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Review

Twenty years on: The inner workings of the shoot apical meristem, a developmental dynamo

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ABSTRACT

The shoot apical meristem of angiosperm plants generates leaf, stem and floral structures throughout the plant's lifetime. To do this, the plant must maintain a population of stem cells within the meristem while at the same time carefully controlling the placement and establishment of new leaf primordia. As there is little cell rearrangement in plants, underlying patterning mechanisms must exert careful control of cell division rates and orientations to achieve the correct final form. It has been twenty years since the first genes controlling meristem development were molecularly cloned. In the intervening decades, our understanding of the inner workings directing meristem development has increased enormously. This review summarizes our current knowledge of how the meristem functions as a persistent organ generating center. The story that emerges is one in which transcription factor activity combines with the action of the classic plant growth regulators auxin and cytokinin and with the action of more recently discovered small peptides to control proliferation and cell fate in the shoot apical meristem.

Plants form new organs nearly continuously throughout their lives. This strategy allows them to replace body parts lost to disease, environmental stress and predators. It also allows them to disperse as tiny plants, with a minimal number of leaves and a mere stub of a root, packed tightly into a seed. Once the seed has reached its destination, the seedling germinates and its meristems become active. The above-ground organs of the plant are generated by shoot apical meristems while the below-ground organs are made by root apical meristems. Each type of meristem contains a small mass of stem cells that divide to produce new cells to be used in the constant formation of new organs.

In 1989 and 1990, the first genes involved in control of apical meristem function were molecularly cloned—the *Knotted1* gene of maize and the *AGAMOUS* gene of *Arabidopsis* (Hake et al., 1989; Yanofsky et al., 1990). The two decades since have seen a rapid accumulation of knowledge about the molecular mechanisms responsible for the action of the apical meristems. In this review I summarize the developmental paradigms that have emerged to explain the inner workings of the amazing developmental dynamo that is the shoot apical meristem.

Organization and growth pattern of the shoot apical meristem

Most of what we know about shoot apical meristems comes from studies carried out on a few genetically tractable model organisms—snapdragon, tomato, *Arabidopsis*, petunia, maize and rice (Table 1). Important advances have been made in all of these. For the most part, similar principles apply to all of the species and it is often possible to identify orthologous genes with similar roles in meristem development. Progress in *Arabidopsis* has been particularly rapid. This is in part due to completion of the genome sequence and to the generation of comprehensive mutant collections (The Arabidopsis Genome Initiative, 2001; Alonso et al., 2003; Daxinger et al., 2007).

Both shoot and root apical meristems are made during embryogenesis. In dicotyledonous plants, such as *Arabidopsis*, the shoot apical meristem forms centrally, between the two seed leaves, or cotyledons. In monocotyledonous plants, such as maize or rice, the shoot apical meristem forms laterally, at the base of a single cotyledon. The shoot apical meristem produces a variable number of leaves before the seed matures and dries down. For instance, two barely perceptible leaves are made in *Arabidopsis* (Conway and Poethig, 1997) while about five are made in maize (Evans et al. 1994). Upon germination, the shoot apical meristem becomes active and resumes leaf production. The most common pattern of leaf arrangement, or phyllotaxy, is spiral, as is seen in *Arabidopsis*, but other patterns are possible. In maize and rice, leaves develop alternately and on opposite sides of the meristem (distichous phyllotaxy). At some point in development, typically in response to an environmental stimulus, the meristem receives a signal to flower and the shoot apical meristem switches from being a leaf-producing vegetative meristem to being a flower-producing inflorescence meristem. In some cases, this involves a transition from one pattern of phyllotaxy to another (Table 1). In maize and rice, for instance, the vegetative meristem produces leaves in a distichous arrangement (alternating leaves on opposite sides of the meristem) while the inflorescence meristem produces floral meristems in a spiral pattern.

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Table 1
Attributes of plants used to study meristem development.

