant development begins as a single fertilized cell in the ovary within the carpel of the flower. The initial growth and divisions of this cell to form the embryo are known as embryogenesis. This stage of development is deterministic (has a set end point) and culminates in the production of a mature embryo with a root and shoot apical meristem and embryonic leaves (cotyledons). The shape and size of a mature plant is dependent on postembryonic growth. Upon seed germination the RAM and SAM are activated and a small number of founder cells generate essentially all of the adult plant body. Postembryonic development is discussed in more detail in Lectures 3 and 4.

Construction of the Arabidopsis body plan during embryogenesis.
Empirically, the plant body is progressively built by a regular series of cell divisions which build - cell by cell, an increasingly complex and asymmetric structure.
(1) After fertilisation, the Arabidopsis zygote is already polarised, with the apical end of the cell being more cytoplasmically dense, with more vacuoles present at the basal end.
(2) The first division of the Arabidopsis zygote is asymmetric [A]. The zygote undergoes an asymmetric division to produce an apical cell (ac) which will give rise to the bulk of the embryo proper, and a basal cell (bc) which gives rise to the suspensor connecting the embryo and maternal tissues.
(3) The embryo divides to produce a radially symmetric ball of 2, 4 and then 8 cells above the suspensor [B]. The suspensor also continues to divide longitudinally.
(4) Dermatogen or protoderm stage [C]. Transverse divisions produce a single outer layer of cells. This outer layer is termed the protoderm and will give rise to the epidermis of the plant.
(5) Globular stage embryo [D]. Continued radial divisions give rise to the central vascular initials within the body of the developing embryo. A lens shaped cell derived from the upper portion of the suspensor cell file and shown dark coloured, divides to give rise to the central cells of the root apex.
(6) Heart stage embryo [E]. Proliferation of cells in the upper half of the embryo gives rise to cotyledon primordia. This is the first appearance of bilateral symmetry.
(7) Further cell divisions produce the "torpedo" stage embryo with further elaboration of the root and shoot apices and growth of the cotyledons.

(8) After ten days, the embryo consists of about 20,000 cells, is about 0.5 mm in length and has developed a body plan similar in miniature to that of the Arabidopsis seedling [F]. The embryo is converted to a quiescent state and is desiccated prior to seed dispersal. After seed dispersal and germination, development of the embryo is reactivated.





Adult plant growth is due to the activity of meristems established during embryogenesis.
Meristems are organised cellular structures capable of indeterminate growth. The embryo contains root and shoot apical meristems. Each contains an organised core of undifferentiated "stem" cells which can divide and differentiate to produce adult tissues, while maintaining and regenerating the meristem. An apical meristem is formed during embryogenesis, and contains cells which will give rise to the aerial portion of the plant. After germination, the Arabidopsis apical meristem gives rise to many small primordia which develop at the meristem periphery. These primordia undergo cell division and differentiation to develop into organs such as leaves or into additional meristems. After a phase of vegetative growth, the shoot apex changes to become an inflorescence meristem, which in turn produces many floral meristems. Each floral meristem produces primordia which form the various floral organ, such as sepals, petals, stamens and carpels. The Arabidopsis root meristem is a highly ordered cell assembly. After germination, files of cells are laid down by cell division behind the root meristem. These cells expand and differentiate to form the adult root.

Cell fate determination in plant development: position or lineage?
Cells must adopt particular identities during the construction of a regular body plan in embryogenesis, and during adult growth. Every cell arises by cell division, and adoption of any new fate could be governed by (1) its parent cell (i.e. the cell's lineage) or (2) the position of the cell within the embryo. The first model relies on the regular asymmetric segregation of cell fate determinants at cell division during development. While such a mechanism can be seen in some animal systems, cell fate determination in plants appears largely independent of cell division patterns. Evidence for this comes from studies of cell ablation and patterning mutants. Therefore it is more likely that developing plant cells primarily sense their different positions within the tissue and develop according to regulatory signals exchanged between neighbouring cells.

Cell ablation
The role of cell lineage has also been tested in the Arabidopsis root by using surgery or lasers to ablate (kill) individual cells. In these experiments particular cells within an organised tissue are ablated and the ensuing development is observed. Generally, plants show a high capacity for regeneration and death of a cell will lead to compensating division of a neighbour. Daughter cells are seen to adopt the fate of the lost cell and rebuild the pattern of the damaged tissue. These observations indicate that lineage cannot be the sole determinant of cell fate during development, and confirm that positional cues play a major role in controlling cell fate.

Genetic screening of Arabidopsis plants for developmental defects.
Mutations can be induced by conventional chemical or ionising mutagens or by the random insertion of foreign DNA sequences, that may disrupt existing host genes and provide a unique "tag" for the mutant gene. In either case, the mutant gene can be isolated and characterised to gain a more direct view of the mechanisms of development. For example, if an Arabidopsis seed is subjected to chemical mutagenesis, one or a small sector of cells in the mature embryo may contain a particular mutation in one copy of a gene. If the mutation causes a recessive defect in

gene activity, the cells will be phenotypically normal since there remains a second unmodified copy of the gene in the diploid cells. After the seed is germinated, the mutated cells will divide and contribute to a clonal sector of mutant cells within the adult plant. The extent of this sector will depend on the initial position of the progenitor mutant cell within the embryo. If mutant cells come to reside within the shoot apex, it is possible that the mutant clone will extend into some flowers of the adult plant. Arabidopsis flowers are self-fertilised. If both pollen and female gametophyte tissue are derived from a heterozygous mutant sector, there is a 1:4 chance of producing a homozygous mutant plant (m/m), and 1:2 of the seed will carry the mutation as a heterozygote (m/+). So, if any of the seed from a plant show a developmental defect, sibling plants can be grown to seed again to rescue any developmental defect. The mutant gene can be maintained in heterozygous plants, even if lethal as a homozygote. After two generations, mutant lines can be identified and analysed.

Shared features of the Arabidopsis body plan in embryos and adult plants.
The regular pattern of cell divisions during embryogenesis produces a simple correspondence between embryo and adult body plans. Gerd Jurgens' group in Tubingen used this feature as the basis for a screen for Arabidopsis mutants with defective pattern formation. i.e. seedlings were screened for defects in organisation of the plant body, as a way of finding lesions that affect early embryo development. Such an approach had been highly successful in identifying gene regulators of early Drosophila development.

Seedlings were screened for loss or distortion of root, hypocotyl or cotyledon regions – which were shown to result from defects during embryogenesis. Mutants were grouped as: having disrupted organogenesis - *knolle* (*kn*), *keule* (*keu*), *fass* (*fs*), *knopf* (*knf*), *mickey* (*mic*) lacking body segments - *gurke* (*gk*), *fackel* (*fk)*, *monopteros* (*mp*) disturbed radial symmetry - *gnom* (*gn*). There have been several important conclusions from this work:
1. Disruption of the normally regular patterns of cell division in the Arabidopsis embryo does not necessarily interfere with proper cell fate determination.
2. The embryo mutants fall into two major classes, which have been found which contain genetic disruptions in: (i) basic processes required for cell division, secretion and wall synthesis, and (ii) components required for polar transport of auxin and auxin response. This is a marked contrast to the discovery of patterning genes in a similar screen for Drosophila.

Precise cell division is not required for pattern formation.
Example 1: Mutations in the FASS gene produce disordered patterns of cell division.
The normal, very regular patterns of cell division during embryogenesis are highly disrupted in *fass* mutants. Even the direction of the first plane of cell division is perturbed. The *fass* mutants show an inability to form microtubule preprophase bands, which predict the site of future cell wall deposition in plant cells. FASS gene product is required for proper microtubule dynamics and the accurate positioning of cell walls during division. However the full range of appropriate cell types is present in the *fass* mutant plants and complete, albeit disordered, seedlings are produced despite the inability to undergo the precise cell divisions normally found during embryogenesis.
This suggests that lineage-dependent segregation of cell fate determinants does not play a crucial role in patterning.

(Other mutants, such as *knolle* (kn) and *keule* (keu) also disrupt cell division and shape. At the cellular level, mutant embryos are characterised by incomplete cross walls and enlarged cells with polyploid nuclei. KNOLLE is homologous to syntaxin, and is a t-SNARE involved in vesicular targeting and fusion during secretion. The mutant gene causes defects in cell wall deposition during cytokinesis. Despite this profound defect, embryogenesis still proceeds, proper cell fates are negotiated, and a seedling is formed.)

Example 2: Genetic defects in auxin traffic or perception produce plants with altered body plans.
A number of the embryo patterning mutants possess defects in hormone traffic or response. These are covered in more detail in Lecture 2. For example, the *monopteros*, *bodenlos* and *gnom* mutants affect the polarity of early cell divisions in the embryo. MONOPTEROS and BODENLOS regulate auxin-mediated gene expression. GNOM encodes a membrane-associated ADP ribosylation GTP exchange factor (ARF GEF) that is required proper secretion and localisation of auxin efflux transporters. The plant hormone auxin plays a critically important role in coordinating the initiation and maintenance of growth in plants.

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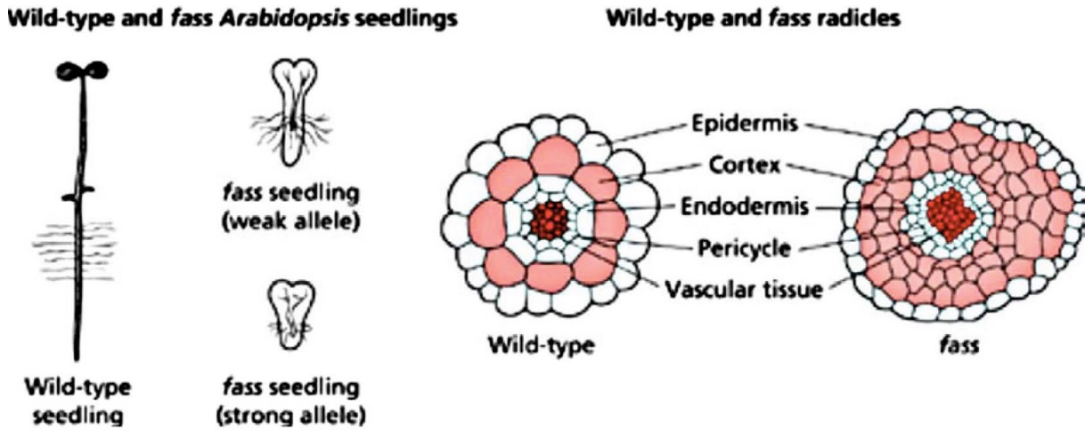
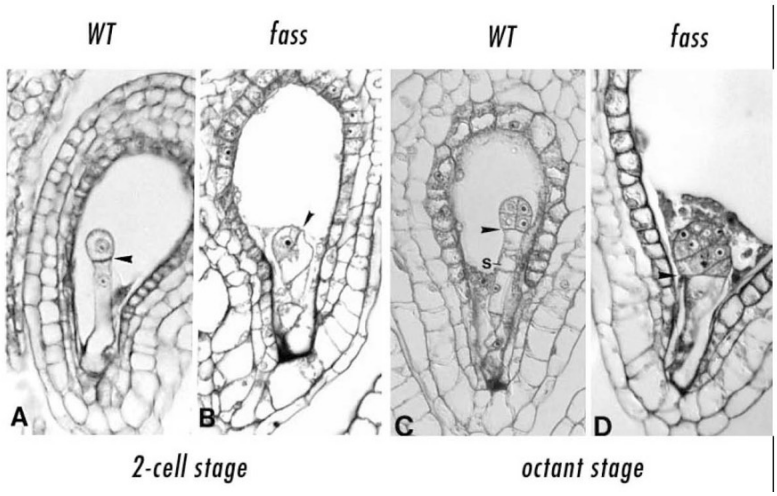




Auxin as an example: how cell polarity and fates are established in plants.
Hormones are known to regulate many aspects of plant growth. For example, cytokinins are associated with cell division in many tissues and may interact directly with regulators of the cell cycle. Auxins (e.g. indole acetic acid, IAA) control a number of developmental processes in plants, including cell elongation, and the formation of vascular tissue. Auxin plays a pivotal role in initiating and maintaining apical-basal polarity in plant tissues – and we will focus on this growth regulator to better understand its role and the mechanisms of action that allow coordination of cell fates at both long and short ranges.

