CELL SIGNALLING AT THE SHOOT MERISTEM

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The regulation of cell differentiation at meristems is crucial to developmental patterning in plants. Rapid progress has been made in identifying the genes that regulate differentiation and the receptor-mediated signalling events that have a key role in this process. In particular, we are now learning how the CLAVATA receptor kinase signalling pathway promotes stem cell differentiation in balance with the initiation of stem cells by the transcription factor WUSCHEL.

MERISTEMS
Locations on a plant where stem cells are maintained and organogenesis occurs. Root meristems, shoot meristems and flower meristems fit this description.

ORGAN PRIMORDIA
An organ (for example, a leaf, flower or petal) at an early stage of development, immediately after its initiation.

ANGIOSPERMS
Most extant plants are angiosperms, or flowering plants. Non-angiosperms include gymnosperms (for example, pine and cycads), ferns and mosses.

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Plant development is fundamentally different from developmental patterning in most animals in that very little of the plant body plan is established during embryogenesis. Embryogenesis in higher plants establishes a very simple structure that contains two stem-cell populations — the shoot meristem and the root meristem. Post-embryonic developmental patterning at these MERISTEMS is responsible for the morphology of the adult plant (FIG. 1). The shoot meristem is ultimately responsible for all of the 'above-ground' organs formed during the plant's lifespan. In *Arabidopsis thaliana*, the shoot meristem initiates the leaves, flowers, vasculature and other tissues of the stem.

The shoot meristem is able to form organs continuously by carefully balancing two activities. The first is the maintenance of undifferentiated stem cells at the very centre of the shoot meristem. The second is the direction of appropriately positioned progeny cells towards differentiation, so that they are competent to form ORGAN PRIMORDIA. The breakdown of either of these processes would be a morphological disaster for the plant, so the balance must be maintained even when variations in light, temperature or nutrient supply drive differences in growth and organ formation rates.

In ANGIOSPERMS, the cells of the shoot meristem are found in three clonally distinct populations of cells called cell layers[1,2] (FIG. 2). The outermost cell layer in *Arabidopsis*, referred to as the L1 layer, is the epidermal cell layer. Within the meristem, cells of this layer divide in a strictly ANTICLINAL fashion. As a result, the L1 cell layer in the meristem is one cell thick and remains so during organogenesis. Cells in the first subepidermal layer, the L2, also divide in a largely anticlinal fashion, forming a completely separate population of cells from the other cell layers. Within developing organs, the L2 divides anticlinally and PERICLINALLY, but it still remains largely separate from the underlying L3 layer. The L3 layer is different, in that whereas the apical edge of the cell layer — the boundary between the L2 and L3 layers — is clearly defined, L3 cells frequently divide in various orientations. The net flow of cells is from the centre and the apex to the flanks and the basal regions of the shoot meristem (FIG. 2). This pattern of cell division indicates that cell signalling is required. First, a small number of stem cells give rise to all the differentiated cell types of the adult plant, ruling out any important role for cell lineage patterns in regulating cell fate. Second, the organs initiated on the flanks of the meristem are composed of cells from all three clonally distinct layers[3,4], so these layers must communicate to execute organ formation in a coordinated fashion.

Key events in the differentiation of cells at the shoot meristem include the commitment to differentiation, initiation of organ primordia and the establishment of polarities within each organ primordium. As a stem cell divides, leaving one daughter in the centre of the meristem and one daughter towards the flanks of the meristem, positional information must distinguish between these cells, such that the central daughter retains stem cell identity, and the peripheral daughter differentiates. Cells on the flanks of the meristem form either organ primordia or internodes. The PHYLLOTAXY of the individual plant species determines which peripheral cells of the meristem form organs[1,2]. In *Arabidopsis*, the sites of