Species	Genome size/ sequenced?	Vegetative phyllotaxy	Inflorescence phyllotaxy	Meristem size (height by width)	Leaf type
Dicotyledonous plants					
Arabidopsis[a] *Arabidopsis thaliana*	200 Mb/yes	Spiral	Spiral	40×60 μ	Simple
Tomato[b,c] *Solanum lycopersicon*	950 Mb/in progress	Spiral	Sympodial	75×175 μ	Compound (pinnate)
Petunia[d,e] *Petunia hybrida*	1.2 Gb/no	Spiral	Sympodial	75×150 μ	Simple
Snapdragon[f,g] *Antirrhinum majus*	430–860 Mbp/no	Opposite, decussate[h]	Spiral	40×60 μ	Simple
Monocotyledonous plants					
Maize[i,j] *Zea mays*	2.5 Gbp/anticipated 2009	Alternate, distichous[k]	Spiral	200×200 μ	Simple
Rice[l] *Oryza sativa*	400 Mbp/yes	Alternate, distichous[k]	Spiral	50×80 μ	Simple

[a] The Arabidopsis Genome Initiative, 2001.
[b] Reinhardt et al., 2001.
[c] Kimura and Sinha, 2008.
[d] Stuurman et al., 2002.
[e] Arumuganathan et al., 1991.
[f] Hudson et al., 2008.
[g] Waites and Hudson, 1995.
[h] Two leaves per node, opposite each other. Leaf pairs perpendicular at successive nodes.
[i] Ohtsu et al., 2007.
[j] Strable and Scanlon, 2009.
[k] One leaf per node. Alternate leaves on opposite side of stem.
[l] Kurakawa et al., 2007.

The organization of the *Arabidopsis* vegetative shoot apical meristem is typical of most dicotyledonous plants. It is a roughly triangular-shaped dome, about 60 μm across at its widest point and consists of a few hundred cells (Fig. 1). Meristem size varies substantially between species (Table 1). Evidence to date points to conserved signaling systems operating within the meristem to control development. How similar signals operate across such different scales is not known.

The *Arabidopsis* meristem is made up of three distinct cell layers. The two outermost layers (L1 and L2) grow as two-dimensional sheets of cells with cell divisions (mitotic spindles) oriented parallel to the meristem surface. The third and innermost layer (L3) contains cells that divide in all orientations. The meristems of the monocots maize and rice are similarly organized but tend to be taller and more finger-shaped. Although three is the most common, the number of layers within the meristem can vary. Maize, for instance, has only two meristem layers with a single sheet-like layer (L1) overlaying the L2 layer with its less regularly oriented cell divisions.

Stem cells reside in the center of the meristem in the central zone (Figs. 1C, E). Clonal analysis experiments indicate that there are approximately nine long-lived stem cells (three per meristem layer) in the *Arabidopsis* shoot apical meristem (Furner and Pumfrey, 1992;