Lecture 2: Polarity, auxin traffic and auxin response.

A number of mutations have been found to disrupt cell division patterns without greatly affecting morphogenesis. In contrast, patterning mutants are caused by defects in the intercellular exchange of regulatory molecules. Positional information, rather than lineage, is the major determinant of plant cell fate. A high degree of intercellular communication allows plant cells to develop in a precise way and to maintain developmental plasticity. The selective traffic of a plant hormone, auxin, is used to control cell polarity in plant tissues. A combination of influx and highly selective efflux transporters coordinate the flow of auxin within the plant. and directly control cell fates. A core mechanism for nuclear auxin responses has been identified. This mechanism involves binding of auxin to both the SCFTIR1/AFB ubiquitin ligase and its AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) substrate protein. The subsequent ubiquitination and degradation of Aux/IAA proteins releases interacting DNA-binding AUXIN RESPONSE FACTOR (ARF) transcription factors from inhibition and allows these to regulate gene transcription and cell fate.

Extended reading:

Structure and function of auxin transporters.
Hammes, UZ & Pederson, BP. Annu. Rev. Plant Biol. 75:185-209 (2024).

Over 25 years of decrypting PIN-mediated plant development.
Luschnig, C & Friml, J. Nat Comm. 15:9904-9916 (2024).

Transcriptional responses to the auxin hormone.
Weijers, D and Wagner, D. Annu. Rev. Plant Biol. 67:21.1–21.36 (2016)

Structural biology of nuclear auxin action.
Dinesh DC, Calderón Villalobos LIA and Abel, S. Trends in Plant Science, 21:302-315 (2016).

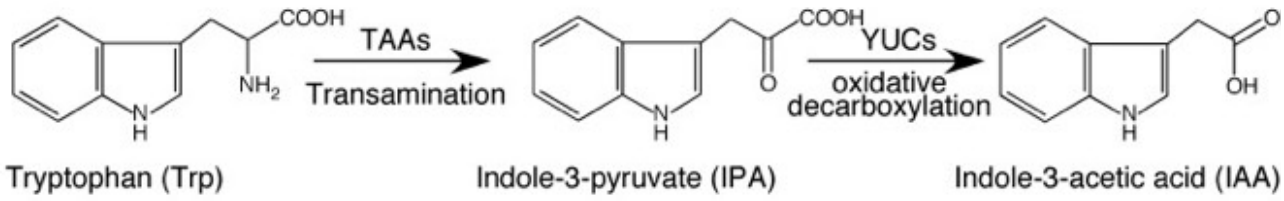
To bind or not to bind: how Auxin Response Factors select their target genes.
Rienstra J, Hernández-García J & Weijers D. J Exp. Bot. 74:6922-6932 (2023).

Auxin synthesis.

Auxin is synthesized from the amino acid tryptophan in two steps via TAAs and YUCCA enzymes. Unlike animals, where hormones are often produced in specialised glands before being transported globally, plant hormones are synthesised throughout the plant body in many different cell types and positions. However, although auxin is produced locally, polar transport is still vital for its function.

Isolation of genes for auxin transport in Arabidopsis.

1. The gene for the AUX1 influx carrier was isolated because loss of the gene confers resistance to the herbicide 2,4-D, an auxin mimic. Auxin influx appears to be non-specific.



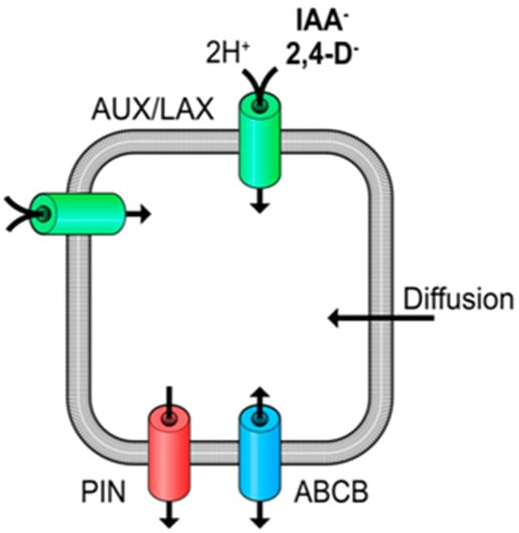
2. In contrast, the PIN1 gene encodes a specific auxin efflux carrier. Loss of gene activity results in the formation of a "pin-like" bolt with complete loss of lateral organs.

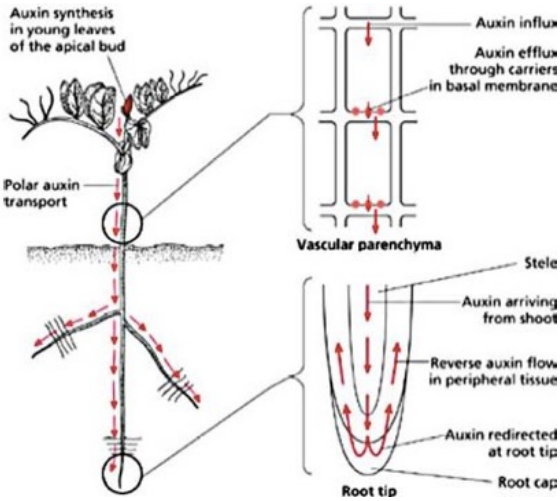
3. Additional auxin efflux carriers, PINs 2, 3, 4 and 7 are required for regulated growth of the plant.

The PIN genes are part of a small family of transmembrane carriers. For example, PIN1 is a 67K protein with 10 predicted transmembrane spans. Immunolocalisation showed that the protein is localised at the basal side of cells in the centre of the root, positioned at the plasma membrane. In contrast, the PIN2 protein is localised on the apical side of cells in the outer portion of the root tip. The location of these efflux transporters is consistent with known polar transport of auxin from the shoot to the root tip. At the root tip, auxin flux is redirected upwards, through the outer cells of the root. It is thought that asymmetric redistribution of this auxin flux controls cell elongation, and is required for bending of the root. Accordingly, loss of PIN2 causes loss of gravitropic response.

Auxin signal reception

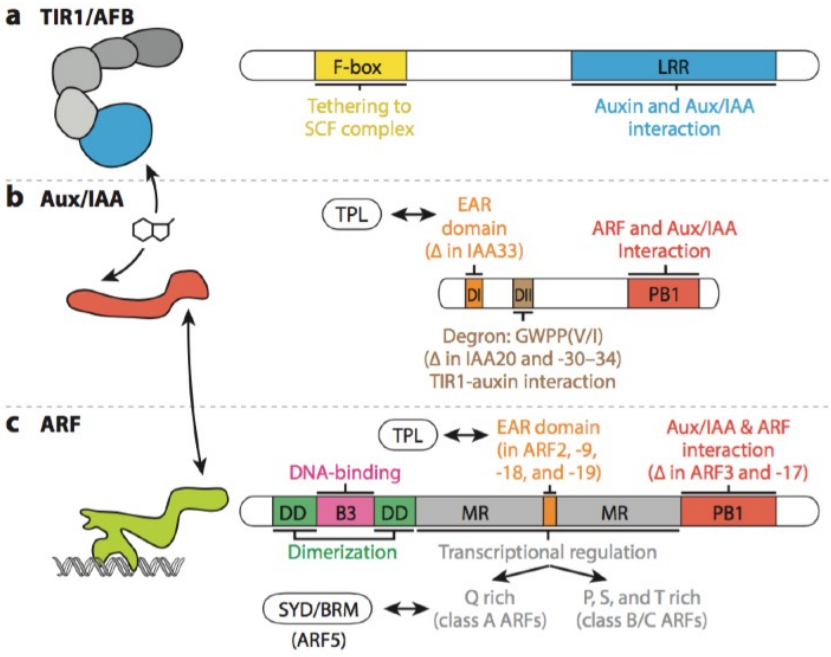
The path from auxin signal perception to altered gene expression is short. The key components of this pathway are the TRANSPORT INHIBITOR RESISTANT (TIR) F-box proteins, the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA)





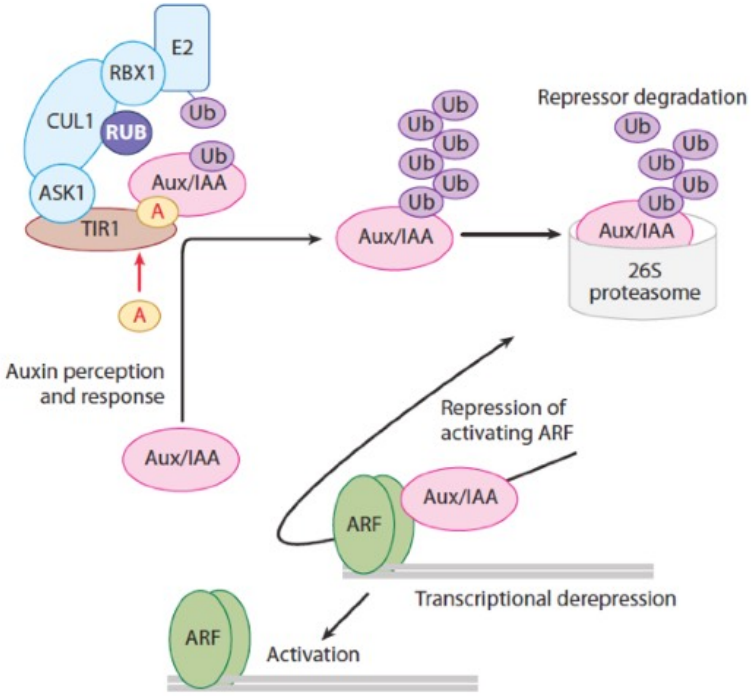
transcriptional coregulators, and sequence-specific binding proteins called AUXIN RESPONSE FACTORs (ARFs). A coreceptor comprises a TIR1 F-box protein and an Aux/IAA transcriptional coregulator that senses auxin. Auxin promotes the interaction between TIR1 and Aux/IAA, thereby triggering ubiquitin-mediated degradation of the Aux/IAA proteins via the proteasome. Aux/IAA proteins generally act as corepressors to prevent auxin-responsive transcription. Arabidopsis has 6 and 29 paralogous members in the TIR and Aux/IAA families, respectively.

