Patterning and Plant Development
Cell and Developmental Biology Part 1B

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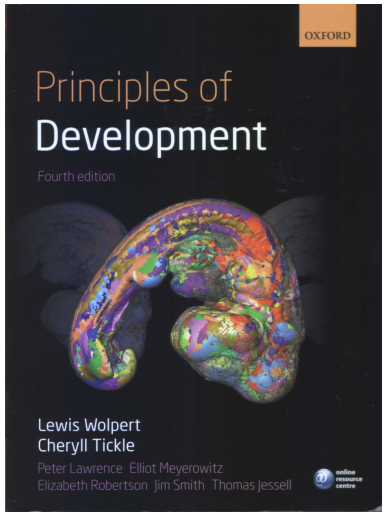
Summary:
In these six lectures we will look at some striking features of biological self-organisation and morphogenesis using examples from the model plant, *Arabidopsis thaliana*. Plant cells are immobile, constrained by a rigid cell wall – yet plant development is plastic and indeterminate. Communication between neighbouring cells controls plant cell fate, and plays a major role in shaping plant growth.

Lecture 1: Plant architecture and embryogenesis.
Lecture 2: Polarity and auxin flow.
Lecture 3: Regulation of gene expression by auxin.
Lecture 4: Patterning of indeterminate growth.
Lecture 5: Formation and specification of lateral organs.
Lecture 6: Morphogenesis.

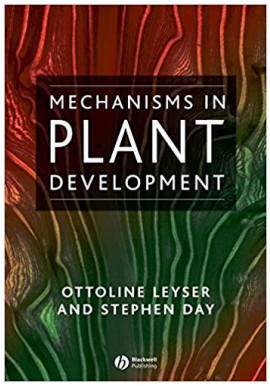
Web resources:
An electronic version of the lecture slides, a colour version of these notes and additional teaching materials including review papers and essay topics can be found on the web site: **http://haseloff.plantsci.cam.ac.uk** (click the "education" menu choice and navigate to the CDB Part 1B resources section).

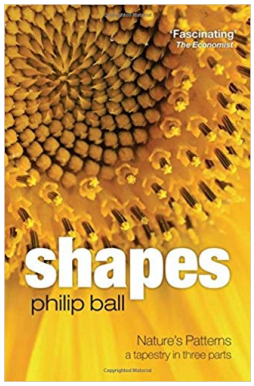


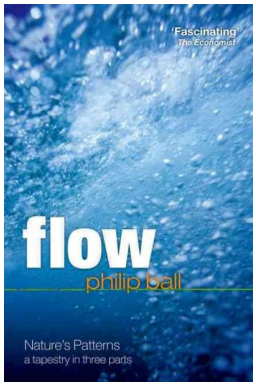
Recommended Text books:
For an integrated overview of animal and plant development see:
***Principles of Development*, Lewis Wolpert and Cheryll Tickle, Oxford University Press, 2011.** Chapter 7 provides a concise overview of the lecture content.

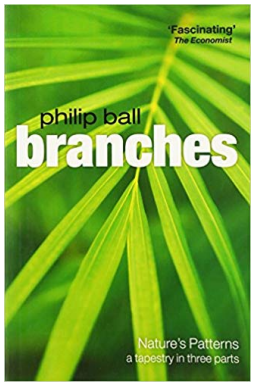
For more coverage of plant development see:
***Mechanisms in Plant Development*, Ottoline Leyser & Stephen Day, Blackwell Science, UK, 2002.**

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 architecture and embryogenesis.

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.

Background reading:

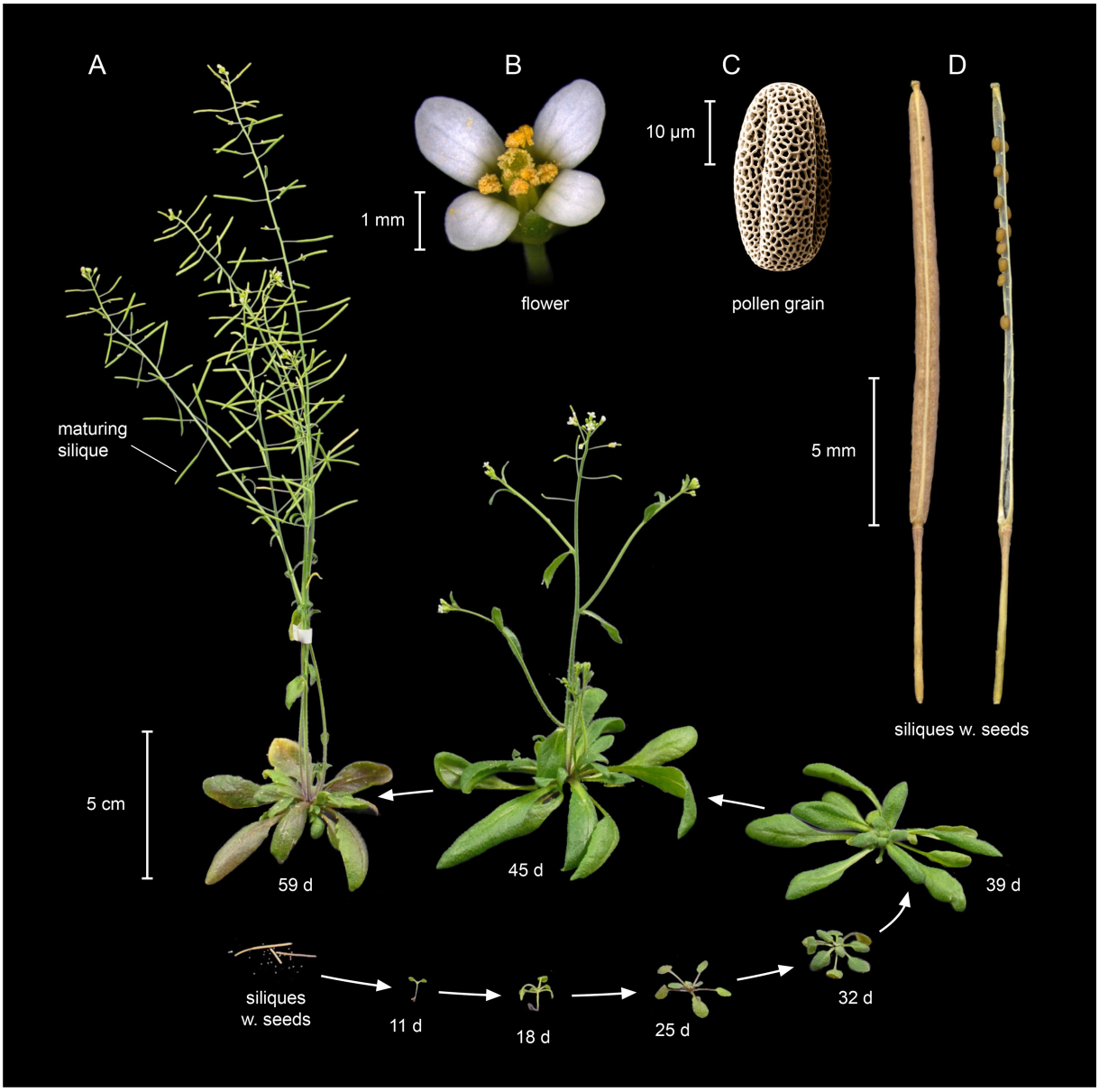
Field guide to plant model systems. Chang, C., Bownam, J.L. and Meyerowitz, E.M. Cell 167:325-339, (2016).

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).

Evolution of plants: adaptation to a terrestrial environment

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, but their green algal ancestors are thought to have appeared nearly 400 million years earlier. Shared features comprise the major evidence that vascular plants, possibly also bryophytes, evolved from green algae: both synthesise



chlorophylls a and b, both store true starch in plastids; both have motile cells with whiplash flagella, and both (but only a few green algae) are characterised by phragmoplast and cell plate formation following mitosis.

The first, indisputable vascular plants were characterised by a conducting system containing xylem and phloem, a waxy cuticle, epidermal stomata, and a reproductive system that produced trilete spores (spores with a triradiate scar resulting from their development in spherical tetrads) and probably containing sporopollenin in the walls. Such plants appear first in the late Silurian, but *Aglaophyton major* from the Lower Devonian, which has morphologic and structural features of both some bryophytes and primitive vascular plants, provides perhaps the best available model of a vascular plant precursor. Aglaophyton was a small plant, probably no more than 180 mm high, composed of dichotomous, upright axes that branched from rhizomes on the surface of the substrate. The epidermis of all axes was covered by a cuticle and contained stomata. In its small size and free-sporing reproduction, and water- and photosynthate-conducting cells it closely resembles mosses. It is likely that vascular plants evolved from this or plants of similar morphology and anatomy.

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.

Plant 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. Cell fate decisions are negotiated in the presence of constant neighbours.

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.

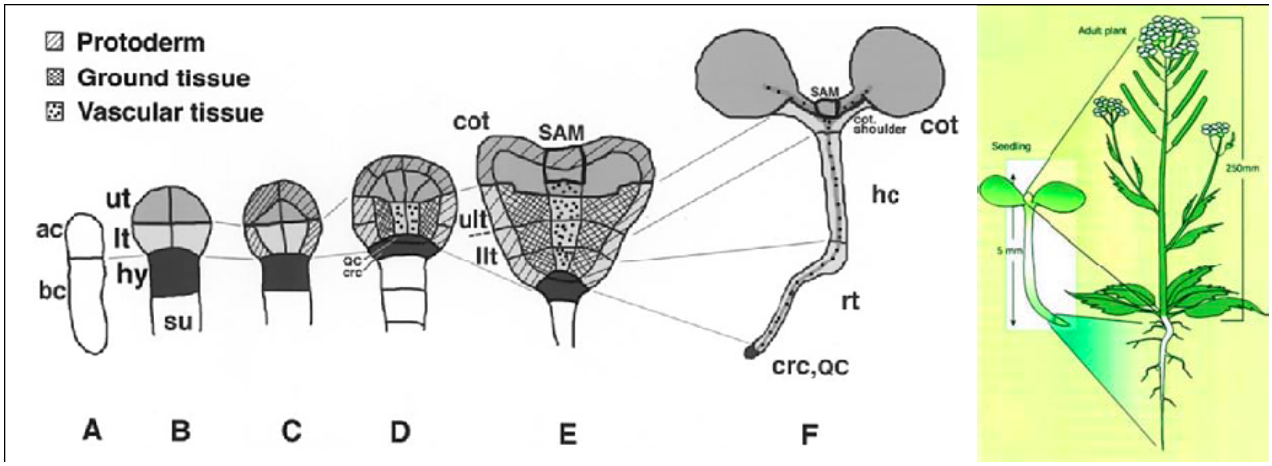
(4) Dermatogen stage [C]. Transverse divisions produce a single outer layer of cells. This outer layer is termed the protoderm and will give rise to the epidermis of the plant.

(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.