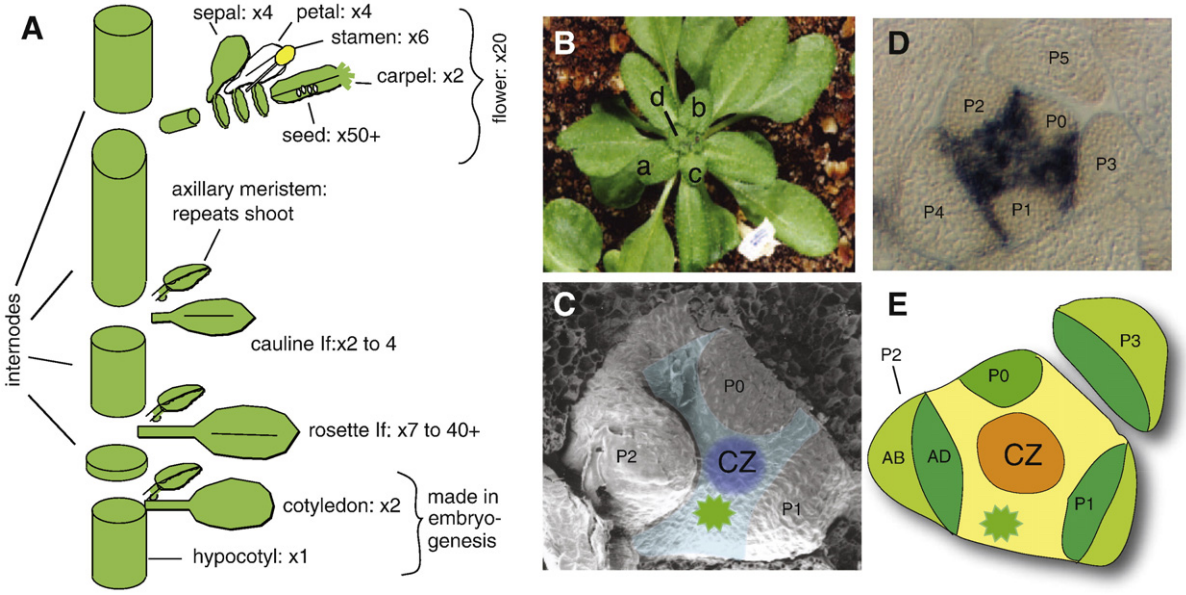

Fig. 1. Overview of *Arabidopsis* shoot development. (A) Parts list for the *Arabidopsis* shoot. All above ground parts of the plant, except the hypocotyl and cotyledons, are made from the shoot apical meristem. Leaves are located at nodes with stem segments, or internodes, between them. The number of rosette leaves depends on ambient environmental conditions that influence time to flowering. Axillary meristems are made in the leaf axil—the junction of leaf and stem. (B) Vegetatively growing *Arabidopsis* plant as seen from above. Note that leaves are made in a spiral with approximately 137° between successively formed leaves. The leaf labeled "a" in the image was made before the leaf labeled "b" and so forth. Leaves in vegetatively growing *Arabidopsis* plants are separated by very short internodes giving the plant a "rosette" appearance. (C) Scanning electron micrograph of a vegetative shoot apical meristem. Cells in the central zone (CZ—darker blue) act as stem cells. They are surrounded by peripheral zone cells (lighter blue). Leaf primordia form in the peripheral zone and are labeled from youngest to oldest. The youngest leaf primordium, P0, is not morphologically distinct from the rest of the meristem but can be detected using molecular markers. The site of the next leaf primordium to develop is labeled with a green burst. (D) Expression of *SHOOTMERISTEMLESS* gene expression (a *KNOX* gene) in the vegetative apex showing downregulation in early primordia. (E) Schematic of organization of the *Arabidopsis* shoot apical meristem as seen in transverse cross section. Leaves develop ad/abaxial polarity early in their development, possibly in response to a signal from the shoot apical meristem (Husbands et al., 2009). Site of next primordium to develop is labeled with a green burst.

Irish and Sussex, 1992; Schnittger et al., 1996). Surrounding the central zone is the peripheral zone. As the stem cells in the central zone divide, their descendants are pushed outward into the peripheral zone. It is in the peripheral zone that leaves are generated. Subtending the central zone is the rib zone. Stem cell descendants pushed downward into the rib zone generate stem tissues.

Leaf primordia are detected as transcriptionally distinct groups of leaf founder cells before they become morphologically distinct from the shoot apical meristem. This was first clearly demonstrated as the disappearance of *KNOTTED1* transcripts from young leaf primordia (Smith et al., 1992; see also Fig. 1D). In *Arabidopsis*, the leaf primordium grows first laterally, away from the center of the meristem. As it does so, the leaf primordium acquires a distinct polarity along the ad/abaxial axis. (See Long and Barton (2001) for a description of early events in *Arabidopsis* leaf development and Husbands et al. (2009) for a review of the development of ad/abaxial polarity in the leaf.) The adaxial domain of the leaf is toward the center of the meristem. The abaxial domain is away from the center of the meristem. The adaxial side of the primordium will become the upper part of the leaf, and the abaxial side will become the lower part. As the primordium grows away from the meristem, a crease forms at the boundary between the meristem and the primordium, producing a clear physical boundary between the developing leaf and the meristem. Subsequently the leaf primordium grows upward, initially curving over the top of the meristem before later growing outward and away from the meristem.