TIR proteins are incorporated into a four-subunit SCFTIR1/AFB complex. Both this complex and the small Aux/IAA proteins are localised in the nucleus. All TIR proteins bind auxin. TIR family members have been shown to promote auxin responses, and mutants in these factors are either subtly or strongly auxin resistant and display morphological defects consistent with a role in auxin perception. Mutants in other components of the SCFTIR1/AFB ubiquitin ligase complex, such as ARABIDOPSIS SKP1 HOMOLOGUE (ASK1), CULLIN 1 (CUL1), or RING-BOX 1 (RBX1), also cause auxin resistance. Members of the TIR1/AFB family have an N-terminal leucine-rich-repeat region and a C-terminal F-box domain. The crystal structure of TIR1- ASK1 in a complex with auxin and a small Aux/IAA peptide has been solved, revealing that the leucine-rich-repeat domain of TIR1/AFB contains the auxin-binding pocket, whereas the F-box domain contacts ASK1. The Aux/IAA peptide is also in contact with the leucine-rich-repeat domain at the auxin-binding site. These results suggest that auxin stabilises the interaction between TIR1/ASK and the Aux/IAA proteins.



The TIR1/AFB proteins also contact the CUL1 subunit of the SCFTIR1/AFB complex via the F-box domain, which is linked to autocatalytic degradation.

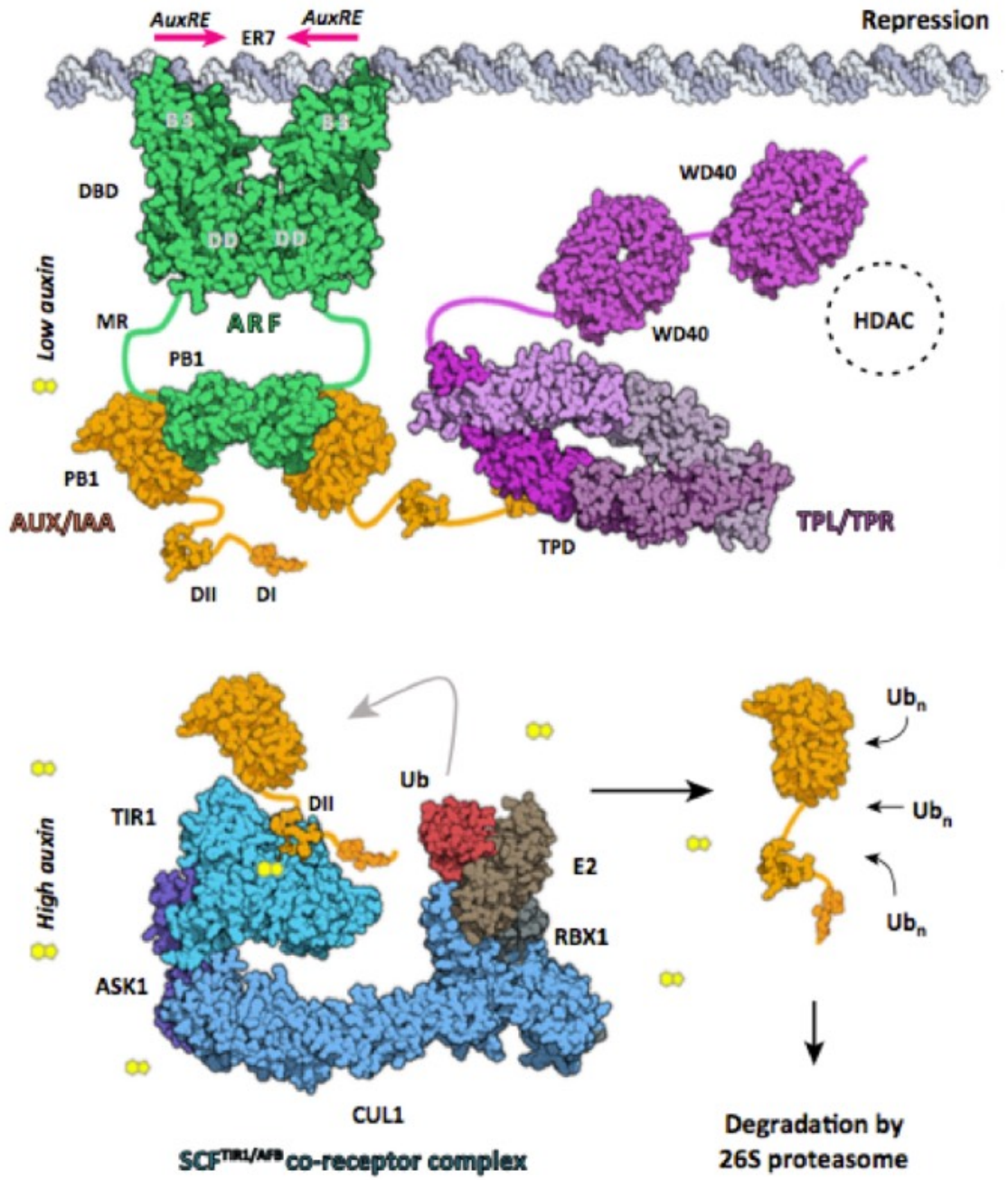
Domain architecture of the central components of auxin-dependent gene regulation.
Auxin responses are mediated by interactions (arrows) between three core components: (a) TIR1/AFB auxin receptors, (b) Aux/IAA transcriptional repressors, and (c) ARF transcription factors. TIR1/AFB proteins contain an F-box domain for tethering to the other subunits in the SCF E3 ubiquitin ligase complex and a leucine-rich-repeat (LRR) domain that carries the auxin-binding pocket and Aux/IAA contact site. Aux/IAA proteins consist of domain 1, which harbours an EAR motif that mediates interaction with TOPLESS (TPL); domain 2, which carries the degron [the conserved amino acid sequence GWPP(V/I), which acts as the contact site with TIR1/AFB and auxin]; and a PB1 domain, which mediates oligomerisation and Aux/IAA-ARF heterodimerisation. ARFs have an N-terminal B3 DNA-binding domain flanked on either side by a dimerisation domain (DD), followed by a middle region (MR) that mediates transcriptional regulation. This domain can contain an EAR motif for interaction with TPL; it is glutamine (Q) rich in class A ARFs but proline (P), serine (S), and threonine (T) rich in class B and C ARFs. In ARF5, this domain mediates the interaction with SYD and BRM. At their C termini, ARFs have a PB1 domain for oligomerisation and Aux/IAA-ARF heterodimerisation. (Protein abbreviations: ARF, AUXIN RESPONSE FACTOR; Aux/IAA, AUXIN/INDOLE-3-ACETIC ACID; BRM, BRAHMA; EAR, ETHYLENE- RESPONSIVE ELEMENT BINDING FACTOR–ASSOCIATED REPRESSOR; PB1, Phox and Bem 1; SYD, SPLAYED; TIR1/AFB, TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX; TPL, TOPLESS).



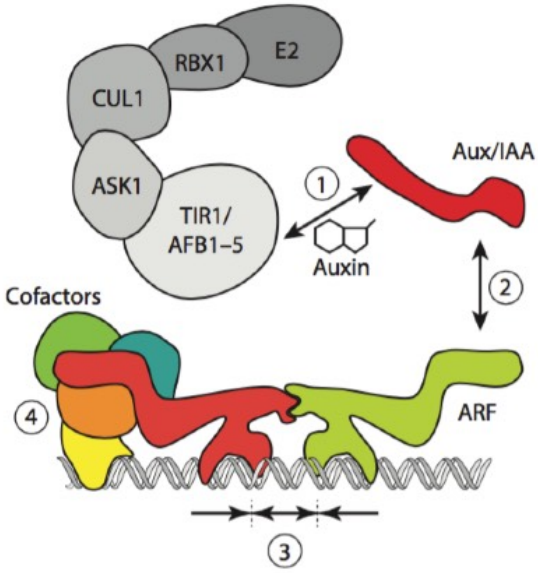
Auxin responsive promoters
Auxin rapidly induces (2–30 min) primary response genes of three families known as AUX/IAAs, GH3s, and SAURs. Select members of each family were established as experimental models to study their function and transcriptional regulation by auxin. GH3 promoter deletion and linker scanning analyses identified the canonical TGTCTC-type AuxRE found in many early auxin genes. However, the core hexamer TGTCTC motif confers auxin responsiveness only when at least duplicated (direct, inverted, or everted repeats) or coupled to a second, different promoter element in an overlapping or disjointed arrangement (composite AuxRE). A comparison of several transcript profiling studies revealed that the early response to auxin (<30 min) comprises mostly upregulated mRNAs. Computational analyses of the genome-wide distribution of TGTCTC-type AuxREs showed a strong association with the transcriptional start sites or proximal promoter regions of auxin-induced genes and recognised the presence of several coupling elements to form composite AuxREs, including additional TGTCTC-type elements or the binding sites of bZIP and MYB transcription factors

ARF transcription factors

Using multiple tandem copies of inverted TGTCTC repeats as a bait, the founding member of the Arabidopsis ARF family, ARF1, was selected in a yeast one-hybrid screen and shown to bind *in vitro* to distinctly spaced palindromic TGTCTC motifs. Early work showed that the ARFs tested bound with specificity to palindromic AuxREs; however, robust DNA recognition required ARF dimerisation and the first four nucleotides of the TGTCTC motif.



ARF proteins can be grouped into three classes from the early land plants onward. Class A comprises ARFs with a glutamine (Q)–rich middle region that are classified as transcriptional activators based on transient gene expression assays in protoplasts. The Q- rich domain is present in all class A ARFs. Characterisation of an allelic series of *monopteros* (mp) mutant alleles in the Arabidopsis Columbia ecotype has highlighted the importance of this domain. The remaining ARFs are classified as repressors based on the same protoplast assay or sequence homology and can be divided into the microRNA 160 (miR160)– targeted ARFs (class C) and the remaining ARFs (class B).



ARF-mediated repression of transcription

Transcriptional regulation by auxin involves chromatin-level control to sustain the repressed state and promote the activated state. Repression involves histone deacetylation upon recruitment of the respective enzyme by the TOPLESS (TPL) corepressor and the Aux/IAA repressor. Activation requires recruitment of SPLAYED/BRAHMA (SYD/BRM) chromatin remodelers to the ARF transcription factor.

Aux/IAA inhibits activating ARFs bound at their target loci by recruitment of corepressor complexes. The EAR repressor motif in domain 1 of the Aux/IAA proteins physically interacts with and recruits Tup1/Groucho/TLE family proteins called TOPLESS (TPL) or TOPLESS RELATED (TPR). Repression of auxin response gene expression in low auxin further requires histone deacetylases (HDACs). Loss of HDAC activity partially rescues the phenotypes associated with gain-of-function mutations in Aux/IAA-encoding genes, and both TPL and HDACs are recruited to activating ARF-binding sites specifically in low-auxin conditions. TPL recruits HDAC complexes in plants, as has been reported for its metazoan counterparts. HDACs remove acetyl groups from lysines on histones (primarily histones H3 and H4), which leads to a more compact chromatin state and reduced accessibility of the genomic DNA for transcription factors or the general transcriptional machinery.

Another class of chromatin regulatory proteins, the SWITCH/ SUCROSE NONFERMENTING (SWI/SNF) chromatin-remodeling ATPases, helps overcome this repressed chromatin state upon auxin sensing. SWI/SNF chromatin remodeling complexes use the energy derived from ATP hydrolysis to alter the occupancy or positioning of nucleosomes on the DNA, thereby changing the accessibility of the genomic DNA in the context of chromatin.

Specificity of response

Despite the short auxin response pathway, transcriptional, post-transcriptional, and post-translational control over core components allows tuning of the pathway by feedback regulation, during development, or by other hormonal or environmental signals. Specificity in response is critical for the ability to trigger multiple, distinct responses in different contexts during plant development.