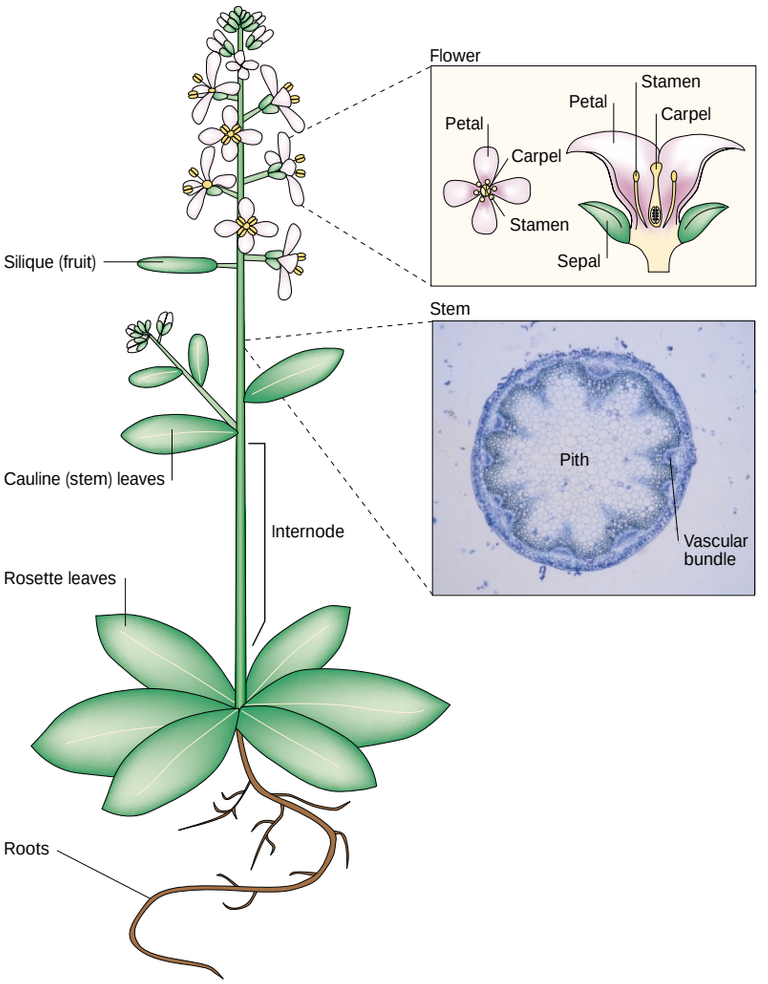

Figure 1 | **An adult *Arabidopsis* plant.** A diagram of an adult *Arabidopsis* plant, showing organs such as roots, rosette leaves, cauline leaves and flowers. Internodes are the regions of differentiated tissue between successive organs. Shoot meristems are found at the tip of each active shoot, as well as in the axils of each leaf. Root meristems are found at the tip of the primary root and each lateral root.

organ initiation form a spiral around the centre of the meristem (FIG. 3a). Once organs are initiated, the proximal/distal, medial/lateral and adaxial/abaxial (dorsal/ventral) asymmetries must be established within each organ primordium (FIG. 3b,c). This review focuses on our emerging understanding of the mechanism of these differentiation events, with particular emphasis on the signalling pathways that regulate meristem development and organ formation.

Stem-cell maintenance and differentiation

Screens in *Arabidopsis* for mutants that lack stem cells or that accumulate ectopic stem cells have uncovered regulators of organogenesis. Many of the corresponding genes have been cloned, and a signal-transduction pathway that regulates stem-cell behaviour is beginning to emerge.

The CLAVATA signalling pathway. The differentiation of stem cells in the shoot meristem is regulated by the *CLAVATA* genes (*CLV1*, *CLV2*, *CLV3*)[5–7], which seem to code for components of a signal-transduction pathway (TABLE 1). Plants mutant for any of the *CLV* loci progressively accumulate undifferentiated stem cells as development proceeds. Plants that are homozygous for strong loss-of-function alleles of *CLV1* and *CLV3* accumulate over 1,000-fold more undifferentiated cells than wild-type plants. Genetic analysis has revealed that these genes function in the same pathway[6,7].

CLV1 encodes a receptor kinase, with an extracellular domain composed of tandem leucine-rich repeats (LRRs)[8]. These LRRs are very similar in structure to several animal receptors, including thyroid-stimulating, luteinizing, and gonadotropin hormone receptors, although CLV1 contains more repeats (21 repeats) than the corresponding animal receptors (7–11 repeats)[9]. Early experiments established that the CLV1 kinase domain, when expressed in *Escherichia coli*, *trans*-phosphorylates multiple serine residues[10,11]. Although this is certainly consistent with the hypothesis that CLV1 acts as a receptor kinase, it far from establishes that CLV1 acts as a receptor, nor does it tell us anything about how CLV1 might interact with intracellular proteins.

When purified from *Arabidopsis*, CLV1 is found in two protein complexes, one of ~185 kDa and a second of ~450 kDa (REF. 12). An attractive interpretation is that the 185-kDa complex contains inactive CLV1, and that the 450-kDa complex is composed of activated CLV1 that is associated with downstream signalling proteins. Genetic studies are consistent with this hypothesis. The *clv1-1* mutant contains a missense mutation in the kinase-domain-coding region. This allele shows a partial loss-of-function phenotype, and the kinase domain has less than 50% of the autophosphorylation activity of wild type when expressed in *E. coli*. Similarly, the *clv1-10* allele contains two missense mutations in the kinase-domain-coding region, shows a null or near-null phenotype, and has no autophosphorylation activity when expressed in *E. coli*. Extracts from *clv1-1* plants have 50% less of the 450-kDa complex (and a corresponding increase in the accumulation of the 185-kDa complex), whereas *clv1-10* extracts have no detectable 450-kDa complex. So, formation of the 450-kDa complex depends on the kinase activity of CLV1, and the 450-kDa complex is very likely the active form of CLV1.

CLV2 is similar to CLV1 in terms of the structure of the extracellular domain, although it is not very similar

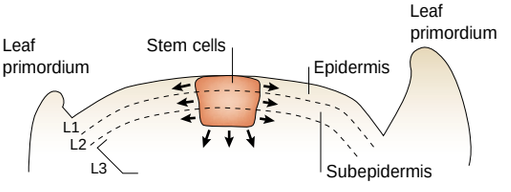

Figure 2 | **Cells layers and cell divisions.** A longitudinal section through a shoot meristem, revealing the organization of the meristem into cell layers (L1, L2, L3). The location of the stem cells in each layer is indicated. The flow of cells as a result of cell growth and cell division is indicated with arrows. On the flanks of the meristem, cells form organ primordia, which become apparent (leaf primoridia) after rapid cell growth and division.