Genetic screening of Arabidopsis plants for developmental defects.

Mutations can be induced by conventional chemical or ionizing 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.

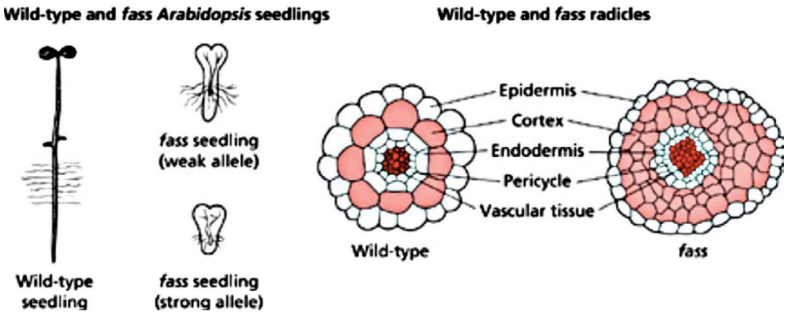
The regular pattern of cell divisions during embryogenesis produces a simple correspondence between embryo and adult body plans. Gerd Jurgens' group in Tubingen used this feature as the basis for a screen for Arabidopsis mutants with defective pattern formation. i.e. seedlings were screened for defects 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.

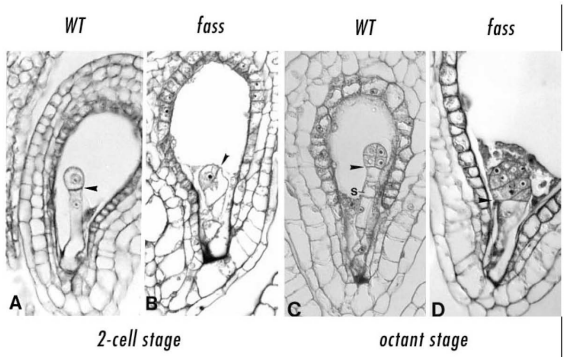
Seedlings were screened for loss or distortion of root, hypocotyl or cotyledon regions – which were shown to result from defects during embryogenesis. Mutants were grouped as: having disrupted organogenesis - *knolle* (kn), *keule* (keu), *fass* (fs), *knopf* (knf), *mickey* (mic) lacking body segments - *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 disrupted in the *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 appears to be required for 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 the auxin efflux transporter.

Lecture 2: Polarity and auxin flow.

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.

Background reading:

Auxin: A major regulator of organogenesis. Bohn-Courseau, I. Comptes Rendus Biologies, 333:290-6 (2010).

Polar targeting and endocytic recycling in auxin-dependent plant development. Kleine-Vehn, J. and Friml, J. Ann. Rev. Cell Dev. Biol. 24:447-473, (2008).

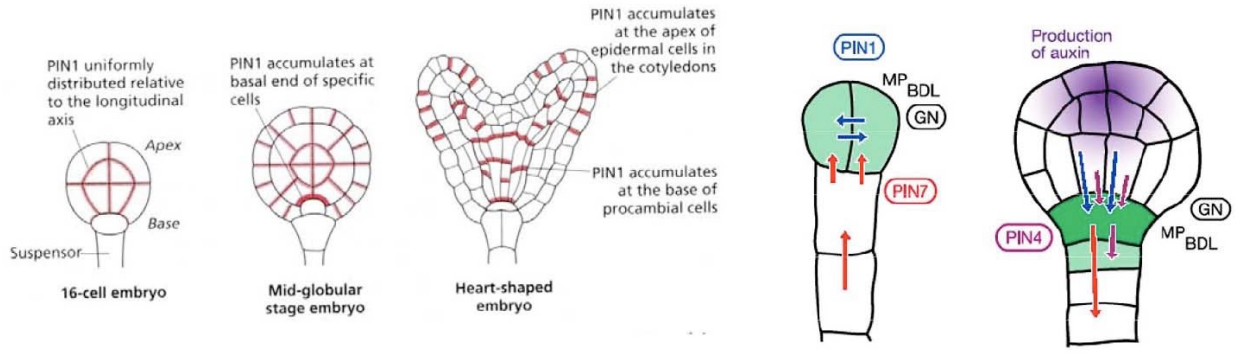
The march of the pins: Developmental plasticity by dynamic polar targeting in plant cells. Grunewald, W., & Friml, J. The EMBO Journal, 29:2700-14 (2010).

Cell fate determination

Cells must adopt particular identities during the construction of a regular body plan in embryogenesis. Every cell is produced 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 would rely 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 Arabidopsis embryos appears independent of cell division patterns. Therefore it is more likely that daughter plant cells primarily sense their different positions within the tissue and develop according to regulatory signals exchanged between neighbouring cells.

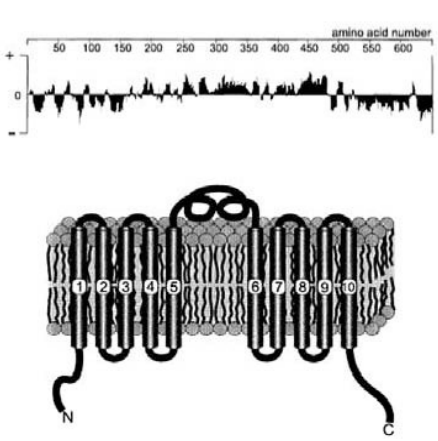
Genetic defects in hormone traffic or perception produce plants with altered body plans.

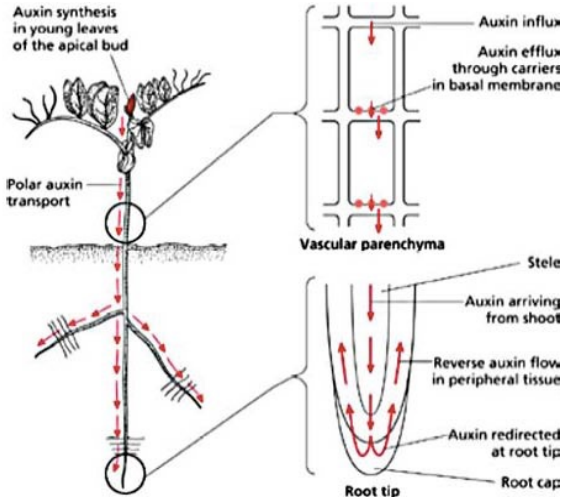
A number of the embryo patterning mutants isolated by Jürgens and colleagues possess defects in hormone traffic or response. For example, GNOM encodes an ADP ribosylation GTP exchange factor (ARF GEF) and regulates traffic of membrane vesicles. Mutant gnom embryos show a loss of apical-basal polarity. 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.



Auxin and the establishment of cell polarity and fate.

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 its unique mechanism of action allows coordination of cell fates at both long and short ranges.





Genetic screens in Arabidopsis have allowed the isolation of genes for components of the auxin influx and efflux carriers.

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.

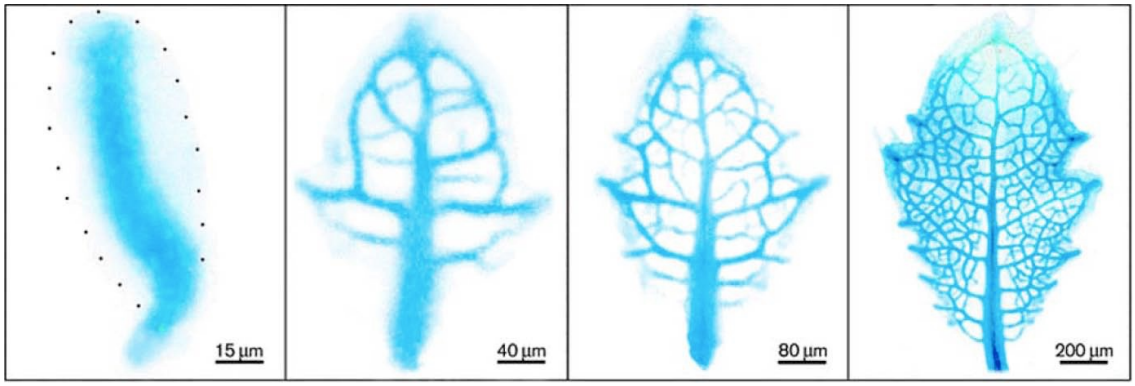
The PIN genes are part of a small family of transmembrane carriers. For example, PIN1 is a 67K protein with 10 predicted transmembrane spans. Immunolocalisation showed that the protein is localised at the basal side of cells in the centre of the root, positioned at the plasma membrane. In contrast, the 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.

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 leads to differentiation of surrounding parenchyma cells as replacement xylem and phloem cells to form a "bypass" around the broken connection.

Auxin transport is required for formation of plant vascular tissues.

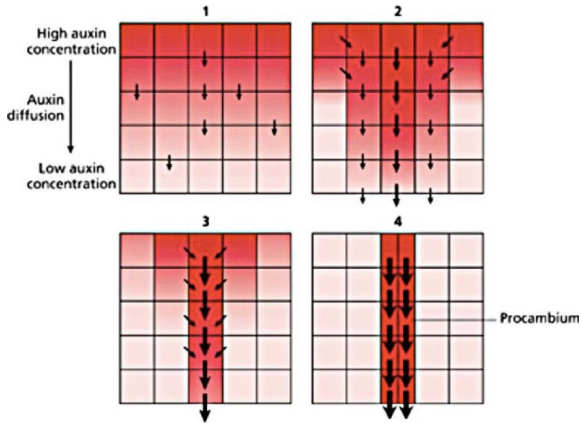
1. Application of synthetic auxin to undifferentiated tissue causes the formation of vascular cells.
2. Auxin transport inhibitors disrupt the pattern and connectivity of the vasculature in Arabidopsis leaves.
3. Arabidopsis mutants with defects in auxin traffic or perception have a disrupted vasculature.



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 may reflect a progressive refinement, or "canalisation" of auxin transport.

Feedback regulated traffic of auxin.

Current models for auxin based regulation in the Arabidopsis embryo, root and shoot rely on a "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.



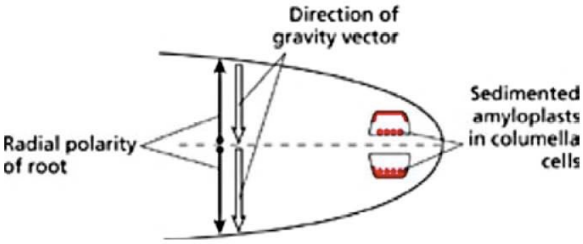
Polar flux of auxin is required for the proper development and growth of the Arabidopsis root meristem.

The Arabidopsis root meristem forms a ideal system for studying cell-cell interactions in plants. It has a simple and stereotypical architecture, with very regular arrangements of cell files around a central group of initials, or stem cells. It grows indeterminately, and is exposed, small, and transparent. These properties make it ideal for microscopy of the intact organ. The root meristem has been used for laser ablation studies. Individual cells within the meristem can be killed by laser illumination, and the response of neighbouring cells can be precisely gauged.