Transcriptional auxin output depends on interactions and regulation at various levels, ultimately leading to either quantitatively or qualitatively different gene expression profiles. (1) The affinity of the TIR1/AFB-auxin-Aux/IAA interaction depends on the identity of the receptor, the type of auxin molecule, and the identity of the Aux/IAA protein and can thus vary by orders of magnitude. (2) Aux/IAA-ARF interactions through their homologous C-terminal domains are likely selective. Aux/IAAs preferentially interact with class A ARFs, although interactions with class B and C ARFs have also been demonstrated. The affinities among the families likely depend on the exact pairs. (3) The selection of DNA target sites by ARF-DNA interactions can be selective not only by direct recognition of binding sites, but also by the spacing between two adjacent inverted binding sites to which ARF dimers can bind with high affinity. Although ARFs bind nearly identical motifs in vitro, there may be more selectivity in vivo. The optimal spacing between binding sites differs, at least in vitro, between ARFs, which adds selectivity. Furthermore,

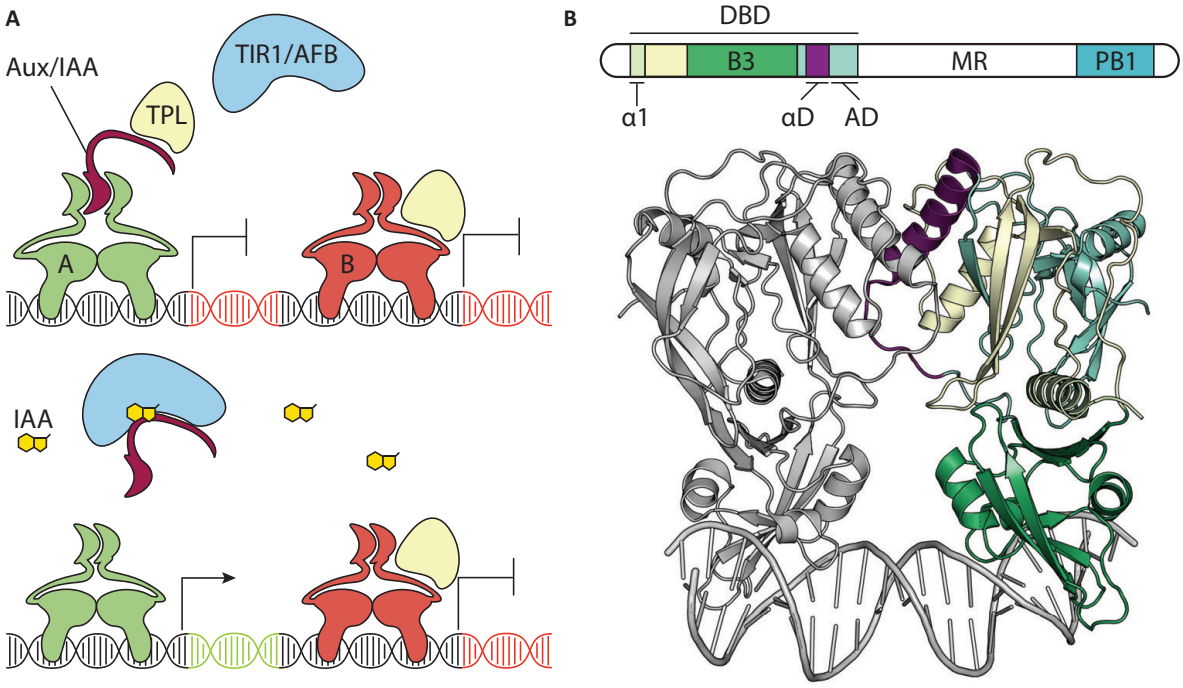


Fig. 1. Overview of the nuclear auxin pathway and the anatomy of an ARF. (A) The minimalistic nuclear auxin pathway. Under low auxin conditions (upper panel), both A-ARFs (green) and B-ARFs (red) act as repressors. A-ARFs bind with a repressive cofactor, Aux/IAA (bordeaux), that recruits TPL, while B-ARFs recruit TPL directly via their middle region. Under increasing auxin conditions [indole-3-acetic acid (IAA) gold], IAA acts as a molecular glue and allows TIR1/AFB to sequester Aux/IAA away from the A-ARFs, which then become transcriptional activators. B-ARFs act as repressors in either condition. (B) The anatomy of an ARF. Top panel shows the general genetic sequence of an ARF, the lower panel shows the atomic structure of the DNA-binding domain in complex with an IR7 motif (pdb: 6ycq). Domains indicated and discussed in this review are: α1 (pear), the α-helix tethering the B3 domain and acting as a molecular hinge; N-terminal dimerization domain (yellow); B3 (green), the domain interacting with the DNA; αD (purple), the α-helix and loop that facilitates dimerization; and the C-terminal dimerization domain and ancillary domain (AD, cyan). The middle region (MR, white) and Phox and Bem1 domain (PB1, blue) are omitted.

ARFs may theoretically heterodimerise, further expanding the range of binding specificities. (4) ARF-interacting cofactors can alter ARF activity or DNA-binding specificity.

Given the profound impact of auxin output on plant growth and development, it likely that this output must be buffered and balanced to prevent excessive response. Feedback control has been demonstrated at the level of auxin transport: PIN-FORMED (PIN) auxin efflux carrier genes are transcriptionally upregulated by auxin so that, when cellular auxin levels rise, excess auxin is transported out of the cell. A similar mechanism operates in auxin biosynthesis regulation. The YUCCA (YUC) auxin biosynthesis enzyme genes are transcriptionally repressed by auxin. Hence, high cellular auxin levels stall endogenous synthesis, and lower auxin levels lift transcriptional repression and elevate cellular auxin levels. Finally, Aux/IAA genes were initially identified because they are transcriptionally upregulated by auxin treatment, which suggested intrinsic feedback control. This feedback regulation has now been formally demonstrated using the MP/ARF5 protein: MP/ARF5 triggers activation of a subset of the 29 Aux/IAA genes through direct interaction with their gene promoters. The Aux/IAA proteins encoded by these same genes directly interact with MP/ARF5 and inhibit its activity.

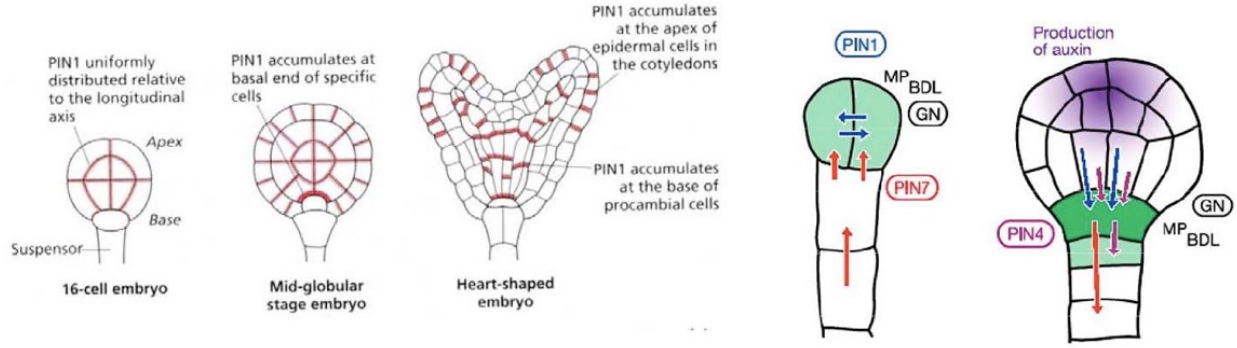
Analysis of the auxin response system in early-diverging land plants has shown that the mechanism of signalling has deep roots, going back at least to the liverworts. Simpler auxin response networks appear to share the same regulatory principles, and the presence of single copies of the 3 classes of ARF in the liverwort *Marchantia polymorpha* suggests that auxin responses may have evolved earlier.

Lecture 3: Regulation of root initiation and polar growth by auxin.
The initiation of the root meristem occurs after that of the shoot meristem progenitor during Arabidopsis embryogenesis. Dynamic shifts in PIN-directed transport of auxin and hormone-responsive gene expression accompanies the formation of meristem progenitor cells. Auxin is subject to cell-cell traffic throughout the plant, allowing long-distance coordination of root growth, vascular patterning and balance of root-shoot growth.

Extended reading:
Building a plant: cell fate specification in the early Arabidopsis embryo.
ten Hove CA, Lu KJ and Weijers D. Development 142:420-430 (2015).
Self-organizing periodicity in development: organ positioning in plants.
Bhatia N and Heisler MG. Development (2018) 145:1-11, (2018).
Connecting emerging with existing vasculature above and below ground.
Blanco-Touriñán N & Hardtke CS. Curr Opin Plant Biology. 76:102461 (2023).
The interplay between extracellular and intracellular auxin signaling in plants.
Tang W, Yu Y and Xu T. J Genetic & Genomics 52:14-23 (2025).

Evolution of plants: adaptation to a terrestrial environment and root formation
Vascular plants are by far the dominant groups on the Earth comprising around 400,000 species in contrast to about 22,000 species of bryophytes and approximately 20,000 species of algae. The first vascular plants appear in the fossil record in the late Silurian, about 420 million years ago. Their green algal ancestors are thought to have appeared nearly 400 million years earlier. The first, indisputable vascular plants were characterised by a conducting system containing xylem and phloem, a waxy cuticle, epidermal stomata. Fossils of *Rhynia* and *Aglaophyton* species, first discovered in chert rock formations in Rhynie, Scotland, share morphological and structural features with some bryophytes and simple vascular plants, and provide perhaps the best available model for a vascular plant precursor. These were a small plants, probably no more than 180 mm high, composed of dichotomous, upright stems that branched from rhizomes that grew on the surface of the soil. The epidermis of the stems was covered by a cuticle and contained stomata. In its small size, spore-based reproduction and water- and photosynthate-conducting cells these early plants resemble mosses. It is likely that vascular plants evolved from this or plants of similar morphology and anatomy. These early plants lacked an elaborate root system. Instead, root hair-like rhizoids emerged from modified stems, and made contact with the soil. The formation of root meristems capable of forming whole organ systems by growth through soil, appears to have been a later evolutionary development than shoot meristematic growth.