The first histological signs of leaf initiation occur after the leaf founder cells become transcriptionally distinct. Instead of dividing with spindles parallel to the meristem surface, a small number of cells in the L2 layer divide with spindles perpendicular to the meristem surface. This causes the formation of a bump. Cells from the L1 and L3 layers surrounding this initiation point participate in primordium formation; the leaf thus consists of cells derived from all three meristem layers. While cell division patterns in the plant are not invariant, the L1, L2 and L3 layers nevertheless make fairly predictable contributions to the leaf: L1 cells typically contribute the epidermis; L3 cells typically contribute the vasculature and associated cells and L2 cells contribute most of the rest of the leaf (Poethig, 1987).

While the shoot apical meristem appears as a mound of cells lacking distinct morphological features, it is in fact a very heterogeneous and highly organized structure. As genes affecting meristem function have successively been identified and their domains of expression determined, it is clear that regions of the meristem have distinct transcriptional profiles. Improvements in technology have allowed researchers to identify regional transcriptional differences on a more global scale. Yadav et al. (2009) have developed a cell sorting approach to identify regionally expressed transcripts. These authors generated lines carrying distinct fluorescent markers for the central zone, peripheral zone and rib meristem. The lines were based on the *CLAVATA3*, *FILAMENTOUS FLOWER* and *WUSCHEL* genes respectively. Meristematic cells carrying these markers were dissociated and mRNA harvested from pools of fluorescent cells was used to probe *Arabidopsis* microarrays. Besides identifying several new genes whose expression is localized to distinct domains of the meristem, this study also showed that the central zone is enriched in transcripts encoding proteins involved in DNA repair and in chromatin modification suggesting that these processes are especially important for plant stem cell function.

Laser micro dissection is another technique used to identify transcripts localized to particular regions within the meristem. Maize workers have leveraged the relatively large size of the maize shoot apical meristem to identify transcripts located in specific parts of the meristem. Similar to Yadav et al. (2009) working in *Arabidopsis*, Ohtsu et al. (2007) found the maize shoot apical meristem to be enriched for mRNAs encoding proteins involved in DNA repair and chromatin modification. These workers also found an over-representation of mRNAs from genes involved in transcriptional regulation, RNA binding, RNA processing, and gene silencing and, surprisingly, of mRNAs from retrotransposons. When divided into two regions – incipient primordia and the shoot apical meristem proper – genes involved in cell division/growth, cell wall biosynthesis, chromatin remodeling, RNA binding and translation were especially abundant in P0 and P1 stage leaf primordia while transcripts involved in DNA repair were more abundant in the shoot apical meristem proper (Brooks et al., 2009). More recently, Nogueira et al. (2009) micro dissected subsections of the maize meristem and identified specific regions enriched in particular microRNA precursors.

It is interesting that groups working in both *Arabidopsis* and in maize have noticed enrichment for genes involved in chromatin modification within the shoot apical meristem. Indeed, mutations in genes encoding proteins that act to generate repressive chromatin can have a profound affect on the ability of the shoot apical meristem to retain its ability to grow indeterminately. The *EMBRYONIC FLOWER1*, *EMBRYONIC FLOWER2* and *TERMINALFLOWER2* loci all encode proteins associated with the formation of silent chromatin (Yoshida et al., 2001; Takada and Goto, 2003; Calonje et al., 2008). Loss-of-function mutations in any of the three loci cause cells in the central zone of the shoot apical meristem to be consumed in the production of a terminal flower. Several examples of chromatin alterations at specific developmental regulatory loci have also come to light (see below). One of the challenges in understanding growth and development at the shoot apical meristem, then, will be to understand the dynamic regulation of chromatin structure in the stem cells and their descendants.