ANTICLINAL
During anticlinal cell divisions, the new cell wall forms perpendicular to the layer of cells. This maintains cells in a single layer.

PERICLINAL
In periclinal cell divisions, the new cell wall forms parallel to the cell layer, effectively thickening that cell layer.

PHYLLOTAXY
The pattern of organ initiation by a shoot or flower meristem.

at the level of primary sequence[13]. However, CLV2 lacks a cytoplasmic domain, so how can it participate in CLV1 signalling? Genetic experiments established that CLV2 functions in the same pathway as CLV1 to regulate meristem development, and that CLV2 is required for the accumulation of CLV1 (REF. 13). This raises the possibility that CLV1 and CLV2 directly bind to each other. Consistent with this, the masses of CLV1 (105 kDa) and CLV2 (80 kDa) add up to 185 kDa, indicating that the inactive CLV1 complex might be a heterodimer of CLV1 and CLV2 (FIG. 4).

CLV3 also acts in the same genetic pathway as *CLV1*, and encodes a small, probably secreted protein[14], which is likely to act as the ligand for CLV1 (FIG. 4). In the absence of CLV3, CLV1 is detected only in the inactive, 185-kDa complex[12], indicating that CLV3 is required for the formation of the active 450-kDa complex. Additional evidence that CLV3 acts upstream of CLV1 came from overexpression studies. Ectopic expression of *CLV3* gives rise to the opposite phenotype to *clv1* or *clv3* mutants — a failure to maintain stem cells — and this phenotype depends on the presence of both CLV1 and CLV2 (REF. 15). *In vivo* biochemical and cell-culture experiments have shown that CLV3 is indeed the ligand for CLV1 (REF. 16). First, CLV3 and CLV1 immunoprecipitate together *in vivo* with the 450-kDa CLV1 complex, indicating that CLV3 binds only to active CLV1. Second, when intact yeast cells expressing CLV1 and CLV2 are incubated with plant extracts, CLV3 binds CLV1. Now that this ligand has been identified, it should be easier to manipulate CLV1 signalling *in vivo* and in cell culture.

CLV-interacting proteins. The CLV1 450-kDa complex presumably has several associated proteins, a subset of which are probably bound to phosphoserine residues in the kinase domain. The first protein identified that binds CLV1 was the kinase-associated protein phosphatase (KAPP)[10,11]. KAPP had originally been identified because it bound the kinase domain of an *Arabidopsis* LRR-containing receptor-like kinase, RLK5/HAE, in a phosphorylation-dependent manner[17]. KAPP contains three domains: a type I signal anchor, a kinase-interaction domain and a functional type 2C protein phosphatase domain. The kinase-interaction domain has been shown to contain a forkhead-associated (FHA) domain, which is a phosphothreonine/phosphoserine-binding domain[18]. KAPP binds directly to CLV1 *in vitro*[10,11] and is a component of the 450-kDa CLV1 complex *in vivo*[12]. The presence of a phosphatase domain supports the hypothesis that KAPP negatively regulates CLV1 (FIG. 4). This was shown through two complimentary approaches. First, KAPP overexpression in wild-type plants recreates a weak *clv*$^-$ phenotype[10]. Second, when KAPP expression was suppressed in *clv1-1* plants, the mutant phenotype was suppressed, depending on the level of KAPP suppression[11].

The second known component of the 450-kDa CLV1 complex is a Rho/Rac-GTPase-related protein. Although plants lack a protein that is clearly orthologous to the monomeric GTP-binding protein Ras, which carries out

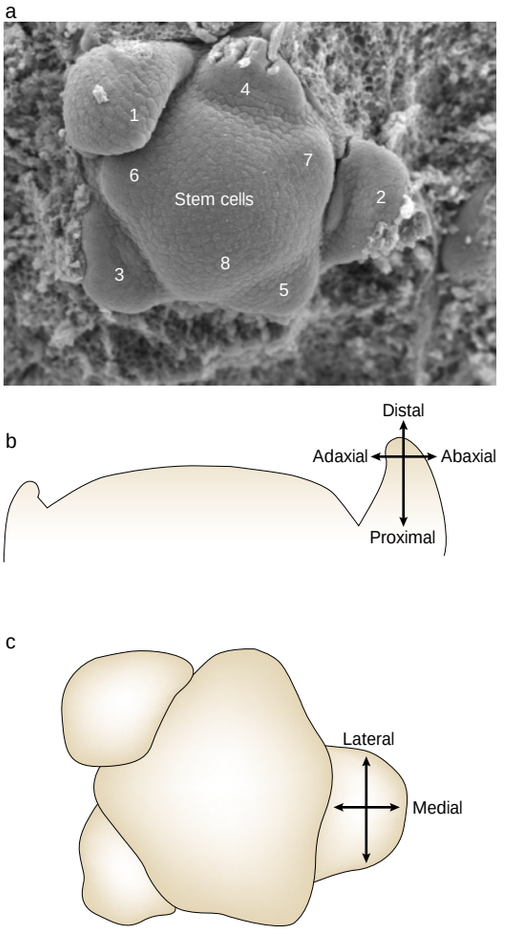

Figure 3 | **The *Arabidopsis thaliana* shoot meristem.** A scanning electron micrograph of an *Arabidopsis* shoot meristem initiating leaf primordia is shown. The region containing stem cells is indicated. Organs (1–7) and incipient organ primordium (8) are numbered from oldest to youngest. Note the spiral arrangement of their initiation. **b** | Indicates the distal/proximal and the adaxial/abaxial axes. **c** | Indicates the lateral and medial axes.

many of the receptor-mediated signalling events for animal receptor tyrosine kinases (RTKs), they do have a family of small GTPases of the Ras superfamily, called ROP, which are similar to animal Rho/Rac proteins[19,20]. Using an antibody that cross-reacts with all of the *Arabidopsis* ROP isoforms tested, an appropriately sized cross-reacting protein was identified in the CLV1 450-kDa complex[12]. So plants might have evolved a unique role for Rho proteins as a relay for RTK signalling.