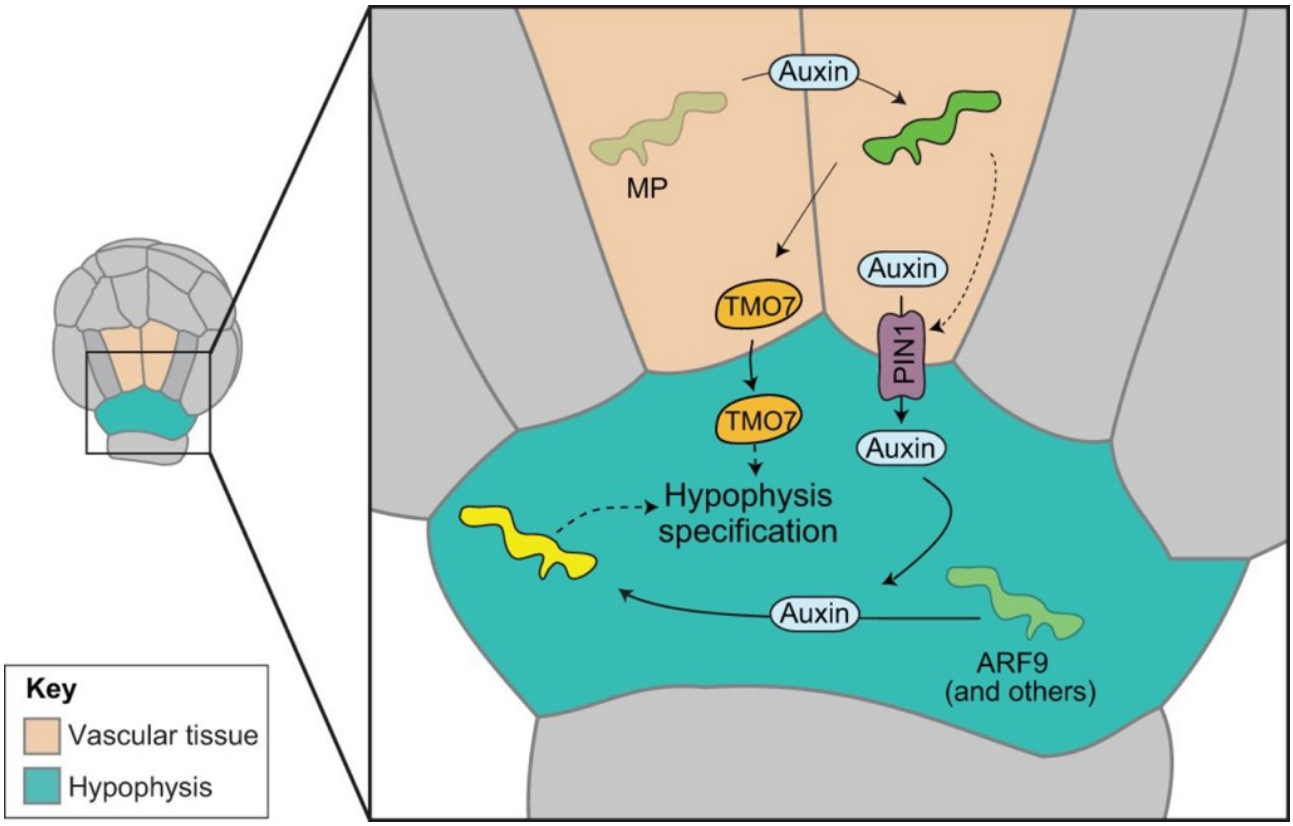
Auxin dynamics during embryogenesis
The progenitors of the shoot and root meristems are formed separately during Arabidopsis embryogenesis, with root meristem formation starting later. Both meristems are initiated via a series of characteristic cell divisions, accompanied by changes in auxin traffic and accumulation. The first division of the zygote cell is asymmetric, and gives rise to an apical lineage corresponding to the proembryo and a basal lineage generating a short file of extra-embryonic cells known as suspensor. The cellular initials of the shoot apical meristem become evident at the 16-cell dermatogen stage. At the 32-cell early globular stage, the uppermost derivative of the suspensor changes cell fate to become the hypophysis, which by mid-globular stage undergoes asymmetric division to give rise to the quiescent centre of the primary root meristem. These key steps in organisation of the plant body plan are accompanied by changes in PIN protein distribution and the formation of foci for auxin accumulation.



Genetic defects in hormone traffic or perception produce plants with altered body plans
A number of the embryo patterning mutants isolated by Jürgens and colleagues possess defects in hormone traffic or response. For example, GNOM encodes an ADP ribosylation GTP exchange factor (ARF GEF) and regulates traffic of membrane vesicles. Mutant gnom embryos show a loss of apical-basal polarity. The GNOM protein is required for proper localisation of the PIN1 auxin efflux carrier. Inhibition of vesicle traffic by application of brefeldin A also causes loss of proper PIN1 localisation. Polar localisation of the efflux carrier protein is a steady state that requires BFA-sensitive membrane trafficking for maintenance.

The formation of the root primordium during Arabidopsis embryogenesis requires the auxin-dependent release of the transcription factor MONOPTEROS (MP, also known as ARF5) from its inhibition by the Aux/IAA protein BODENLOS (BDL, also known as IAA12). Auxin-insensitive *bdl* mutant embryos and *mp* loss-of-function embryos fail to specify the hypophysis, giving rise to rootless seedlings.

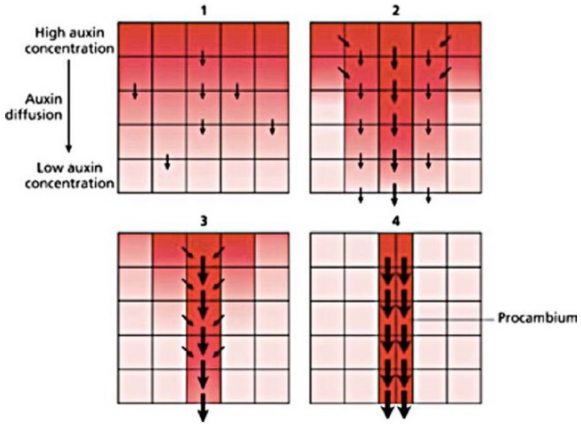
Auxin response during root meristem initiation
The targeted flux of auxin to the base of the embryo triggers degradation of the Aux/IAA protein BDL (IAA12), and the auxin response factor MP (ARF5) is released and in turn activates its target genes including MP itself and its inhibitor BDL. This feedback loop enables the MP–BDL module to be switched on in response to rising auxin concentration, which in turn mediates cell specification and primary root meristem initiation. This local feedback, mediates auxin response in inner cells of the proembryo that is required before the asymmetric division of the adjacent hypophysis. This promotes PIN1-dependent auxin transport to the hypophysis and also leads to degradation of Aux/IAA inhibitors, release of ARF transcription factors and regulation of target genes. This includes the gene for bHLH transcription factor TMO7 (TARGET OF MONOPTEROS 7). The expressed protein subsequently acts non-cell-autonomously, moving to the adjacent hypophysis cell.



Feedback regulated traffic of auxin.
Current models for auxin based regulation in the Arabidopsis embryo, root and shoot rely on "bucket brigade" style polar traffic of auxin, via specifically localised efflux carriers throughout the plant. Some form of positive feedback

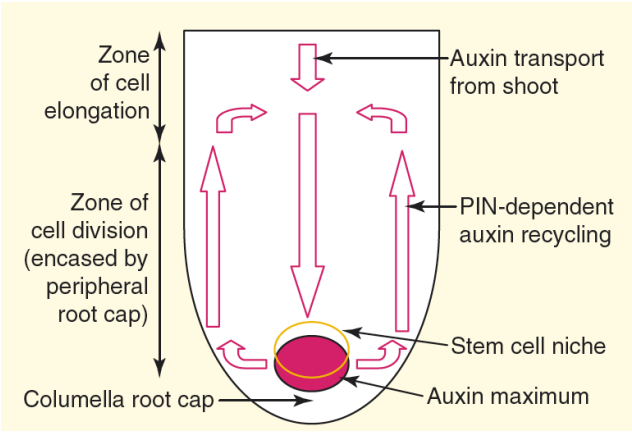
between efflux carrier and auxin flux would allow the self-organisation of long-distance pathways for auxin traffic. The same mechanism also provides a route for local control of cell fates through cellular responses to auxin.

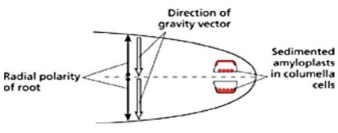
There is now direct evidence that (i) auxins inhibit endocytosis and that auxin application can reverse the effect of brefeldin A, which blocks exocytosis. The PIN class of auxin efflux components are maintained at the plasma membrane by the balance of exocytosis and endocytosis. Therefore an increase in auxin concentration will increase the plasma membrane localisation of PIN proteins, and increase the polar flux of auxin from the cell. This is a possible basis for auxin-triggered flow of auxin across tissues.

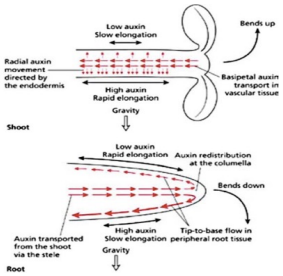


Polar flux of auxin is required for the proper development and gravitropism of the root meristem.
The PIN class of auxin efflux carriers are required for gravitropism in Arabidopsis. PIN1 is localised at the basal side of cells in the centre of the root, positioned at the plasma membrane. In contrast, the PIN2 protein is localised on the apical side of cells in the outer portion of the root tip. The location of these efflux transporters is consistent with known polar transport of auxin from the shoot to the root tip. At the root tip, auxin flux is redirected radially by the action of PIN3, and then upward via the action of PIN2, through the outer cells of the root.

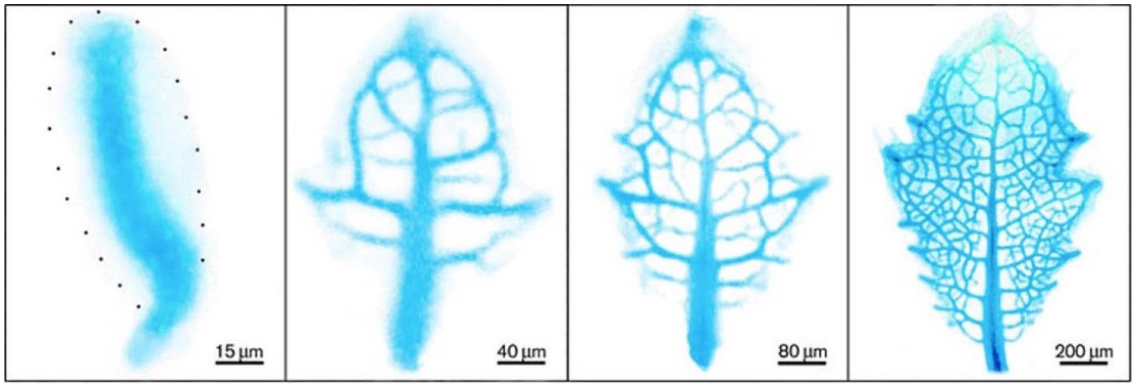
PIN3 acts as a gravity-controlled switch, and will direct auxin to the lower surface of a tilted root. A higher concentration of auxin will inhibit cell elongation on the lower side of the root, and cause it to bend towards the vertical. Loss of PIN2 causes a loss of the gravitropic response. A similar redistribution of auxin is seen in the shoot, except that higher levels of auxin stimulate cell expansion, and the shoot bends in the opposite direction.