1. Death of a central cell in the "quiescent centre" triggers the differentiation of a neighbouring columella initial, i.e. loss of an inhibitory signal.
2. If an endodermis/cortex initial is separated from the more mature cells in its file, it remains arrested in an immature fate.

Laser ablation experiments indicate that interactions between cells in the root meristem are very precise, and suggest that short-range signals control patterning and the balance between cell proliferation and differentiation.

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.


Direction of
gravity vector
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of root
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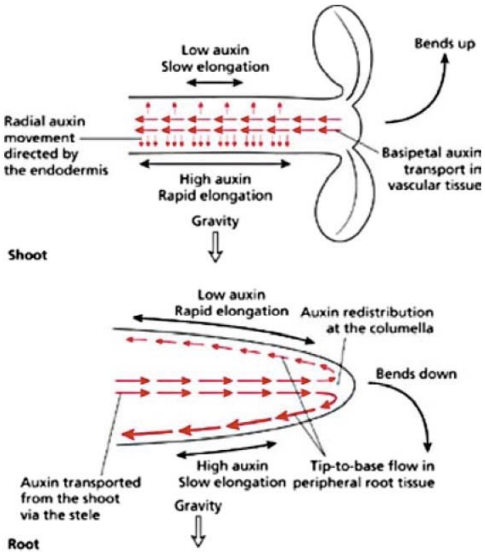

Low auxin
Slow elongation
Bends up
Radial auxin
movement
directed by
the endodermis
Basipetal auxin
transport in
vascular tissue
High auxin
Rapid elongation
Gravity
Shoot
Low auxin
Rapid elongation
Auxin redistribution
at the columella
Bends down
Auxin transported
from the shoot
via the stele
High auxin
Slow elongation
Tip-to-base flow in
peripheral root tissue
Gravity
Root

Lecture 3: Regulation of gene expression by auxin

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.

Background reading:

Mechanisms of auxin signalling. Lavy, M. and Estelle, M. Development 143:3226-3229, (2016).

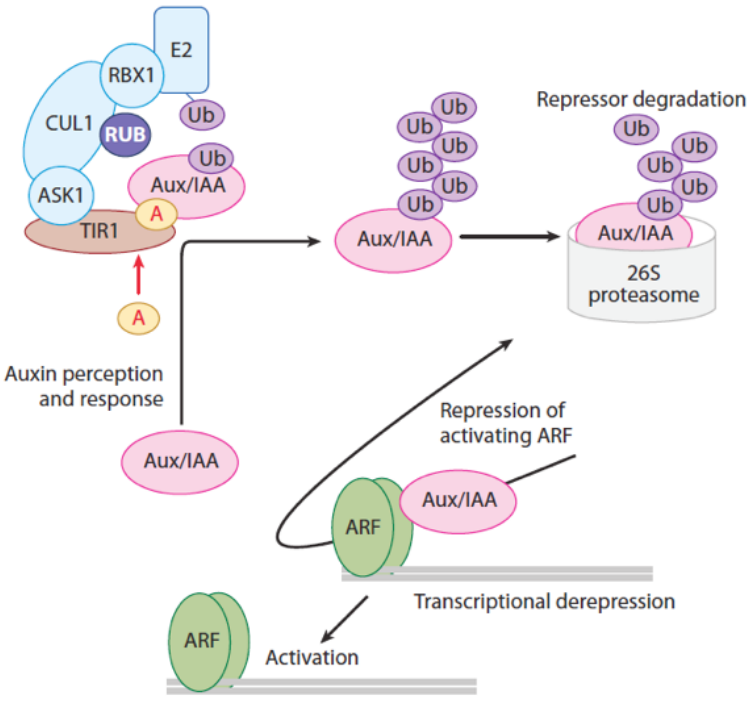
Transcriptional responses to the auxin hormone. D. Weijers and D. Wagner, Annual Rev. Plant Biol. 67:21.1–21.36 (2016)

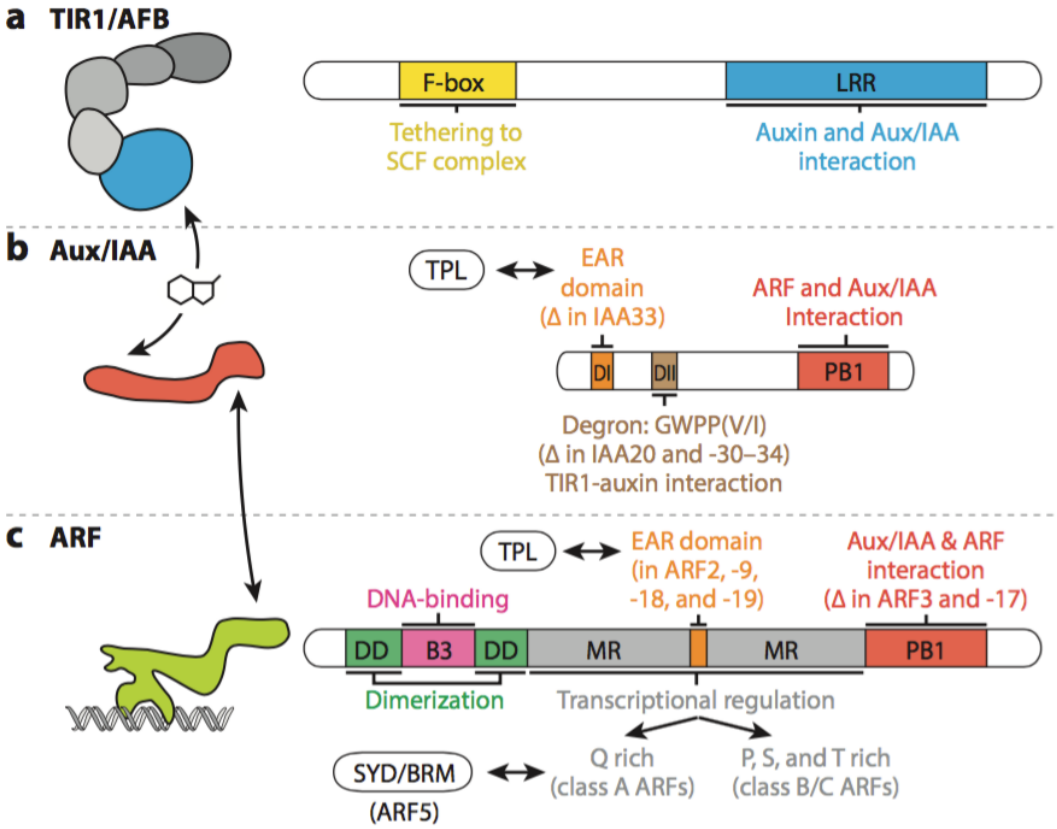
Structural biology of nuclear auxin action. D.C. Dinesh, L.I.A. Calderón Villalobos and S. Abel, Trends in Plant Science, 21:302-315 (2016)

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 1/AUXIN SIGNALING F-BOX (TIR1/AFB) 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 comprising a TIR1/AFB F-box protein and an Aux/IAA transcriptional coregulator senses auxin. Auxin promotes the interaction between TIR1/AFB 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 TIR1/AFB and Aux/IAA families, respectively.

TIR1/AFB 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 TIR1/AFB proteins bind auxin. TIR1/AFB family members have been shown to promote auxin responses, and mutants in these factors are either subtly auxin resistant as single mutants (tir1, afb2, and afb3) or strongly resistant as higher-order mutants (afb1). The tir1/afb mutants also 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 was also in contact with the leucine-rich-repeat do- main 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.





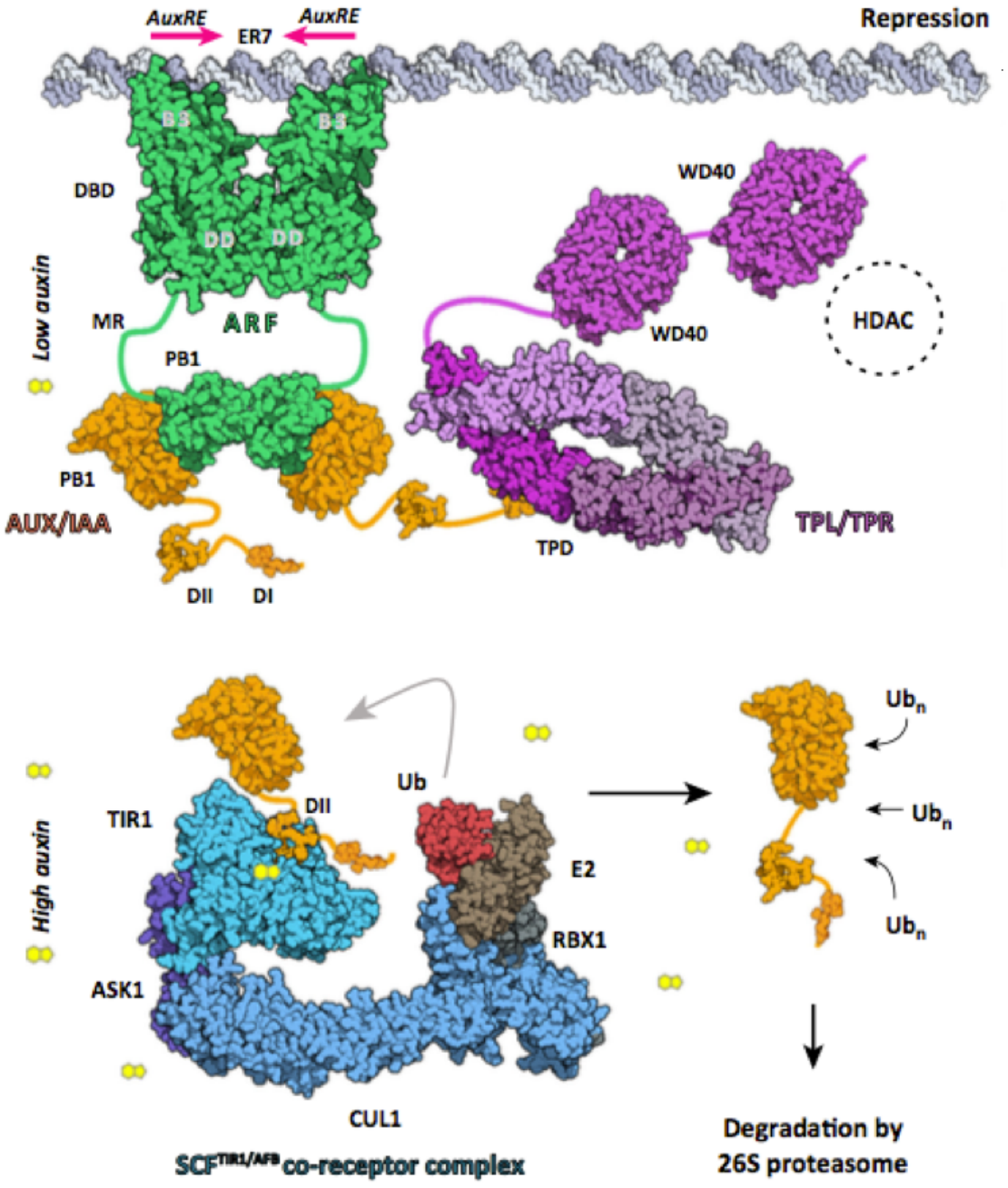
Domain architecture of the central components of auxin-dependent gene regulation.
Auxin responses are mediated by interactions (arrows) between three core components: (a) TIR1/AFB auxin receptors, (b) Aux/IAA transcriptional repressors, and (c) ARF transcription factors. TIR1/AFB proteins contain an 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 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