Regulating stem cell activity in the shoot apical meristem—the *clv3* signaling system

Clavata mutants were among the first morphological mutants described in *Arabidopsis*. Named for their short, club-shaped siliques, these mutations also cause enlargement of the shoot apical meristem accompanied by over proliferation of cells in the central zone. Molecular cloning of these genes and ascertainment of their expression patterns suggested early on that the CLAVATA gene products were involved in a signaling pathway. The *CLAVATA3 (CLV3)* gene was found to encode a small protein expressed in the central zone (Fletcher et al., 1999) while the *CLAVATA1* and *2* genes were found to encode receptor-like proteins expressed primarily in the L3 layer of the shoot apical meristem (Clark et al., 1997; Jeong et al., 1998). *CLAVATA1* encodes a fully functional leucine rich repeat receptor kinase (LRR receptor kinase). The CLAVATA2 protein by comparison lacks the intracellular kinase domain and is thus thought to need to interact with a membrane associated kinase to carry out its function. These findings led to the hypothesis that the small CLAVATA3 protein, made in the central zone of the meristem, interacts with the CLAVATA1 and 2 receptors in the underlying L3 tissue to inhibit cell division within the central zone.

Only recently has the CLAVATA3 ligand been shown to interact directly with the CLAVATA1 receptor. In part, this is because it has taken time to determine the exact nature of the CLAVATA3 ligand. The CLAVATA3 protein is made as a prepropeptide. It is 96 amino acids long and contains an 18 amino acid signal sequence that targets it to the extracellular space (Sharma et al., 2003). Kondo et al. (2006) used *in situ* MALDI-TOF mass spectrometry on callus tissue overexpressing CLAVATA3 to determine the structure of the secreted CLAVATA3 peptide. It was necessary to express the CLAVATA3 protein in callus tissue because overexpression in seedlings resulted in meristem arrest. Kondo et al. identified a processed form of the CLAVATA3 peptide that was only 12 amino acids in length. The corresponding synthetic peptide reduced the size of the meristem when applied to wild-type seedlings. These 12 amino acids are found near the C terminus of the CLAVATA3 protein and are conserved in the larger

family of CLE peptides (Cock and McCormick, 2001; Ito et al., 2006; Jun et al., 2008). While synthetic CLE peptides have activity when applied to the apical meristem, Ohyama et al. (2009) used nano-LC-MS/MS to identify an endogenous form of CLAVATA3 that is 13 amino acids long and is arabinosylated at hydroxyproline 7. This peptide, when purified from plant cultures, is far more potent in its ability to rescue the *clv3* mutant phenotype than the synthetic, non-arabinosylated form.

Once the mature form of the CLAVATA3 ligand had been identified, it became possible to show that the CLAVATA3 ligand binds directly to the CLAVATA1 receptor (Ogawa et al., 2008). Because overexpressing the CLAVATA1 kinase results in growth inhibition, Ogawa et al. overexpressed a truncated form of CLAVATA1 that lacked the intracellular kinase domain. Microsomal fractions from cells expressing this CLAVATA1 "ectodomain" could bind synthetic CLAVATA3 specifically. Since then, the arabinosylated form of CLAVATA3 has been shown to interact more strongly with CLAVATA1 than the non-arabinosylated form (Ohyama et al., 2009).