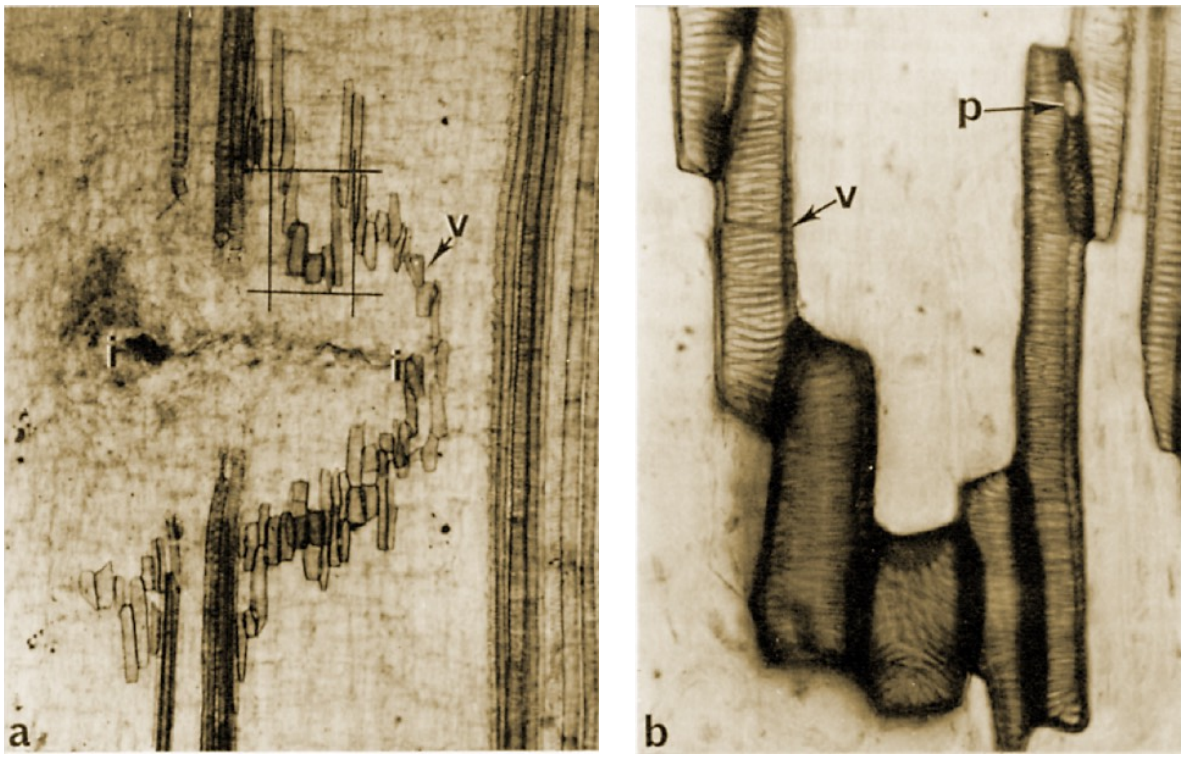


Auxin transport is required for formation of plant vascular tissues.
Auxin traffic is required for correct patterning of plant vascular tissues. (i) Application of synthetic auxin to undifferentiated tissue causes the formation of differentiated vascular cells. (ii) Auxin transport inhibitors disrupt the pattern and connectivity of the vasculature in Arabidopsis leaves. (iii) Arabidopsis mutants with defects in auxin traffic or perception have a disrupted vasculature.



The expression of a reporter gene associated with vascular cell fate (derived from a homeodomain transcription factor, ATHB-8) is progressively refined during leaf development. This reflects a progressive refinement, or "canalisation" of auxin transport. In canalisation, a provascular cell must attract slightly more auxin than its neighbouring cells, which enhances its own polar auxin transport capacity and leads to the export of auxin from the cell. The increase in auxin levels in the adjacent cell then initiates a repetition of the process, ultimately resulting in the formation of narrow pathways for auxin flow across a tissue, and formation of cell strands that differentiate into vascular tissues Although the key components for polar auxin efflux, asymmetrically localised PIN-FORMED (PIN) auxin efflux carriers have been identified, it remained unclear how the auxin signal could influence PIN polarity in neighbouring cells.

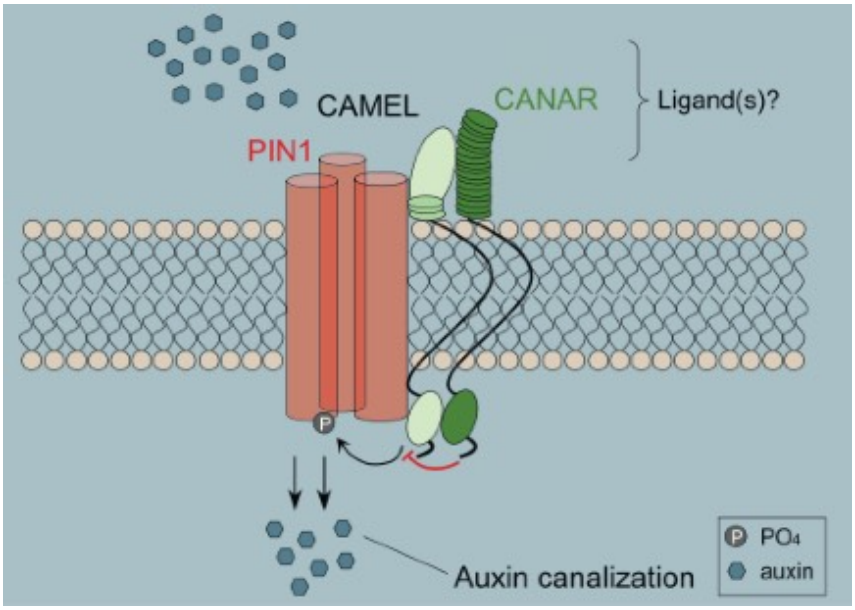
Plant cell fates are flexible, even within adult tissues.
Plant cells adjacent to a wound will re-differentiate to effectively repair disrupted tissue. For example, puncture of Coleus vascular traces causes disruption of existing auxin flow through the tissue. The altered flow of auxin triggers



the differentiation of surrounding parenchyma cells (forming replacement xylem and phloem cells) to form a "bypass" around the broken connection.

In Arabidopsis, the regulation of PIN-FORMED (PIN) proteins and the polar transport of auxin is known to be mediated by several key kinases and phosphatases. An example of this is found with the CANALIZATION-RELATED AUXIN-REGULATED MALECTIN-TYPE RLK (CAMEL receptor-like kinase), a cell-surface transmembrane receptor. CAMEL forms a complex with the CANALIZATION-RELATED RLK (CANAR), which together modulate auxin-mediated PIN trafficking and polarity through the phosphorylation of PIN proteins.

CAMEL possesses kinase activity that directly phosphorylates PIN1, influencing its polar localisation within cells. CANAR functions as a negative regulator of CAMEL by diminishing its autophosphorylation and reducing its kinase activity towards PIN1. Mutations in these genes or the phosphorylation sites of PIN1 have been shown to affect polar distribution of the efflux transporter and lead to defects in cotyledon vein patterning. CAMEL and CANAR are themselves regulated by auxin, and help orchestrate feedback between PIN polarity and auxin transport in Arabidopsis.



Lecture 4: Meristems and patterning of plant growth.

The entire aerial structure of the plant is derived from a few meristematic cells set aside during embryogenesis. Meristems retain the capacity for cell proliferation during the adult life of the plant, and branch to produce specialised organs like leaves and flowers. How is the balance between cell proliferation and differentiation maintained in this small population of cells? Work with Arabidopsis mutants has uncovered extracellular signalling and feedback that controls indeterminate growth of the shoot apical meristem, and regulate the patterning of meristem primordia.

Extended reading:

Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo. Barton, M. K. Developmental Biology, 341:95-113 (2010).

CLAVATA-WUSCHEL signalling in the shoot meristem. Somssich, M., Byoung, J., Rüdiger, S. and Jackson, D. Development 143:3238-3248, (2016).

Patterning at the shoot apical meristem and phyllotaxis. Bihai Shi and Teva Vernoux. Current Topics in Developmental Biology, 131:81-92, (2018).

WUSCHEL in the shoot apical meristem: old player, new tricks. Filipa Lara Lopes, Carlos Galvan-Ampudia, and Benoit Landrein. Journal of Experimental Botany, 72:527–1535, (2021).

Modular growth of the shoot.

Unlike animals, the final body plan of a plant is elaborated after embryogenesis by the activities of meristems, or growing points. The shoot apical meristem (SAM) is a population of cells located at the tip of the shoot axis. It produces lateral organs, stem tissues and regenerates itself. In most plants little or no shoot tissue results from embryogenesis: essentially the entire shoot system derives from postembryonic development in the SAM.

(1). Meristems contain a population of cells with characteristics of stem cells; cell division serves to constantly replenish the meristem and to provide cells that will differentiate into plant organs and tissues. Unlike most types of stem cell in animal systems, cells produced by plant meristems have the capacity to differentiate as any cell type.

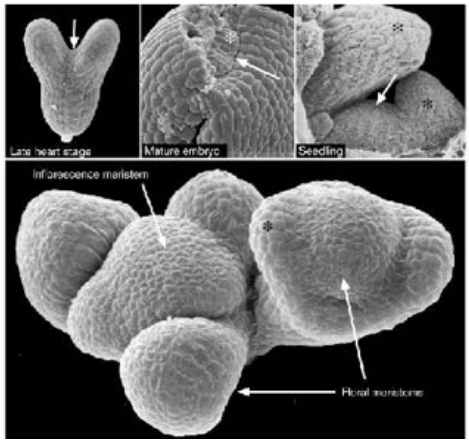
(2). Meristem growth occurs by the production of primordia which develop at the meristem periphery. These primordia undergo cell division and differentiation to develop into structures such as leaves or into additional meristems. After a phase of vegetative growth, the shoot apex changes to become an inflorescence meristem, which in turn produces many floral meristems. Each floral meristem produces primordia which form the various floral organ, such as sepals, petals, stamens and carpels. All shoot growth occurs through the production of lateral organs and secondary meristems by primordia, and this accounts for the characteristic branched appearance of plants.

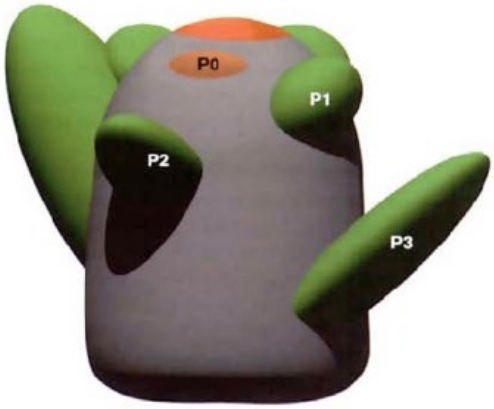
(3). The activity of meristems is often iterative. For example, a vegetative meristem will produce modular units each consisting of a leaf, bud, and internode. Each unit is called a phytomer.

(4). Meristems are self-organising. For example, meristems regenerate after bisection.

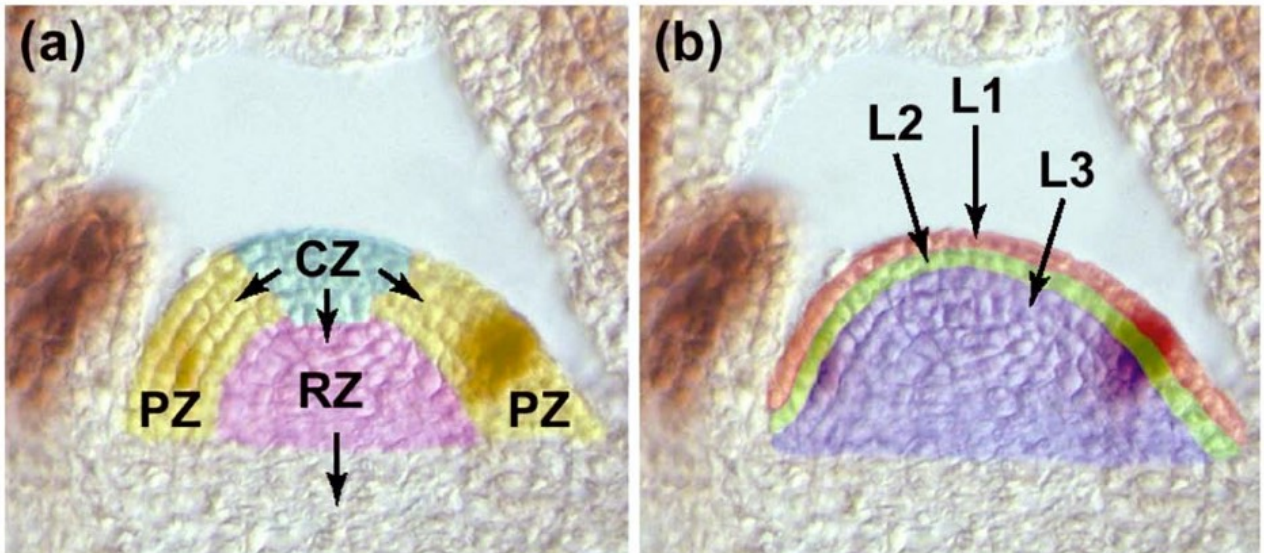
(5). Vegetative meristems are indeterminate, and in some species are capable of growth for thousands of years.