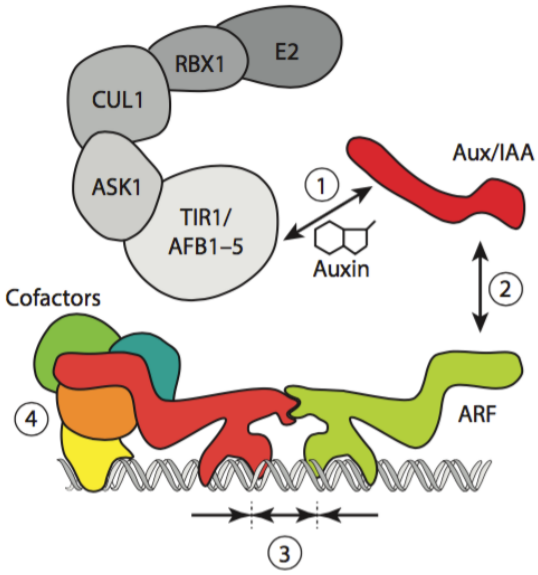
ARF transcription factors

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 dimerization 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) such as HDA19. Loss of HDA19 activity partially rescues the phenotypes associated with gain-of-function mutations in Aux/IAA-encoding genes, and both TPL and HDA19 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.

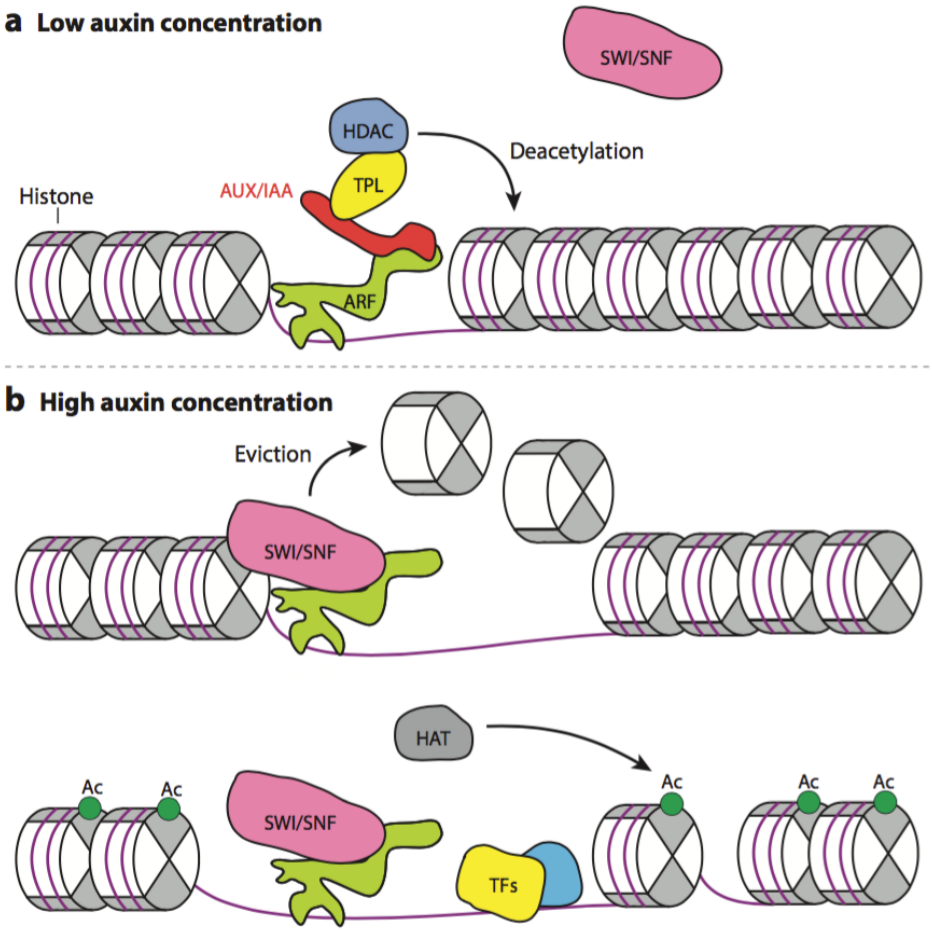
Another class of chromatin regulatory proteins, the SWITCH/ SUCROSE NONFERMENTING (SWI/SNF) chromatin-remodeling ATPases, helps over- come this repressed chromatin state upon auxin sensing. SWI/SNF chromatin- remodeling complexes use the energy derived from ATP hydrolysis to alter the occupancy or positioning 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, 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 seems intuitive 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 such 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 polymorphs* suggests that auxin responses may have evolved earlier.

Lecture 4: Patterning of indeterminate 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 genes that regulate the patterning of meristem primordia.

Background 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).

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.

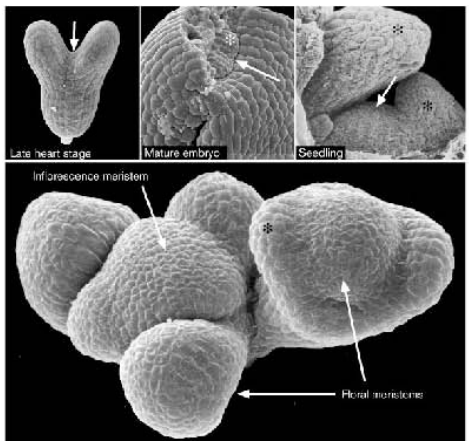
(2). Meristem growth occurs by the production of primordia which develop at the meristem periphery. These primordia undergo cell division and differentiation to develop into structures such as leaves or into additional meristems. After a phase of vegetative growth, the shoot apex changes to become an 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.

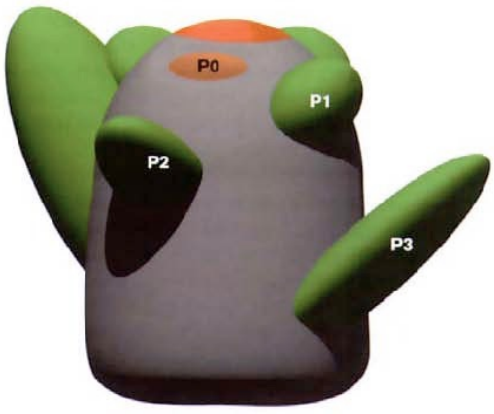
(3). The activity of meristems is often iterative. For example, a vegetative meristem will produce modular units each consisting of a leaf, bud, and internode. Each unit is called a phytomer.

(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.



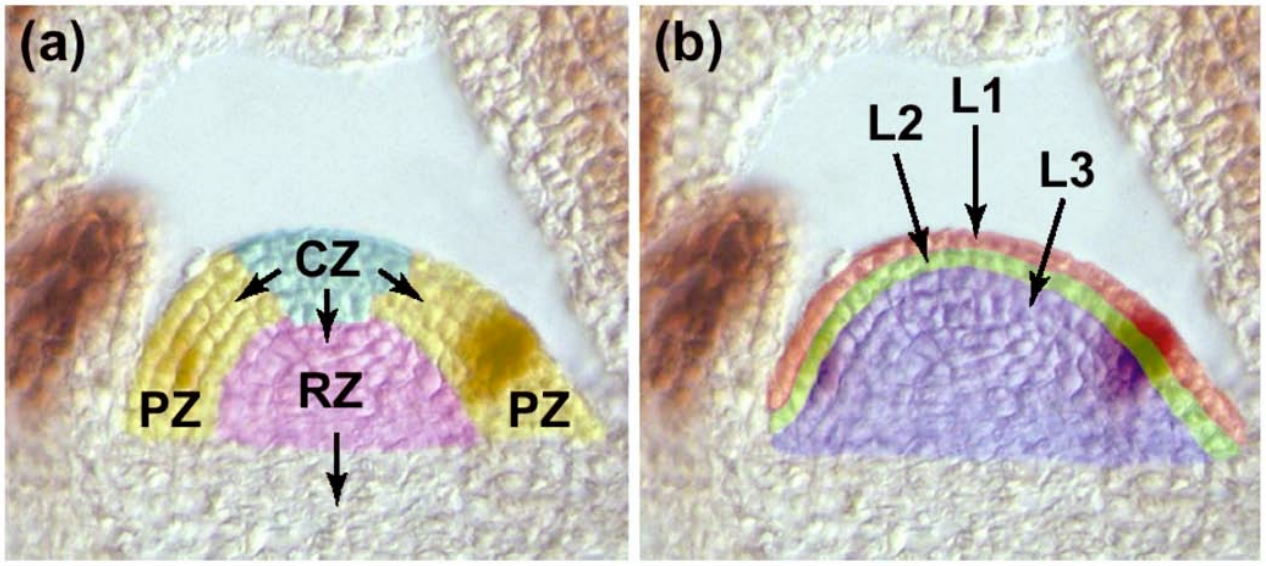


Architecture of the Arabidopsis shoot meristem.

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

WUSCHEL and SHOOT MERISTEMLESS play complementary roles in maintaining the shoot meristem.

(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.