Identification of other interacting proteins, which should be possible using genetic screens combined with biochemical analysis, will allow us to dissect further the mechanisms of CLV1 signalling. For example, screens for suppressors of *clv1* and *clv3* alleles led to the identification of *POLTERGEIST* (*POL*), a gene that seems to function downstream as a negative regulator of the CLV pathway[21]. Characterization of the POL gene product and interacting proteins will certainly clarify the molecular events that relay the signal originated by CLV3.

EPISTATIC
Mutations that are epistatic mask the phenotype of other mutations.

The WUSCHEL/CLV3 feedback loop. The *WUSCHEL* (*WUS*) gene encodes a putative homeodomain-containing transcription factor. *WUS* is involved in initiating stem cells at the shoot meristem during embryogenesis, as *wus* mutants lack stem cells at shoot apices[22,23]. However, *WUS* is not expressed in stem cells, but in the cells underneath[23]. So, *WUS* either signals stem-cell fate to the overlying cells, or the loss of stem cells in *wus* mutants is an indirect consequence of the breakdown in meristem structure.

The CLV proteins regulate the expression of *WUS*, at least indirectly. Genetic interactions revealed that *wus* mutations were largely EPISTATIC to *clv* mutations[22], indicating that *WUS* might act downstream of *CLV* and that *CLV* negatively regulates *WUS*. A look at the expression of *CLV1*, *CLV3* and *WUS* reveals that *WUS* is expressed in a basal and central subdomain of *CLV1*-expressing cells (FIG. 5), whereas *CLV3*, encoding the ligand for CLV1, is expressed in cells adjacent to *CLV1*-expressing cells that do not express *WUS*[14,23]. So CLV3 might be secreted from the apical cells and diffuse to the most apical and lateral *CLV*-expressing cells to repress *WUS* expression. The hypothesis has been well supported in studies showing that *WUS* expression broadens both apically and laterally in *clv* mutants, indicating that the CLV pathway represses *WUS in vivo*, at least indirectly[15,24] (FIG. 5).

How could such a precise distinction between one cell and the next be achieved? *CLV3* is expressed only two cell-lengths away from *WUS*-expressing cells. This suggests that there would need to be a mechanism to limit the range of diffusion of CLV3 to only those immediately adjacent cells. Over 75% of CLV3 is bound to CLV1, consistent with the idea that CLV1 titrates CLV3 from the soluble intercellular phase[16], an effect known as ligand sequestration[25]. So, *WUS*-expressing cells might not detect a significant amount of CLV3 because much of it is sequestered by the overlying CLV1-expressing cells.

Is *WUS* repression the only function for CLV1, and is *WUS* expression sufficient to establish stem cells? Both of these questions were addressed in experiments that tested the effects of misexpressing *WUS* within the meristem and organ primordia. *WUS* expression under

Table 1 | Genes involved in signalling at the meristem

Gene	Type of protein	Function	Plant homologues	Animal homologues
CLV1	Receptor kinase	Promotes differentiation	Large gene family (>150 in *Arabidopsis*)	Kinase and LRR domains separately
CLV2	Receptor-like protein	Promotes differentiation, other functions	Large gene family (>40 in *Arabidopsis*)	LRR domains found in animal receptors
CLV3	Secreted ligand	Promotes differentiation	Putative secreted proteins in maize	No
WUS	Homeodomain transcription factor	Maintain and establish stem cells	Gene family	Yes
STM	Homeodomain transcription factor	Meristem identity, organ separation	Gene family	Yes
KAPP	FHA/phosphatase	Negatively regulates *CLV1*	Only for individual domains	FHA and protein phosphatase 2C domains separately
ROP	Rho/Rac-GTPase	Possible component of active CLV1 complex	Gene family (>11 in *Arabidopsis*)	Yes
PAN	B-zip transcription factor	Regulates organ number in flower	Large gene family (~80 in *Arabidopsis*)	Yes
CUC1	Unknown	Promotes organ separation	–	–
CUC2	NAC transcription factor	Promotes organ separation	Large gene family (>100 in *Arabidopsis*)	No
PHAN	MYB transcription factor	Establishes polarities in leaf primordia	Large gene family (~200 in *Arabidopsis*)	Yes
RS2	AS1 MYB transcription factor	Promotes differentiation of leaf primordia	*PHAN* homology (~7 in *Arabidopsis*)	Yes
AGO1	Novel	Organ polarity, other functions	Gene family	Yes
ZLL	Novel	Organ polarity, other functions	*AGO* homology	Yes
CRC	YABBY transcription factor	Polarity of carpels	Gene family (~6 in *Arabidopsis*)	Zinc finger and HMG domains separately
FIL	YABBY transcription factor	Polarity of organs, flower development	*CRC* homology	Zinc finger and HMG domains separately
REV	HD-zip class III	Polarity of organs, lateral meristems, vascular development	Gene family (~5 in *Arabidopsis*)	HD-zip and START domains separately