Shoot meristems must regulate organ formation by carefully balancing (i) the maintenance of undifferentiated stem cells with (ii) the commitment of appropriately positioned cells towards differentiation. In other words, some mechanism must be in place to maintain the size of the meristem.





Architecture of the Arabidopsis shoot meristem.
Two main architectural features of the shoot apical meristem can be recognised:
(1). The primordia can be divided into regions called, the central zone (CZ), peripheral zone (PZ) and rib zone (RZ). The central zone contains a core of stem cells, while the peripheral zone is the site of production of lateral organ primordia and the rib zone gives rise to differentiated cells of the growing stem.
(2). The shoot apex is divided into three layers. Layer 1 (L1) is a single layer of cells that generally only undergoes anticlinal divisions, and gives rise to the epidermis. Layer 2 (L2) is also a single layer, and gives rise to ground tissue, while the innermost layer (L3) forms the body of new tissues, including vasculature and germline tissue. The three layers generally maintain distinct lineages.



The activities of homeodomain proteins are required to promote shoot meristem activity.
Two genes have been implicated in the maintenance of undifferentiated cells in the meristem: *Shootmeristemless* (*Stm*) and *Wuschel* (*Wus*), both of which encode homeodomain proteins. In strong *stm* mutants, the meristem is absent at the end of embryogenesis; weak *stm* mutants fail to maintain the meristem after germination. The STM mRNA accumulates in both the central zone and peripheral zone of the meristem but is repressed in organ primordia, in accordance with a role in maintaining cells in an undifferentiated state. In *wus* mutants, the meristem is not established during embryogenesis; after germination, axillary meristems are initiated and aborted repeatedly. This repeated termination of the meristem has been attributed to a failure to specify the central stem cells that are required to repopulate the peripheral meristem.

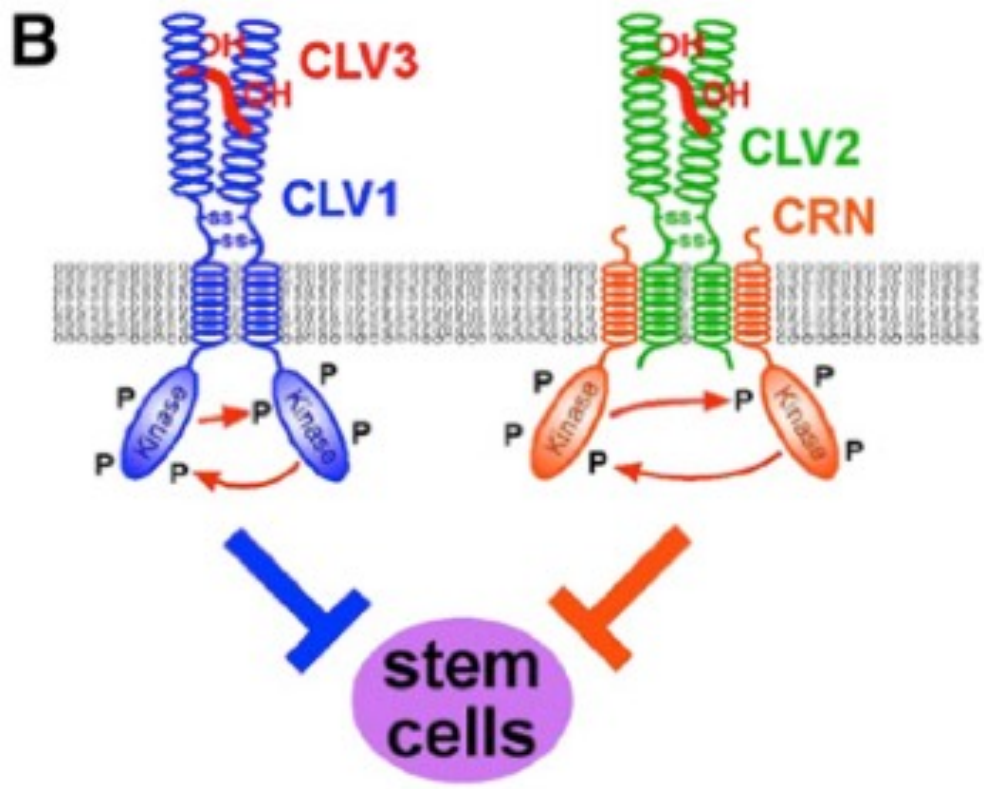
WUSCHEL is expressed at the earliest stages of meristem initiation.
The pattern of WUS gene expression suggests that stem cells in the shoot meristem are specified by an underlying cell group which is established very early during Arabidopsis embryogenesis in the 16-cell embryo and becomes progressively localized to an inner portion of the central zone of the meristem. STM expression commences slightly later, in the globular stage embryo.
(i) The STM gene is thought to prevent premature recruitment of cells into differentiation pathways.
(ii) The WUS gene is required to maintain the pool of stem cells in the central zone of the meristem. Combined expression of both WUS and STM can trigger the initiation of ectopic meristems and organogenesis even in differentiated tissues.

CLAVATA mutants possess enlarged shoot meristems.
In *clavata* mutant plants, vegetative, inflorescence and floral meristems are all enlarged relative to wild type. Flowers of *clavata* plants can have increased numbers of organs in all four whorls, and can also have additional whorls not present in wild-type flowers. The name of the phenotype arises from the "club-like" shape of the siliques (seed pods). The CLAVATA genes are required to limit the size of the meristem.

CLAVATA1 encodes a receptor kinase protein expressed in the shoot meristem. Molecular cloning and expression pattern of the CLV1 gene showed that it encodes a receptor kinase, suggesting a role in signal transduction. The

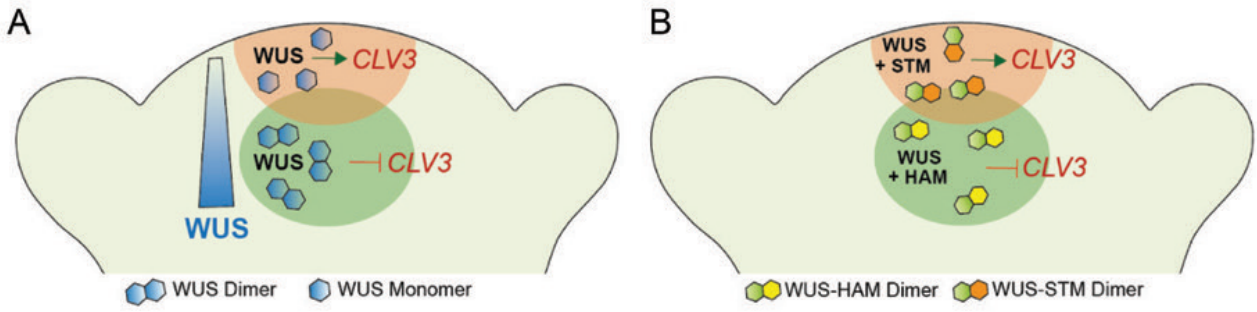
extracellular domain is composed of 21 tandem leucine-rich repeats that resemble the repeats found in pathogen resistance genes in plants and animal hormone receptors. Experiments have revealed additional receptor complexes. CLAVATA2 and CORYN encode receptor-like proteins. The CLAVATA2 gene encodes a receptor-like protein, with a presumed extracellular domain composed of leucine-rich repeats similar to those found in plant and animal receptors, but with a very short predicted cytoplasmic tail. (No protein kinase domain is present). CORYN encodes a receptor kinase domain with truncated extracellular portion, and might create a fully functional transmembrane receptor kinase with CLV2 through dimerisation. The CLV3 signal is probably transduced through two separate receptor complexes, one comprising CLV1 and the other one comprising CRN and CLV2. Unlike CLV1 that has a restricted expression domain, CLV2 and CRN are widely expressed in many plant tissues.

The *clavata3* mutation also produces enlarged shoot and floral meristems. CLAVATA3 encodes a secreted peptide ligand for the CLV1/CLV2/CRN receptor complexes. CLAVATA3 encodes a small, predicted extracellular protein, processed to form an arabinosylated 13 amino acid peptide. CLV3 acts with CLV1/CLV2/CRN (which encode receptor kinases) to control the balance between meristem cell proliferation and differentiation. CLV3 acts non-autonomously in meristems and is expressed in the L1 layer, at the meristem surface overlying the CLV1 domain. These proteins act as a ligand-receptor pair in a signal transduction pathway, coordinating growth between adjacent meristematic regions. CLAVATA1, 2 and CORYNE protein act in a parallel pathway to transmit the CLV3 signal, repress WUS signalling and limit proliferation of indeterminate cells in the centre of the apical meristem.

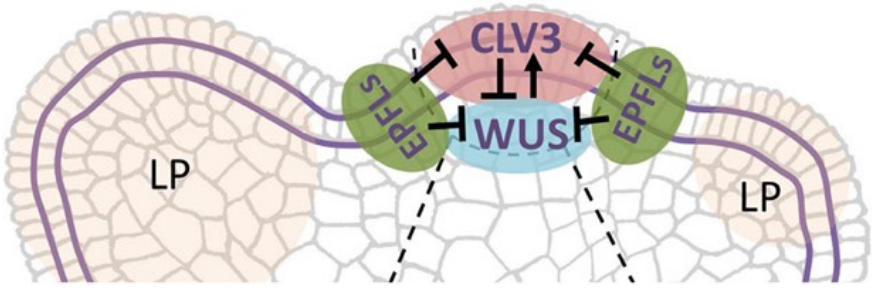


CLAVATA and WUSCHEL form a negative feedback loop.
Maintenance of the shoot meristem depends on the coordination of two antagonistic processes, (a) self-renewal of the stem cell population through cell proliferation and (b) cell differentiation and organ initiation. The WUSCHEL gene is required for stem cell identity, whereas the CLAVATA1, 2, and 3 genes promote organ initiation. (i) WUS expression is sufficient to induce meristem cell identity and the expression of the stem cell marker CLV3. (ii) Both WUS and CLV3 can move/diffuse to surrounding cells in the meristem. (iii) Expression of CLV genes represses meristem maintenance and WUS activity. The interactions between the WUSCHEL and CLAVATA pathways interactions establish a negative feedback loop between the stem cells and the underlying organising centre.

WUSCHEL activity across the meristem is regulated by protein dimerisation.
(i) WUS forms stable homodimers upon binding to DNA in a concentration-dependent manner, which affects its binding to the CLV3 promoter. Current models propose that WUS dimers negatively regulate CLV3 expression in the centre of the meristem, while WUS monomers positively regulate CLV3 expression in the outer part of the meristem.
(ii) In addition to the formation of homodimers,WUS can also form heterodimers with members of the HAIRY MERISTEM (HAM) family of TFs. Given that HAM is only expressed in L3, it was proposed that the formation of heterodimers between WUS and HAM prevents the induction of CLV3 in the central organising centre while the absence of HAM in L1 and L2 allows the induction of CLV3 by WUS in the outer part of the meristem.
(iii) WUS has also been shown to physically interact with STM, and STM binding to CLV3 promoter can enhance the stability of WUS binding to this promoter through the formation of heterodimer in the CZ. These interactions help refine regulatory domains of the meristem along the longitudinal axis of the meristem.