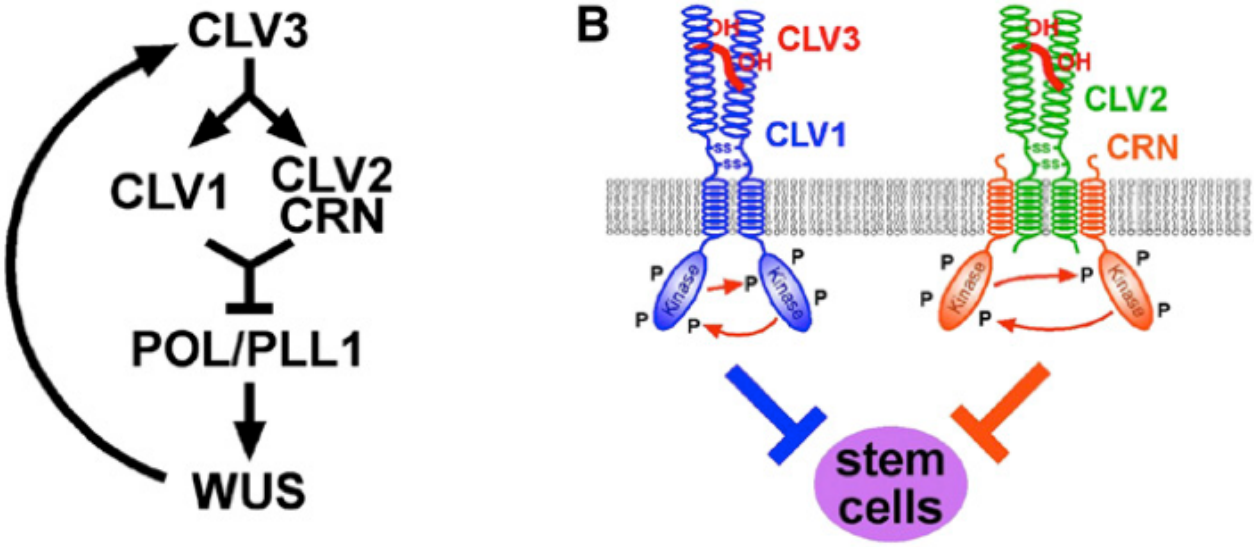
In *clavata* mutant plants, vegetative, inflorescence and floral meristems are all enlarged relative to wild type. Flowers of *clavata* plants can have increased numbers of organs in all four whorls, and can also have additional whorls not present in wild-type flowers. The name of the phenotype arises 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 leucine-rich repeats found in pathogen resistance genes in plants and animal hormone receptors.

CLAVATA2 encodes a receptor-like protein.
Experiments have revealed two receptor complexes, which probably act independently of each other. The CLAVATA2 gene encodes a receptor-like protein (RLP), 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). The clv2 mutants also possess enlarged meristems and distorted organ development.

CLAVATA3 encodes a secreted peptide ligand for the CLV1/CLV2/CRN receptor complexes.
The *clavata3* mutation also produces enlarged shoot and floral meristems. CLAVATA3 encodes a small, predicted extracellular protein. 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.

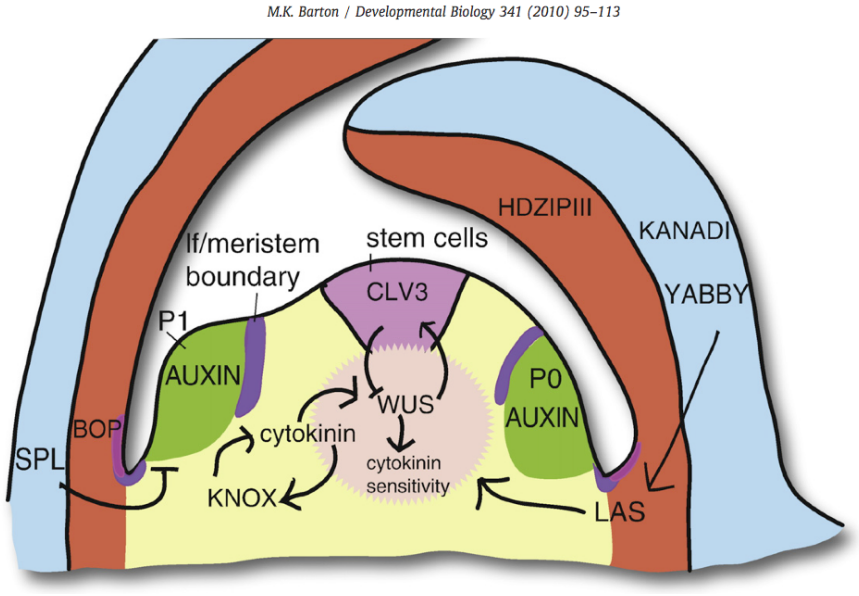
CLAVATA2 acts with the CORYNE protein in a parallel pathway to transmit the CLV3 signal. The crn mutant, like the clv mutants, shows an enlarged SAM and is defective in floral organ development. This suggests that CORYNE is implicated in the repression of WUS signalling. However, whereas the clv1 crn double mutant has an additive effect on carpel number, the clv2 crn mutant has carpel numbers similar to each single mutant, implying that CRN and CLV2 act together, but independently of CLV1. CRN encodes a membrane-bound receptor kinase containing a short non-LRR extra- cellular domain and a cytoplasmic kinase domain. Thus, CRN has a kinase domain which might create a fully functional transmembrane receptor kinase together with CLV2 through dimerisation by the transmembrane domain regions, while the extracellular LRR domain of CLV2 might interact with a putative ligand such as CLV3. 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.



Interaction between the CLAVATA and WUSCHEL regulatory pathways.
Maintenance of the shoot meristem depends on the coordination of two antagonistic processes, organ initiation and self-renewal of the stem cell population. The WUSCHEL gene is required for stem cell identity, whereas the CLAVATA1, 2, and 3 genes promote organ initiation.

1. WUS expression is sufficient to induce meristem cell identity and the expression of the stem cell marker CLV3.
2. 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.



Lecture 5: Formation and specification of lateral organs.

Lateral organs are formed from primordia initiated at the flanks of the apical meristem. The final arrangement of lateral organs like leaves, bracts, florets is due to auxin-mediated competition for space during initiation. The process can be also seen in physical models. Lateral organs adopt adaxial-abaxial polarity due to signals arising from the apex. Interactions between miRNAs and homeodomain gene targets are required for maintenance of adaxial-abaxial polarity. Outgrowth of the leaf blade lamina is observed at the junction of adaxial and abaxial regions. Radial patterning in the root meristem requires intercellular traffic of transcription factor molecules. Combinatorial assembly of transcription factor complexes underpins organ specification in flowers.

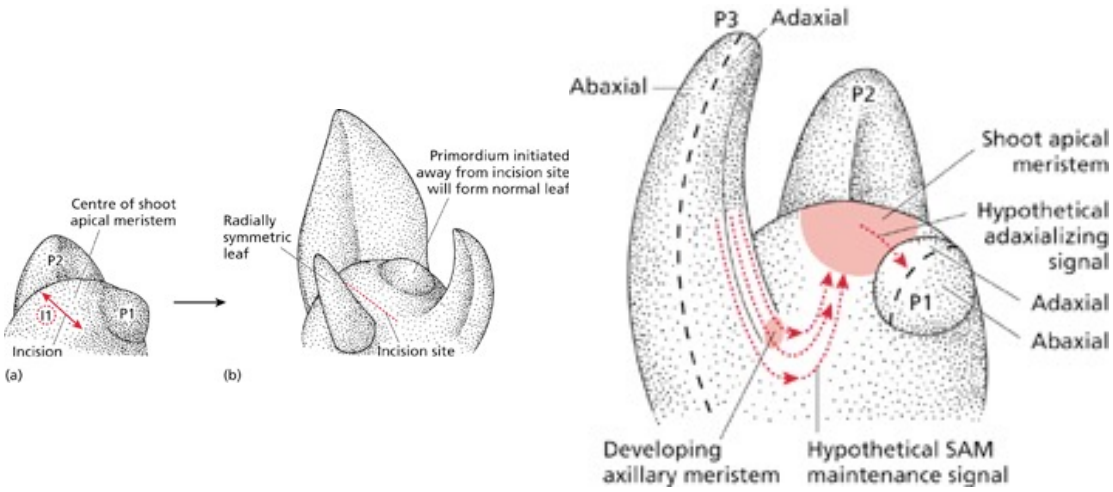
Background Reading:

Perspectives on leaf dorsoventral polarity. Szakonyi, D., Moschopoulos, A., & Byrne, M. E. Journal of Plant Research, 123:281-90 (2010).

Floral organ identity: 20 years of ABCs. Causier, B., Schwarz-Sommer, Z., & Davies, B. Seminars in Cell & Developmental Biology, 21:73-9 (2010).

1. Polarity of lateral organs.

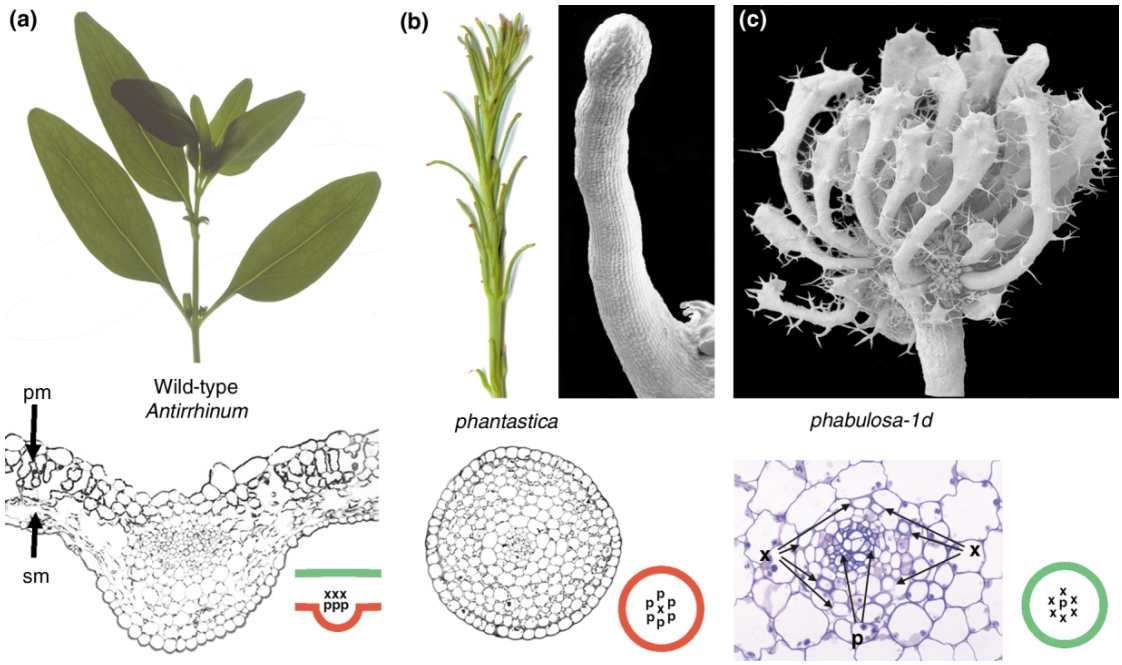
A hallmark of land plant evolution has been development of the leaf. In angiosperms, leaves are typically planar, dorsoventrally flattened structures. Dorsoventrality is specified early in development of primordia. In the initiating leaf, the dorsal, or adaxial, side is immediately adjacent to the shoot apical meristem, whereas the ventral, or abaxial, side is farther from the shoot meristem. In the mature leaf, the adaxial side is usually the upper sun-exposed side of the leaf and the abaxial side is the lower shaded side of the leaf.