AGO, *ARGONAUTE*; *CLV*, *CLAVATA*; *CRC*, *CRABS CLAW*; FHA, forkhead-associated; *FIL*, *FILAMENTOUS FLOWER*; HD-zip, homeodomain plus leucine zipper; HMG, high mobility group; *KAPP*, kinase-associated protein phosphatase; LRR, Leucine-rich repeats; *PAN*, *PERIANTHIA*; *PHAN*, *PHANTASTICA*; *REV*, *REVOLUTA*; *RS2*, *ROUGH SHEATH 2*; START, StAR-related lipid transfer; *WUS*, *WUSCHEL*; *ZLL*, *ZWILLE*.

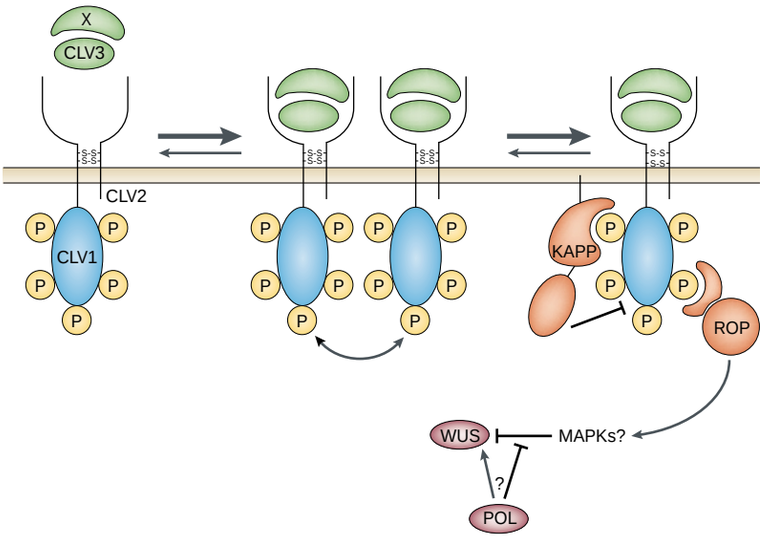

Figure 4 | **The CLAVATA1 signalling pathway.** The CLV3 multimer binds to the extracellular domain of the putative CLV1/CLV2 heterodimer. Ligand binding drives CLV1 phosphorylation, which leads to the binding of the downstream effector molecules, kinase-associated protein phosphatase (KAPP) and ROP. KAPP is a negative regulator of CLV1, whereas the function of ROP is unknown. One possibility is that ROP acts through a mitogen-activated protein kinase cascade (MAPK) cascade to regulate *WUSCHEL* (*WUS*) expression. POLTERGEIST (POL) is another negative regulator of CLV1 signalling that functions downstream of CLV1 and in close association with WUS. P, phosphate.

the control of the *CLV1* regulatory elements recreates the *WUS* expression seen in *clv1* mutants, and mimicks the *clv*⁻ phenotype[24]. So the accumulation of stem cells in *clv1* mutants seems to result largely from *WUS* misexpression. *ANT* is expressed in nascent organ primordia, and *WUS* expressed under the control of *ANT* regulatory elements in incipient organs prevents their differentiation. Plants with *WUS* expression driven by the *ANT* promoter often form only a large mass of stem cells at their apex. These elegant experiments have revealed that *WUS* is sufficient to establish stem-cell fate in adjacent cells. How this occurs remains a mystery.

Several observations point to the existence of a feedback loop between WUS and CLV3 that might aid in maintaining a stable population of stem cells: first, *WUS* and *CLV3* expression is broader in *clv* mutants than in wild-type plants; second, *CLV3* expression is downregulated in *wus* mutants; and last, *CLV3* expression is broader in plants that overexpress *WUS*[15,24]. An attractive possibility is that CLV3 negatively regulates *WUS* expression, and WUS activates *CLV3* expression. Thus, a downregulation of *WUS* would lead to a downregulation of *CLV3*, which in turn would lead to an upregulation of *WUS*. Such a system would move to an equilibrium point at which the expression of *CLV3* and *WUS* would be stable. Imbalances in expression of one gene or the other would tend to return to equilibrium. One could even imagine that, in other species with larger meristems, the equilibrium point is shifted by altering the parameters of *WUS* and *CLV3* interaction.

However, the evidence for this hypothesis could also be interpreted as the indirect consequences of changes in cell identity. For example, the loss of *CLV3* expression from *wus* mutants could simply reflect the lack of stem cells in *wus* mutant plants. Distinguishing between these alternatives will require more subtle and inducible regulation and detection of gene expression.

Continued differentiation

The loss of stem-cell identity by cells on the flanks of the meristem is just the first step in the long road towards differentiation. We must also keep in mind that this is not a one-way street: some cells regain stem-cell status if they are incorporated into lateral shoot and flower meristems. Although all the cells on the flanks of the meristem are competent to form organ primordia, only some do; others form the internodes between organs. So how do the cells that make up organs acquire the correct proximal/distal, lateral/medial and adaxial/abaxial asymmetries?