A network of regulators constrains cell patterning within the meristem.
A signalling pathway, consisting of ERECTA-like (ERL) family of receptors and EPIDERMAL PATTERNING FACTOR LIKE (EPFL) ligands, restricts meristem width. Although ERL receptors are expressed throughout the meristem, EPFL ligands are expressed in its periphery. Genetic analysis of Arabidopsis meristems demonstrated that ERL and CLV3 synergistically regulate the size of the SAM. Activation of ERL signalling with exogenous EPFLs results in a rapid decrease of CLV3 and WUS expression. ERL-EPFL signalling inhibits expression of WUS and CLV3 in the periphery of the SAM, confining them to the centre. These complementary interactions establish stem cell positioning along the radial axis.

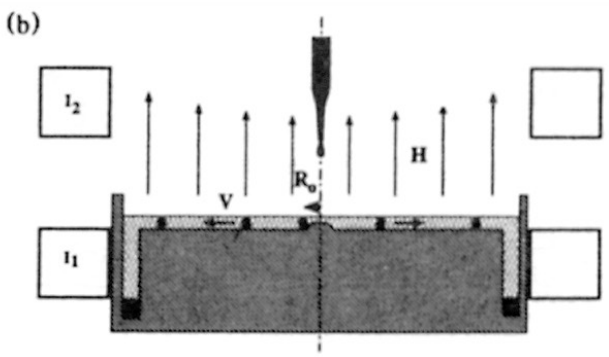


Phyllotaxy and the close-packed arrangement of emerging lateral organs.
Phyllotaxy is the arrangement of leaves on a stem. As a stem grows at its apex, new leaf buds form along the stem by a highly controlled developmental process. Depending on the species, the leaf origins on the stem may be opposite (in which leaves arise in pairs on opposite sides of the stem), whorled (three or more leaves arise from the same locus on the stem), or alternate (leaves are arranged in a helix along the stem).

Most species have alternate leaves. This pattern is often called spiral phyllotaxy because a spiral is formed when an imaginary line is drawn which connects progressively older leaf origins on the stem. The divergence angle of successive leaves determines the developmental spiral of leaves and homologous plant organs, such as the individual florets of a sunflower, and has been intensively studied by botanists and mathematicians since the mid-1800s. Interestingly, the angle between successive leaves on a stem is often about 137.5 degrees, known as the Fibonacci or "golden" angle.

In 1868, German botanist Wilhelm Hofmeister suggested that the mechanisms of plant development might help

explain spiral phyllotaxis. He was studying the growing tips of plants, and proposed that each new primordium develops on the tip of the growing stem in the spot that is farthest from older primordia. As the tip continues to grow from its center, the primordia are pushed outward and form spiral patterns. In recent decades, electron microscope images have added support to the idea that primordia arrange themselves according to Hofmeister's rule.



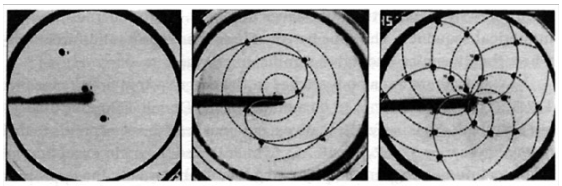


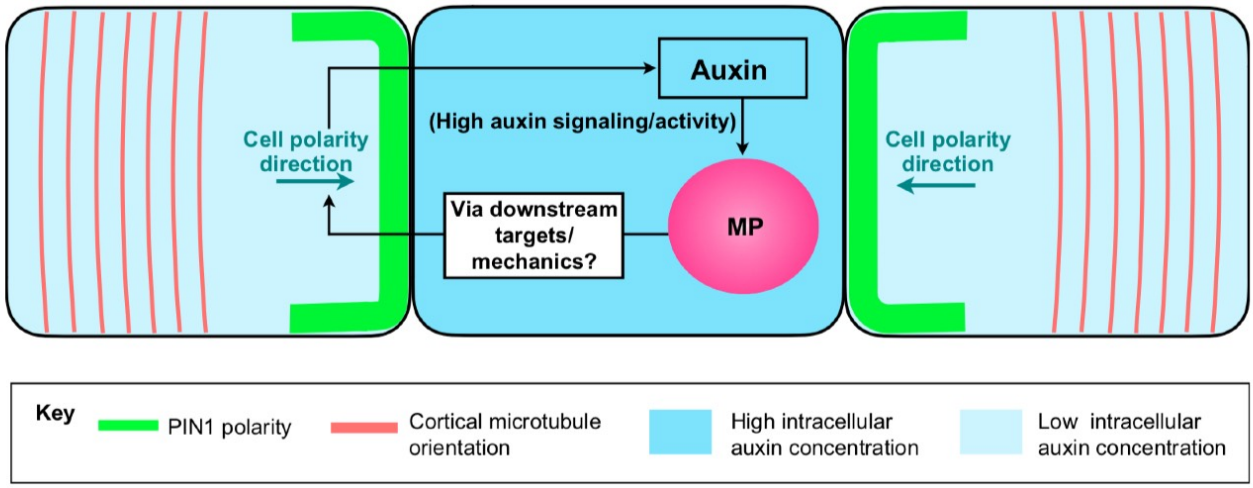
Figure 50
Fibonacci spirals observed in an experiment with electrically charged oil drops.

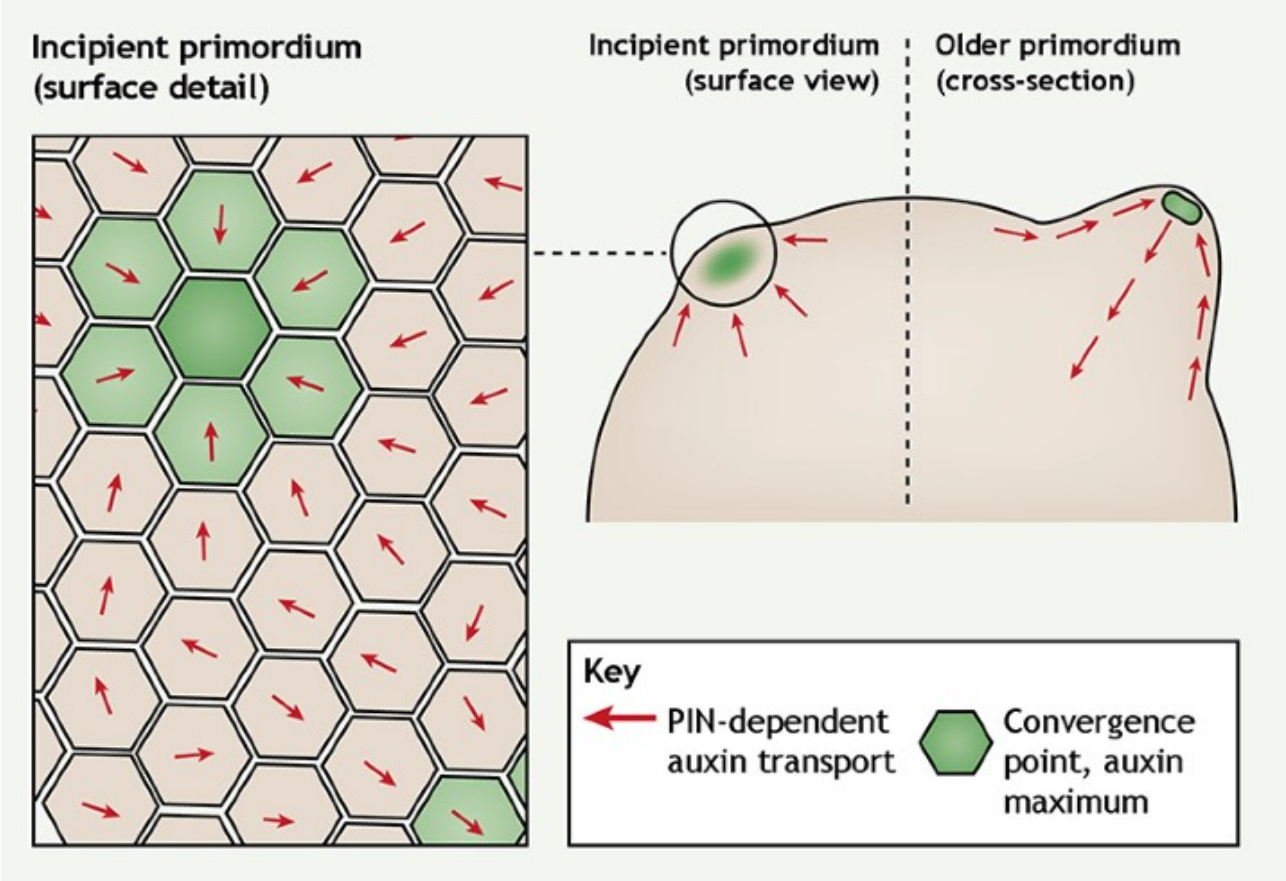
In 1992, physicists Douady and Couder performed an experiment where they let droplets of a magnetized liquid fall into a dish that was filled with silicone oil and magnetized along its outer edge. Magnetic forces attracted the droplets to the edge of the dish but made them repel one another. When Douady and Couder added droplets slowly, each new droplet would move toward the side of the dish, directly opposite from the previously added drop. But when they added droplets faster, the two most recently added droplets would both strongly repel the new one. Instead of marching to one side or the other, the new droplet would move in a third direction—at the golden angle from the line connecting the drop's landing point with the previous droplet. A stream of droplets added in this way formed a spiral pattern. The droplets in their experiment behaved like primordia.

A growing stem continually produces auxin, and a new primordium forms only when the concentration of auxin reaches a critical value. Once a primordium begins to form, more auxin flows into the primordium's cells. This inflow not only stimulates the growth of the existing primordium but also depletes the surrounding stem of hormone and suppresses the formation of new primordia nearby. Auxin is depleted least in the spot on the growing stem that is farthest from the older primordia. As auxin production across the stem tip continues, that farthest spot will be the first to reach the critical threshold to form a new primordium. In this way, the biochemistry of plant growth can explain Hofmeister's rule that new primordia form farthest from older primordia.

Feedback switch

Auxin flux and accumulation can trigger genetic responses through interaction with Aux/IAA and ARF proteins. This is seen in (i) the initiation of primordia in the shoot meristem, and (ii) the initiation of the embryonic root meristem primordium (described in lecture 3), both mediated by the MONOPTEROS (MP) ARF transcription factor. Studies of *Mp+* clonal sectors induced in *mp* mutant shoots demonstrated that MP expression elevated expression of auxin transporters and recruited adjacent cells to form a focus for auxin traffic and cytoskeletal polarity, and likely create a positive feedback loop for auxin response. The mechanism for recruitment of surrounding cells is unclear, but provides a possible basis for the switch-like response to auxin that is seen in these primordia.





Take-home messages:

1. Plant body plans are flexible, and are built step-wise through a series of local interactions.
2. Auxin is a mobile informational molecule and its directed traffic plays an important role in establishing key landmarks during cellular development in plants.
3. Auxin triggers specific genetic responses in cells via Aux/IAA and ARF pathways.
4. Genetic responses can trigger coordinated behaviour in adjacent cells, creating feedback systems e.g. PIN1-MP, WUS-CLV3 across meristems.
5. Competition for auxin can cause lateral inhibition and result in spatial patterning in responsive tissues, e.g. phyllotactic patterning.
6. PIN-mediated transport of auxin results in coupling of cells, and formation of long-distance interactions that can regulate the balance of growth across tissues or the entire plant.