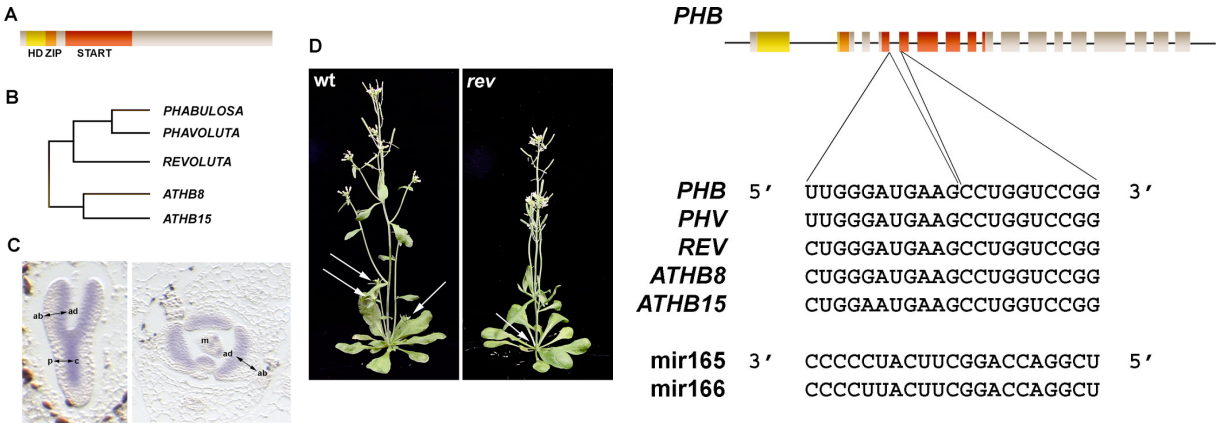
A series of surgical experiments carried out in the 1950s, and elaborated upon more recently, were the initial key to mechanisms that establish leaf dorsoventral patterning. Separation of initiating primordia from the meristem by surgical incision generated a radial, abaxial leaf. This suggested, firstly, lateral organ patterning required an interaction between the initiating organ and the shoot apical meristem and, secondly, that in the absence of this interaction loss of dorsoventrality resulted in radial organs. Thus positional information in the context of the apical meristem specifies dorsoventral patterning and development of a leaf as a planar structure.

Bilateral polarity and leaf mutants in Antirrhinum

The evidence for the molecular basis for dorsoventral patterning came from the aptly named *phantastica* (*phan*) mutant in Antirrhinum. Severely affected leaves in *phan* mutants are abaxial and fully radial, whereas weakly affected leaves have abaxial sectors on the adaxial leaf surface surrounded by ectopic lamina. The phenotypes of *phan* are entirely consistent with a requirement for dorsoventrality in lamina development.



Class III HD–ZIP transcription factors have in common a homeodomain DNA binding motif and a leucine zipper dimerisation motif (HD-ZIP), and are a subset of a much larger group of plant proteins that also include a sterol/lipid binding (START) domain. Although lipid ligands for a small number of START domain proteins have been identified in animals, none to date have been found for plant START proteins. There are five Class III HD–ZIP genes in Arabidopsis, each encoding a protein in the range of 833– 852 amino acids, and sharing between 60% to 85% amino acid homology.



(A) Class III HD–ZIP genes encode 833–852 amino acid proteins with main domains indicated; an N-terminal HD-ZIP domain, and a 213–218 amino acid START domain. (B) Relationship between five Arabidopsis Class III HD–ZIP genes. (C) Representation of expression pattern of PHB in longitudinal section of embryo (left) and transverse section of shoot apex (right). In the embryo, expression is adaxial in cotyledons and in central provasculature. In the shoot apex, expression is adaxial in developing leaves and in the meristem.
ad, adaxial; ab, abaxial; p, peripheral; c, central; m, meristem (D) Phenotype of *rev* mutants. In wild-type, axillary meristems in axils of leaves give rise to lateral branches (arrows). In rev mutants, axillary meristems are frequently absent, and fewer or no lateral shoots are produced. Above right: conserved targets for miRNAs.

Class III HD–ZIP Genes in Arabidopsis

All five Class III HD–ZIP genes in Arabidopsis have well defined tissue-specific expression patterns within the embryo and shoot. PHB and REV are expressed early in embryogenesis and appear throughout the 16-cell embryo. As development proceeds, expression becomes confined to the adaxial domain of the cotyledons and the central region of the embryo, including the shoot apical meristem and provasculature. The Class III HD–ZIP genes play multiple, possibly interdependent, roles in plant development.

Conservation of these genes and expression patterns throughout land plants, in particular in lower land plant species, highlight a critical role in development of the basic plant body. Spatial, temporal, and quantitative regulation of expression appears to involve a number of mechanisms including posttranscriptional and transcriptional gene silencing mediated by microRNAs. The importance of microRNAs as regulators of this gene family is reflected in conservation of miR166 and conservation of Class III HD–ZIP gene function in divergent plant species. An additional layer of regulation may involve modulation of function via a sterol type ligand.

Regulation by microRNAs

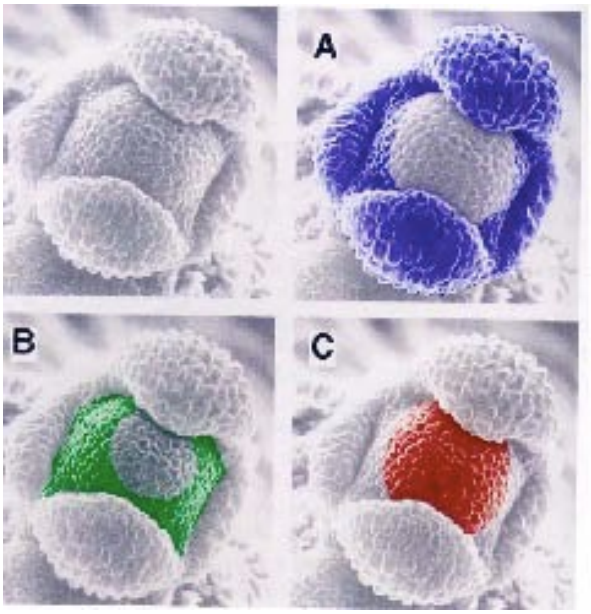
MicroRNAs are approximately 21 nucleotides in length and are generated from longer precursor transcripts. The precursor transcripts notably form a hairpin loop structure that is recognised and cleaved into a double stranded form carrying the microRNA and a complementary sequence. Ultimately, the microRNA as a single strand is guided to the target transcript, a process involving the small RNA binding proteins of the ARGONAUTE family. Subsequently, target transcripts are either cleaved within the region binding the microRNA or are subject to translational inhibition. The Arabidopsis genome encodes two copies of miR165 and seven copies of miR166, which have near perfect match with a sequence conserved within the transcript of all Arabidopsis Class III HD–ZIP genes. Overexpression of microRNAs results in a reduction in Class III HD–ZIP gene transcripts.

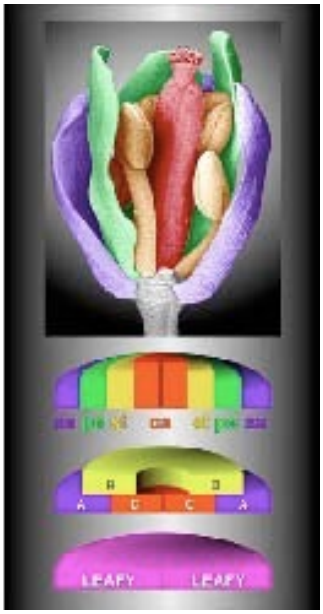
Outgrowth of the leaf lamina.

Boundary ridges surround ectopic clonal sectors of abaxialised tissue on a *phantastica* leaf. These ridges appear to represent secondary leaf laminae, and are produced at the junction of abaxial and adaxial tissues. The juxtaposition of the two tissue types triggers outgrowth of the lamina.

2. Specification of different organs.

The shoot apical meristem undergoes a series of transitions during development of the plant. The plant normally grows vegetatively for a time, before bolting and entering the reproductive phase. The shoot meristem changes from a vegetative meristem (producing leaves) to an inflorescence meristem (producing flowers) to a floral meristem, which is a determinate structure, producing a regular arrangement of floral organs. The Leafy gene plays a major role in integrating various environmental and hormonal cues and controlling this transition. Constitutive expression of LEAFY results in premature flowering.





Arabidopsis flowers and the ABC model.

Arabidopsis flowers consist of four concentric rings of organs: an outer whorl of 4 sepals, followed by 4 petals, 6 stamens and 2 fused carpels. These organs are produced as a series of outgrowths from the meristem. Mutations with altered floral structure have provided insight into the genetic interactions that drive patterning of the floral organs. Three types of patterning information are thought to be the basis for formation of the floral organs. An A function has been proposed to specify sepals; A+B specifies

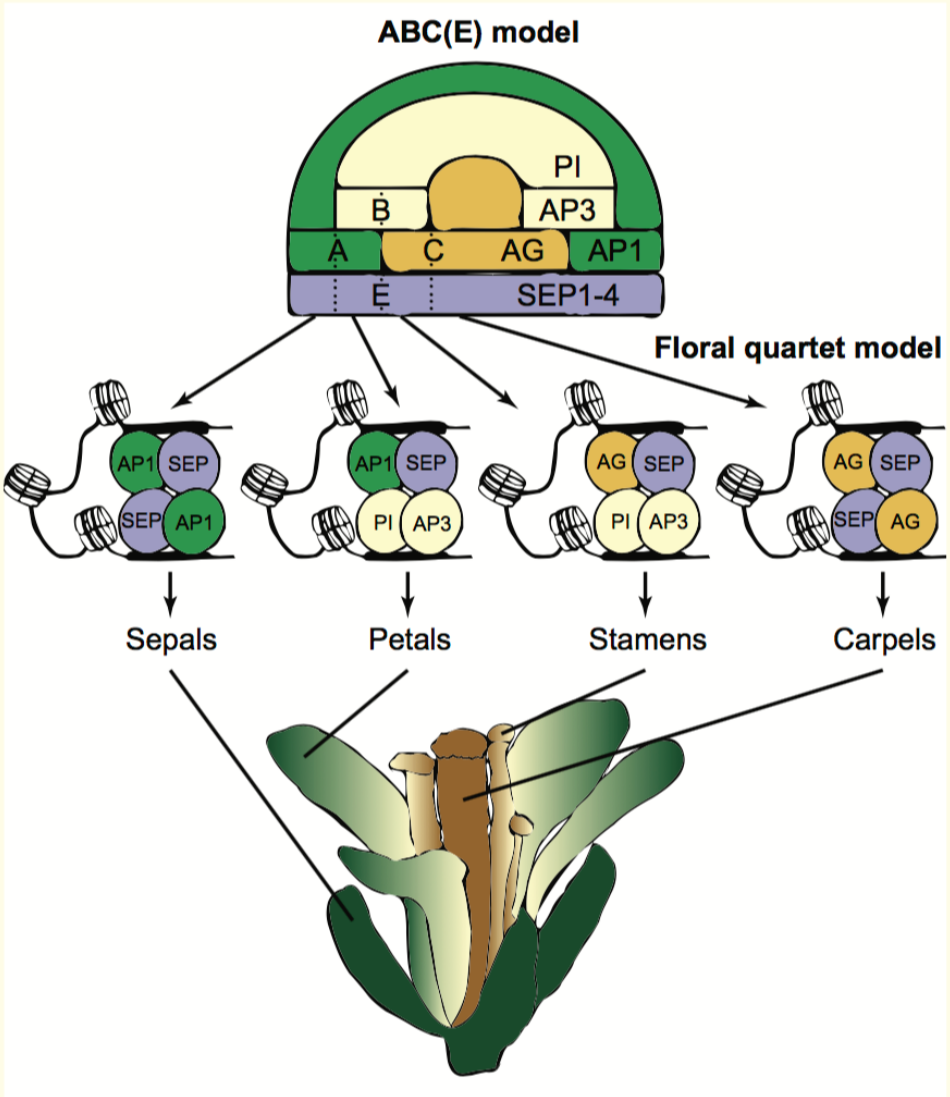
petals;B+C specifies stamens, and C specifies carpels. A and C functions are mutually antagonistic. Loss of A results in C activity in all whorls, and conversely, loss of C results in A activity in all whorls. This simple model is consistent with the properties of a number of mutants (see below) -where *apetala2* (*ap2*)corresponds to loss of the A function, *apetala3* (*ap3*) and *pistillata* (*pi*) correspond to the B function, and *agamous* (ag) is the C function. These genes have been identified as MADS box transcription factors.