Organ initiation. Organs in higher plants are initiated in distinct patterns[1,2]. The *Arabidopsis* phyllotaxy is spiral, with a defined angle of 137° between each subsequent organ (FIG. 3a). Other species initiate organs in rings or in alternate or opposite patterns. The pattern of organ formation can vary between shoot and flower meristems (BOX 1), and between shoot meristems at different stages of development. The mechanisms that regulate the phyllotaxy of organogenesis has fascinated plant biologists for centuries, but they remain a mystery. Two well-discussed hypotheses invoke inhibitory signals from recently initiated organ primordia, and the role of biophysical stresses in marking the site of buckling and hence organ formation, respectively. Theorectical models of each can explain the various patterns of organ formation observed in nature[1,2,26]. But, so far, little experimental evidence is available to support any of the hypotheses.

One recent study showed that nearly all cells on the flanks of the meristem might be competent to form organs. Exogenous application of an inhibitor that blocks transport of the plant hormone auxin to tomato

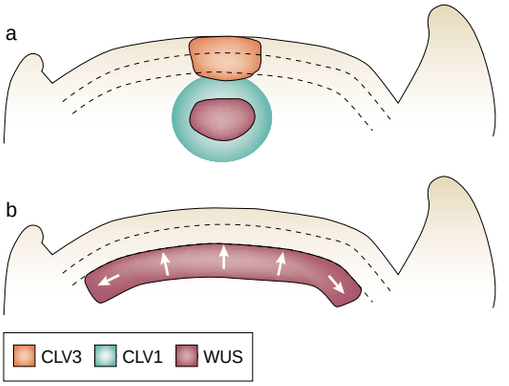

Figure 5 | **Stem-cell regulators. a** | The approximate domains of mRNA expression for *CLAVATA1* (*CLV1*), *CLV3* and *WUSCHEL* (*WUS*) are shown. Note that *WUS* is expressed in the cells immediately underlying the stem cells. The function of CLV1 and CLV3 is to repress *WUS*, as implied by the expansion of *WUS* expression and stem cells in *clv* mutant plants. **b** | Expression of *WUS* in *clv* mutants. Arrows indicate that *WUS* expression expands both apically and laterally.

Box 1 | Flowers and shoots — functional and evolutionary relationships

There are only two sites within a plant where distinct lateral organs can be initiated: the shoot meristem and the flower meristem. At each location, a population of centrally located stem cells give rise to more differentiated progeny on the flanks of the meristem that are then incorporated into organ primordia. The flower meristem is probably a modified shoot meristem, as indicated by similarities in structure and function, the fact that shoots evolved long before flowers, and the fact that flower organs seem to be modified leaves. More recent molecular genetic work on the development of *Arabidopsis* and other species have confirmed this. *LEAFY* and *APETALA 1* in *Arabidopsis* are two genes that are involved in specifying flower meristems. Mutation of both genes converts the flower meristems into shoot meristems[57–59]. Conversely, mutations in the *TERMINAL FLOWER* (*TFL*) gene convert shoot meristems into flower meristems[60,61].

Given that the shoot and flower meristems are functionally very similar, genes that regulate a fundamental aspect of meristem function (for example, stem cell maintenance, differentiation and organ formation) might be expected to have similar functions in shoot and flower meristem development. Indeed, for the genes that are the focus of this review (TABLE 1), mutations result in very similar phenotypes within the shoot and flower meristems. For example, in *clv1* mutants, both shoot and flower meristems accumulate stem cells[5].

However, many differences are found between shoot and flower meristems. The positioning of organs often varies between the two structures. For example, the *Arabidopsis* shoot meristem initiates organs in a spiral pattern, whereas the flower meristem initiates organs in a ring pattern. Whereas the *Arabidopsis* shoot meristem always maintains stem cells, flower meristems inevitably terminate in differentiated organs. Finally, the identity of the organs that are initiated differ widely between shoot and flower meristems.

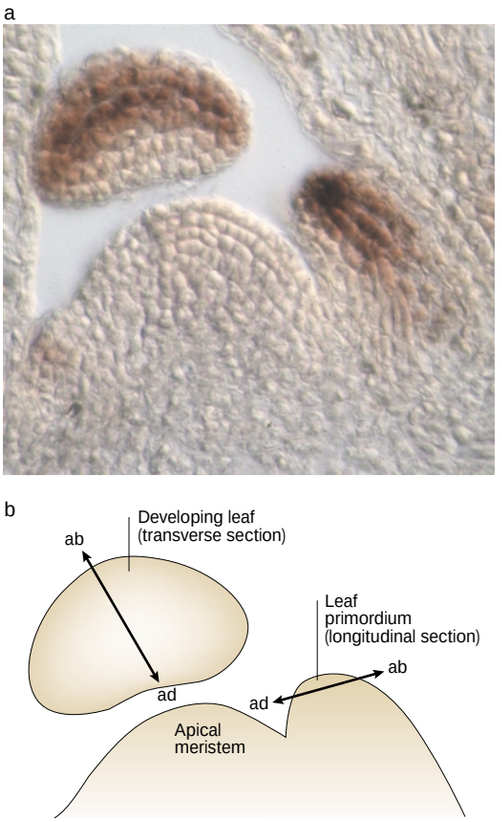

Figure 6 | **Establishment of organ polarities within organ primordia. a** | Longitudinal section through the vegetative apex of an *Arabidopsis thaliana* shoot meristem. The expression of the developmental receptor *FILAMENTOUS FLOWER* (*FIL*) is revealed in longitudinal and transverse sections through developing leaf primordia. *FIL* is expressed specifically within the abaxial region of leaf primordia. **b** | Sketch of **a** indicating the polarity axes. (Ab, abaxial; ad, adaxial.) **a** taken with permission from REF. 41 (1999) © Company of Biologists Ltd.

MOSAIC ORGANS
Organs that have cell types characterisitic of two or more organs.

FUSED ORGANS
Organs fused together at early stages of development, usually at the lateral edges.

apices, or use of an auxin mutant in *Arabidopsis*, resulted in shoot meristems that lack organs on the flanks without affecting differentiation[27]. An organ formed wherever a drop of auxin was placed on the meristem flanks, indicating that all the cells on the flanks were at least competent to form organ primordia.

No mutants that specifically alter shoot meristem phyllotaxy have been identified so far in *Arabidopsis*. All the mutants that alter the pattern of organ initiation also notably alter the entire structure of the meristem, implying that the two processes might be inextricably linked. The only exception to this is the *PERIANTHIA* (*PAN*) gene that functions within the flower meristem. *pan* mutants alter the number of organs initiated in each ring of organs within the flower meristem, without altering the fundamental structure of the flower meristem[28]. Understanding how *PAN* functions might provide the first clear insight into how phyllotaxy is established.

Organ separation. Three genes involved in separating organs at the earliest stages in organ initiation have been identified. Plants mutant for *STM*, *CUC1* or *CUC2* result in the fusion of the earliest organs initiated — the cotyledons[29–31]. In addition, these genes also seem to have a role in separation of all the organs that are initiated by the shoot meristem. *STM*, although it is expressed in a central region of the meristem and is required for meristem maintenance, is also expressed between the early forming organ primordia[31,32]. Indeed, weak alleles of *stm* that form transient shoot meristems show a great deal of MOSAIC and FUSED ORGANS[33,34]. *cuc1 cuc2* plants also give rise to postembryonic organ fusion[30]. *CUC2* encodes a putative plant-specific transcription factor that is expressed between organs. The expression of these genes at the boundaries of organs could prevent these cells from being incorporated into the organ primordia, or could simply inhibit their growth.

Box 2 | Advantages of plants as a model system for studying signalling

Studies of animals and yeast signal transduction have dominated the signalling field since its inception. However, the rapid development of modern molecular genetic approaches in plants, especially *Arabidopsis thaliana*, allows us to make some comparisons of the relative strengths of studying signal transduction in plants and animals.

A curious consequence of the sequencing of the *Arabidopsis* genome was the discovery of nearly 200 receptor-like kinases that are similar to CLV1. This large diversification of receptors in a relatively simple organism might mean that plant receptors are much more specialized than in animals. Indeed, the available evidence on developmental receptors in plants indicates that many might regulate a single developmental process: CLV1 promotes differentiation; RLK5/HAESA promotes ABSCISSION[62]; ERECTA regulates organ length[63]; S-receptor kinase mediates pollen/pistil recognition[64]; and PRK1 regulates gamete development[65]. This has many important consequences for experiments. First, plants with null mutations in these receptors are generally viable. This allows simple genetic screening for interacting factors. Second, the process that is regulated by the receptor is usually a process that continues for much of the lifespan of the plant. For example, all growing plants initiate organs and use CLV1 signalling.

These features combine to provide plants with their greatest advantage as an experimental system for studying signalling — the ability to characterize receptor function through *in vivo* biochemistry. Compare this with animals in which key receptors have a role in a plethora of developmental processes and act during very specific developmental stages. This means that examining the *in vivo* status of protein–protein interaction in a specific organ at a specific stage of development is technically difficult to say the least. Animal developmental biologists have, of course, developed powerful genetic and cell-culture systems in which to study signalling. However, the ability to study signalling *in vivo* in plants might well lead to the characterization of new features that are common to both plants and animals.

Organ polarity. The asymmetric growth at the earliest stages of organ development indicates that organs might rapidly acquire apical/basal, lateral/medial and adaxial/abaxial polarities. The field has progressed rapidly with the isolation of several key genes.

One of the first polarity specification genes was *PHANTASTICA* (*PHAN*) from *Antirrhinum majus* (snapdragon). This predicted MYB TRANSCRIPTION FACTOR is proposed to establish adaxial/abaxial polarity in developing leaves[35]. An interesting hypothesis resulting from *PHAN* analysis was that the outgrowth of leaf blades might be promoted by the juxtaposition of abaxial and adaxial domains at the edges of young leaf primordia. This hypothesis was based on the frequent absence or ectopic formation of blade outgrowths in the leaves of *phan* mutant plants, thought to result from an absence or incomplete establishment of abaxial/adaxial polarity. Mutations in a homologue of *PHAN* from maize, *ROUGH SHEATH 2* (*RS2*), also led to leaf defects, although these have been interpreted as a breakdown in proximal/distal polarity[36]. This difference might arise from the different architecture of leaves in *Antirrhinum* and maize.