MADS box transcription factors and the quartet model.

The family of MADS box transcription factors [MCM1 (*minichromosomemaintenance*), AG (*agamous*), DEF (*deficiens*), SRF (*serum response factor*)] possess conserved protein domains involved in DNA binding, dimerisation and protein-protein interaction. The proteins bind to DNA as dimers and recognise a CArG-box (CC(A/T)6GG) sequence motif. There are about 80 MADS box genes in Arabidopsis. Sequencing of the Arabidopsis genome revealed a additional 3 MADS box genes that are closely related to those involved in floral patterning. The genes work in a redundant fashion and were not detected in early mutant screens. Subsequent analysis of triple mutants revealed that these SEPALLATA 1-3 genes also play important roles in specifying the flower. In addition, MADS box genes are required for specifying ovules within the carpels, and the model has been extended to the ABCDE model.

The molecular interactions between the MADS box proteins is thought to provide an explanation for observed genetic interactions between the proteins. The proteins heterodimerise and tetrameric complexes can be formed between dimers bound to the same promoter sequences. This "quartet model" provides a fundamental basis for the switching of floral primordial identities.

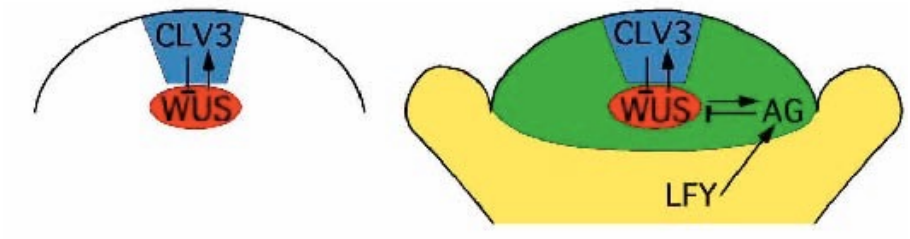
Box 2. ABC and floral quartet models of floral organ specification



Switching off the meristem.

Wild-type flowers are "determinate" structures, producing a fixed number of organs. The *agamous* mutants produce indeterminate flowers, and the C function is required to terminate the growth of the floral meristem. Stem cell regulation and floral patterning in Arabidopsis are closely linked, as *Wuschel* regulates stem cell fate in both shoot and floral meristems.

During flowering, the *Leafy* and *Wuschel* genes are required to trigger the first expression of *Agamous*. *Leafy* provides the "flower specificity" and *Wuschel* provides the "central zone" specificity to ensure C gene activation in the center of floral meristem. Once triggered, *Agamous* represses the expression of *Wuschel*. This negative feedback loop results in termination of meristem growth, and accounts for the characteristic determinate character of flowers.



Lecture 6: Morphogenesis

The final shape of a plant tissue is generated by an interplay between (i) genetic programs that regulate cellular development and (ii) the biophysical consequences that constrain cell growth, and provide instantaneous feedback across the tissue. Advances in our understanding of plant development allow the prospect of engineering morphogenesis.

Recommended Reading:

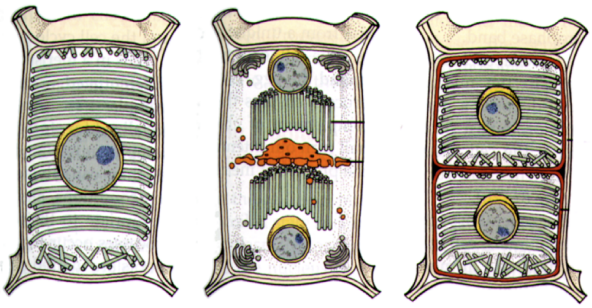
Modeling plant growth and pattern formation. Jonsson, H., & Krupinski, P. Current Opinion in Plant Biology, 13:5-11 (2010).
Stochasicity in the symmetric division of plant cells when the exceptions are the rule. Besson, S. and Dumais, J. Frontiers in Plant Science 5:1-4, (2014).

Cell division and plant form.

Plant cells proliferate within a semi-rigid cell wall matrix. Unlike animal cells, which are free to migrate to their final position within a developing tissue, plant cells are laid down, brick-like, in a sequence of cell division events. For any given cell division, a new wall is deposited and the orientation and position of the daughter cells is locked in place. The final form of a tissue or organ is due to the coordinated patterns of cell proliferation, expansion and differentiation.

Plant anatomists working in the 1800's contributed to formulation of the Cell Theory and to the rapid development of new microscopy techniques. During their studies, the importance of the polarity of cell division during plant morphogenesis was quickly recognised. Hofmeister (1863), Sachs (1878) and Erera (1888) established a series of empirical rules that broadly described the behaviour of dividing plant cells. Hofmeister observed that if a plant tissue grows in different directions, cell divisions are generally perpendicular to the direction of fastest growth, and Sachs stated that a new cell wall meets side walls at a right angle. Further, Errera's rule states that new cell walls follow the shortest path that will divide the parent cell, as if the nascent wall transiently possessed the surface minimisation properties of a fluid. It was clear to these workers that many of the properties of dividing plant cells could have a physical underpinning, and this view was exemplified in Darcy Wentworth Thompson's book, "On Growth and Form".

Plant cells are immobilised.
Morphogenesis is driven by cell division and elongation.



Empirical rules describe cell division

1. Hofmeister's rule (1863)
Cell plate formation normal to the growth axis.

2. Sachs' rule (1878)
Cell plate formation at right angles to existing walls.

3. Errera's rule (1888)
Cell plate of minimal area for cutting the volume of the cell in half.

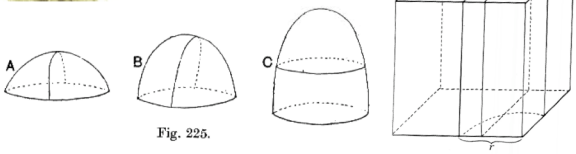


Fig. 225.

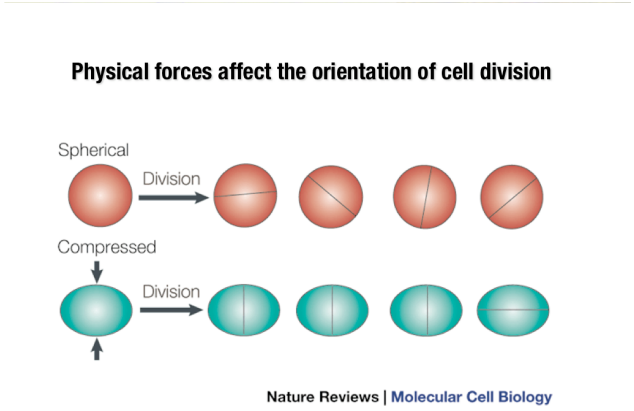
Preprophase bands predict the plane of cell division.

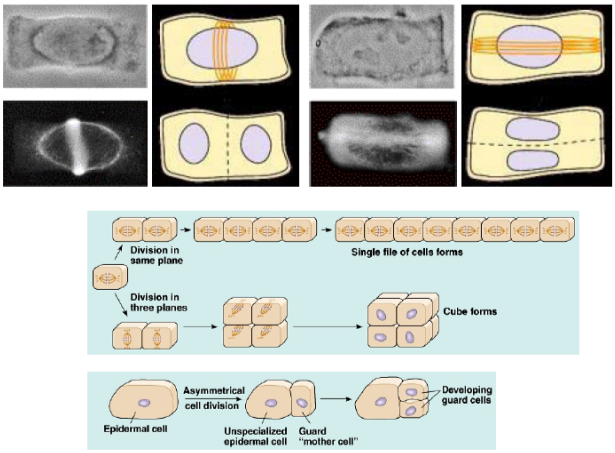
The plane of division in highly vacuolated plant cells is predicted during prophase by a plate-like arrangement of transvacuolar cytoplasmic strands that radiate away from the nucleus, linking the cytoplasm surrounding the nucleus to the cortical cytoplasm. During prophase, cortical microtubules are organised into a belt-like arrangement circumscribing the future division plane called a preprophase band (PPB). More recent studies have shown that F-actin is also a component of the PPB. Plant cells seem to select a division plane early in the cell cycle (before mitosis), which is marked by the location of the PPB, and also by the phragmosome in highly vacuolated cells.

Phragmoplast and cell wall formation.

Cytokinesis in plant cells is achieved through the construction of a new cell wall between daughter nuclei after mitosis. This process is directed by a cytoskeletal structure called the phragmoplast, which is made up in part by two interdigitated discs of parallel

microtubules. Microtubules of the phragmoplast are thought to guide the movement of Golgi-derived vesicles containing cell-wall materials to the equator of the phragmoplast, where these vesicles fuse together, gradually coalescing to form a new cell wall. Actin filaments are also present in the phragmoplast, mostly lying parallel to the microtubules, but their functions are not well understood. The phragmoplast arises between daughter nuclei from the remnants of the mitotic spindle, initially in isolation from the parental wall and plasma membrane, and then expands radially to complete the formation of the new cell wall. The site at which the new cell wall will become attached to the parental wall seems to be governed by an interaction between the phragmoplast and a specialised cortical site, the 'division site', which is left behind when the PPB is disassembled upon entry into mitosis. During cytokinesis, the cortical site previously occupied by the PPB seems to attract the expanding phragmoplast.