The identification of an *Arabidopsis* orthologue of *PHAN*, *ASYMMETRIC LEAVES 1* (*AS1*), provided evidence linking organ differentiation to meristem function[37,38]. The *as1* mutation disrupted leaf morphology, which had also been shown for mutations in *PHAN* and *RS2*. Interestingly, *as1* also suppressed the lack of stem cells in *stm* mutants[37]. *STM*, and its homologue in maize *KNOTTED 1* (*KN1*), had been shown to be necessary for stem-cell maintenance at the shoot meristem[29,39]. How *STM* carried out this activity was unclear, because *stm* mutations showed additive interactions with *wus* and *clv* mutations, indicating that the genes might function in parallel pathways[22,33,34]. The observations that *as1* suppresses the *stm* meristem defect and that *STM* is expressed normally in the *as1* mutant[38], indicates that *STM* carries out stem-cell maintenance by inhibiting the expression of *AS1* in the meristem. Indeed, in *stm* mutant embryos, *AS1* is expressed in the position that the shoot meristem would normally occupy[37]. So stem-cell maintenance requires two activities: *WUS* to promote stem-cell identity, and *STM* to inhibit differentiation.

Later specification of adaxial/abaxial polarity seems to involve several genes in *Arabidopsis*. Several members of the *YABBY* family of putative transcription factors, such as *CRABS CLAW* (*CRC*) and *FILAMENTOUS FLOWER* (*FIL*) are also expressed in the abaxial portion of several organ types, including leaves and carpels[40–42] (FIG. 6). As predicted by their expression patterns, mutant analysis of several *YABBY* genes indicates that these genes establish abaxial fate. Conversely, *REVOLUTA* (*REV*) is expressed in the adaxial region of each primordia[43]. The *ZWILLE*/*PINHEAD* (*ZLL*)[44,45] and *ARGONAUTE 1* (*AGO1*) genes seem to specify adaxial fate as well, and *ZLL* is expressed in the adaxial portion of leaf primordia. *AGO1* has recently been implicated in the process of post-transcriptional gene silencing or RNA interference[46,47]. The *PHABULOSA* (*PHB*) gene, which has not yet been cloned, seems to promote adaxial fate[48]. Adaxial/abaxial polarity within the flower primordia is necessary for the development of ZYGOMORPHIC FLOWERS. Many flowers develop strikingly asymmetric floral organs, especially petals, that are essential for complex interactions with pollinators. In *Antirrhinum*, this asymmetry is established by the position of the flower primordia relative to the meristem, and requires several putative transcription factors[49].

Perspectives

Our understanding of differentiation and organ formation has progressed rapidly over the past few years. Several of the key regulatory elements that determine the fate of stem cells within the shoot meristem have been identified. And much more is now known about the function of receptor kinases in

ABSCISSION
The process by which dead parts of a plant break off naturally (for example, leaves).

MYB TRANSCRIPTION FACTOR
A type of transcription factor first identified in animals. The MYB gene family is greatly expanded in plants, and the proteins that these encode have been shown to control many developmental processes.

ZYGOMORPHIC FLOWERS
Flowers that are asymmetric, and in which the development of specific organs varies depending on the polarities of the flowers. Snapdragon flowers are zygomorphic, whereas roses are not.

plant signalling[15,16,50–53]. In most cases, the investigators benefit from the ability to carry out *in vivo* biochemical experiments in a genetic system. The combination of *in vivo* biochemistry and genetics is a great advantage for studying signal transduction in plants (BOX 2).

But, as always, the results have created as many questions as answers, many of which concern the initial steps towards differentiation. How is CLV1 signalling relayed within the cell? How does WUS signal to the overlying cells to establish stem-cell fate? Which genes are expressed within stem cells to maintain their identity? Curiously, screens for single mutants that specifically affect meristem development have failed to identify many of these factors. Attention must therefore turn to mutations with pleiotropic effects[54–56], and to designing genetically sensitized screens for mutations that do not lead to a phenotype on their own[21]. Only such a comprehensive approach will complete the cast of characters that regulate cell differentiation.

Later differentiation events, touched on briefly in this review, are also being vigorously addressed by many labs. Exciting progress has been made on factors that regulate polarity. However, the interaction between the various factors or the type of genes that these potential transcription factors target has not been sorted out. Nor has an understanding been developed of the signals that establish the earliest polarity events. Although at an early stage of investigation, it is clear that many of the genes already identified will provide excellent starting points for understanding such processes.

Links

DATABASE LINKS *CLV1* | *CLV2* | *CLV3* | thyroid-stimulating hormone receptor | luteinizing hormone receptor | gonadotropin hormone receptor | RLK5 | Rho/Rac | *POL* | *WUS* | auxin | *PAN* | *STM* | *AS1* | *CRC* | *FIL* | *REV* | *ZLL* | *AGO1* | *PHB* | *LEAFY* | *APETALAI 1* | *TFL* | ERECTA

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ENCYCLOPEDIA OF LIFE SCIENCES *Arabidopsis thaliana* as an experimental organism | *Arabidopsis*: Flower development and patterning | Positional information in plant development

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