Primary walls contain three main classes of polysaccharide. The first is cellulose, an unbranched polymer of glucose subunits that is synthesised and deposited into the wall by an enzyme complex (cellulose synthase) in the plasma membrane. Individual cellulose polymers associate into bundles called 'microfibrils'. Cellulose microfibrils are crosslinked together by two other classes of polysaccharide — hemicelluloses and pectins — which are both branched polysaccharides of varying composition synthesised in the Golgi and deposited into the wall through secretion. During cytokinesis,when a new cell wall is initially formed, its composition is different — it contains mainly callose rather than cellulose, a different polymer of glucose subunits that is also synthesised by an enzyme complex in the plasma membrane (callose synthase). After completion of the new cell wall, flattening and rigidification of the wall is associated with replacement of callose with cellulose. After a cell stops growing, more components are added to form thicker and more rigid secondary walls — in woody tissues, for example, deposition of lignin makes cell walls extremely tough and rigid.

Cellular vs. organismal models for morphogenesis

Experimental evidence supporting the idea of a direct link between cell shape and the plane of division has come from studies in which shapes were altered by application of a compressive force. Spherical cells within multicellular clumps of callus, or isolated by suspension of single cells in semi-solid medium, divide in random orientations. However, when compressed into oval shapes, cells become strongly biased towards division in the plane perpendicular to the long axis of the oval. This kind of evidence has given rise to theories of an organismal basis for morphogenesis – that the physical properties of the organism determine its form. The alternative view is that cellular processes build the form of the organism, and that this process is largely genetically controlled.

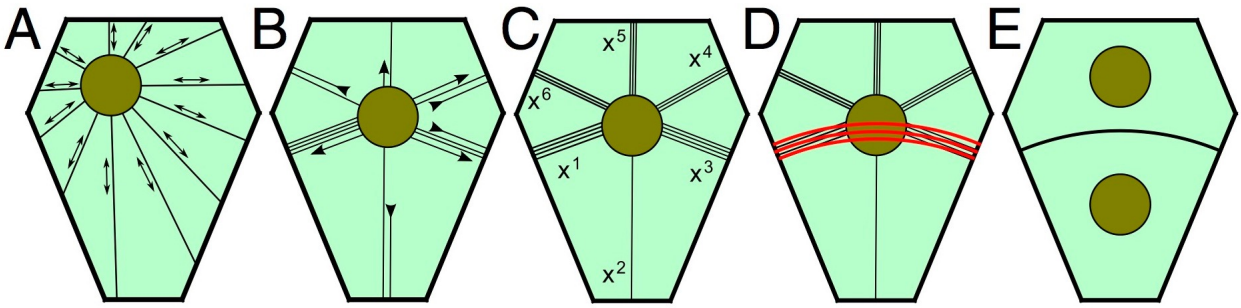
Genetic, molecular and biochemical models have come to dominate thinking in this field over the last century. In particular the explosion of new genetic tools and information has provided large amounts of information about the components that drive plant cell processes, and has contributed greatly to our understanding of what goes on inside cells. However, our understanding of how cellular processes are tied together across a growing tissue has not advanced at a comparable rate. There is still considerable debate over the relative contribution of physical and genetic processes to the coordination of cell growth during morphogenesis. At one extreme, a hypothetical dyed-in-the-wool molecular geneticist would point to DNA control of cell division, elongation and differentiation, and exchange of genetic information between cells, and suggest that this would be sufficient to regulate morphogenesis. At the other extreme, a biophysicist might point to the work of Paul Green suggesting that tissue buckling might

provide a physical basis for organogenesis. In this view of things, the genetic system responds to cues provided by physical interactions between growing cells, rather than the reverse.

It is likely that these conflicting viewpoints represent extremes. The activities of growing cells will produce physical strains across a growing tissue, and these may constrain further cell division and expansion. In this view of things, there is no need to directly sense external forces applied to the cell. Rather, external forces will constrain changes in cell size or shape during development, and cells would simply need a mechanism for sensing their own size and shape. Interestingly, there is increasing genetic evidence for such mechanisms in microbes. There is no opportunity for tissue stresses to play a role in the timing or orientation of cell division for single cell organisms. The *Min CDE* minicell mutants of *E. coli* indicate that feedback regulated interactions between proteins within cells can provide feedback on cell size and shape, and allow the correct partitioning of cells during division. This and other systems are found in other bacteria and plastids. However the question remains whether this type of intracellular reaction-diffusion system is a special case, or whether systems like it might be present in multicellular organisms like plants.

Patterns of cell proliferation and morphogenesis

The division of eukaryotic cells requires the assembly of complex cytoskeletal structures to exert the forces required for chromosome segregation and cytokinesis. In plants, evidence suggests that tensional forces within the cytoskeleton cause cells to divide along the plane that minimises the surface area of the cell plate (Errera's rule) while creating daughter cells of equal size. However, exceptions to Errera's rule cast doubt on whether a broadly applicable rule can be formulated for plant cell division. It has been shown that the selection of the plane of division involves a competition between alternative configurations whose geometries represent local area minima. The probability of observing a particular division configuration increases inversely with its relative area, and this is widely conserved in algae and plant cells, independent of shape and size. The division rule is predicted by the dynamics of the tense cytoskeletal elements that lead to the positioning of the preprophase band. The division plane is selected from the interaction of the cytoskeleton with cell shape, and this may be the default mechanism for plant cell division when internal or external cues are absent.

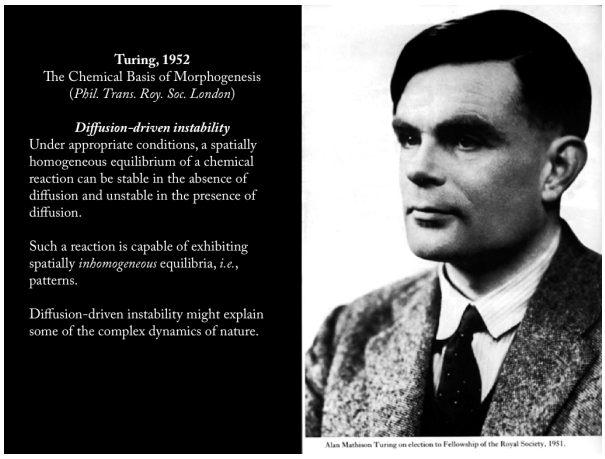


Mechanistic model for the selection of the division plane. (A) Before preprophase, microtubules radiate from the nucleus. (B) Microtubules reorganise into a finite number of configurations corresponding to the shortest distances between the nucleus and cell edges. (C) The equilibrium configuration favours microtubules that are short. (D) The PPB forms on the edges most heavily populated by microtubules. (E) The cell plate forms at the same position as the PPB.

Self-organising genetic systems.

In 1952, the British mathematician Alan Turing proposed a simple mathematical equation capable of generating a wide range of patterns commonly found in the natural world, including stripes, spots, and reticulations. This model, known as the reaction-diffusion model, mathematically demonstrates that the interaction between a local activator and a long-range inhibitor can give rise to various periodic structures in response to differences in their individual diffusion rates.

He demonstrated how a simple model system of coupled reaction-diffusion equations could give rise to spatial patterns in chemical concentrations through a process of chemical instability. He showed that this kind of system may have a homogeneous stationary state which is unstable against perturbations, such that any random deviation from the stationary state leads through diffusion to a symmetry break. This process is called diffusion-driven instability. Since complex spatial patterns are commonly found in nature, it is quite natural to think that such pattern formation could be caused by some general physico-chemical process. Inspired by this notion, Turing systems have been proposed to account for pattern formation in various biological systems



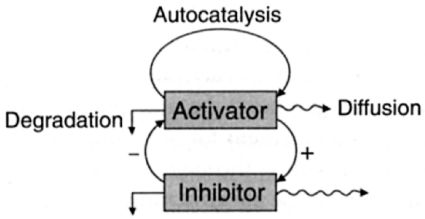


Fig. 4.2 How an activator–inhibitor scheme works. The activator generates more of itself by autocatalysis, and also activates the inhibitor. The inhibitor disrupts the autocatalytic formation of the activator. Meanwhile, the two substances diffuse through the system at different rates, with the inhibitor migrating faster.

after Gierer & Meinhardt, 1972

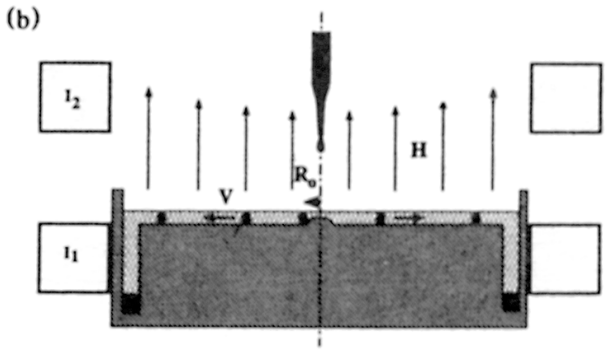
Epidermal patterns are ideal for studies of the molecular basis of the Turing mechanism. They are externally visible, and because many epidermal patterns are not similar to internal structures, it is clear that they form without any pre-pattern. For example, patterns found in animal skins, sea shells, the skin pattern of a certain tropical fish change continuously as a Turing model might predict; this was accepted as the first reliable evidence that Turing's principle was actually working in a living organism.

Phyllotaxy and the close-packed arrangement of 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.



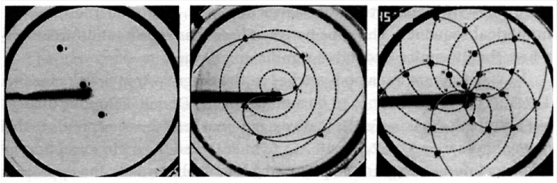


Figure 50
Fibonacci spirals observed in an experiment with electrically charged oil drops.

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.

To date, biological research has focused on the analysis of naturally evolved systems. Living systems are characterised by complexity, non-linearity and parallelism, often involving multicellular organisms with tens of thousands of genetically encoded components and possessing feedback dominated mechanisms for self-organisation, reproduction and repair. They produce functional structures that are many orders of magnitude more complex than the most sophisticated man-made artifacts. A formidable array of biochemical, biophysical and genetic techniques have been assembled for the description of biological systems, and this has given us methods for the comprehensive description of an organism’s genome, gene expression patterns and metabolic activities. New imaging techniques allow non-invasive monitoring of biological activities and precise reconstruction of cellular and tissue architecture.

Increasing knowledge of natural systems is facilitating new engineering approaches for editing and reprogramming genetic systems. This approach also shows great potential for the engineering of multicellular systems. (i) The greatest diversity of cell types and biochemical specialisation is found in multicellular systems, (ii) the molecular basis of cell fate determination is increasingly well understood, and it is feasible to consider creating new tissues or organs with specialised biosynthetic or storage functions by reorganising the distribution of existing cell types. Of all multicellular systems, plants are the obvious first target for this type of approach. Plants possess indeterminate and modular body plans, have a wide spectrum of biosynthetic activities, can be genetically manipulated, and are widely used in crop systems for production of biomass, fuels, food, polymers and drugs.