The receptor side of the CLAVATA signaling pathway is more complex than initially appreciated. The CLAVATA1 and 2 receptors belong to a large family of LRR receptor kinases. Closer examination revealed that the original *CLAVATA1* alleles were dominant negative alleles and that null alleles at the *CLAVATA1* locus have a very weak phenotype suggesting that there are additional receptors that act redundantly with CLAVATA1 (Dievart et al., 2003). It is satisfying then that Müller et al. (2008) have identified a novel kinase encoded by the *CORYNE* locus that acts in parallel to *CLAVATA1*. The CORYNE receptor is unusual in that it lacks the extracellular domain typical of LRR receptor kinases and consists principally of a transmembrane domain linked to an intracellular kinase domain. Genetic studies are consistent with *CORYNE* and *CLAVATA2* acting together and *CLAVATA 1* acting in parallel to them (Müller et al., 2008). The LRR receptor kinases have been proposed to form dimers through their transmembrane domains. Thus, Müller et al. proposed a model in which the two "partial" receptors – CLAVATA 2 with its extracellular domain and CORYNE with its intracellular kinase domain – interact to transduce the CLV3 signal while CLAVATA1 homodimers do so in parallel. Knocking out either complex causes only a weak loss of control of cellular proliferation in the meristem while knocking out both complexes yields a phenotype more similar in strength to mutations that disrupt the *CLAVATA3* gene. The dominant negative effect of the original *CLAVATA1* alleles, many of which are caused by mutations in the intracellular kinase domain, could be explained if the dominant negative CLAVATA1 truncated peptides interfere with the CLAVATA2/CORYNE receptor complex. The CORYNE mRNA is not limited to the L3 layer of the meristem as *CLV1* is, but appears to be in all layers (Müller et al., 2008). A corollary of this is that all cells in the shoot apical meristem would be competent to respond to a *CLAVATA3* signal making localization of the CLAVATA3 product critical.

The CLAVATA3 signaling system is conserved widely throughout the plant kingdom. (See Thompson and Hake (2009) for a review comparing meristem development in the grasses to meristem development in *Arabidopsis*.) In maize, the *fasciated ear 2* and *thick tassel dwarf1* genes cause over-proliferation of cells in inflorescence meristems when mutant and encode LRR receptor kinases that are most similar to the *Arabidopsis CLAVATA2* and *CLAVATA1* genes respectively (Taguchi-Shiobara et al., 2001; Bommert et al., 2005). Rice *FLORAL ORGAN NUMBER1 (FON1)*, a *CLAVATA1* ortholog, increases the size of floral meristems when mutant (Suzaki et al., 2004). Mutations in *FON1* have little effect on vegetative or inflorescence meristems despite the fact that *FON1* is expressed in them, suggesting the presence of redundantly acting receptors. Interestingly, instead of being limited to the interior portions of the meristem, as in *Arabidopsis*, *FON1* mRNA is expressed throughout the rice meristem (Suzaki et al., 2004). Two *CLAVATA3*-like genes regulate meristem size in rice—*FON2* (also known as *FON4*) and *FON2-LIKE CLE PROTEIN* (*FCP*) (Chu et al., 2006; Suzaki et al., 2006; Suzaki et al., 2008). *FON2* acts through the *FON1* receptor in inflorescence and floral meristems but appears to lack activity in vegetative meristems as overexpression of *FON2* has no effect on these. *FCP*, by contrast, causes termination of vegetative meristems when overexpressed and does not appear to act through the *FON1* receptor. Thus, the FON2 and FCP ligands may have diversified to act in different tissues and on different receptors. One amino acid differs between the predicted mature forms of FON2 and FCP. Suzaki et al. (2008) speculated that this may explain the different action of the two peptide signals.

The increase in central zone size in *clavata* deficient meristems could happen in at least two distinct ways. The rate or number of divisions occurring in cells specified as central zone cells could be increased. In this scenario, the *CLAVATA* loci play a role in regulating the rate of cell division of a particular cell type. Alternatively, the size of the domain specified as central zone within the meristem could be increased. In this case, the *CLAVATA* loci play a role in partitioning the meristem into central and peripheral regions. To test this, Reddy and Meyerowitz (2005) knocked down *CLAVATA3* function in developing inflorescence meristems using an inducible RNA interference system. They then monitored cells within the meristem for the expression of a fluorescent central zone specific marker. They found that peripheral zone cells adjacent to the central zone became respecified as central zone cells within 24 h of treatment. These cells had not divided during this time and therefore did not result from increased cell division of central zone cells. Cell division did increase in treated meristems but not until later than respecification, and at a distance from the meristem center. Thus, the primary role of the *CLAVATA3* signaling system appears to be to position the boundary between the central zone and the peripheral zone within the meristem. This study illustrates the power of real-time imaging to distinguish early, direct consequences of experimental manipulation from later, less direct consequences that can confound the analysis.

Regulation of the *wuschel* gene and its product

One of the outcomes of decreased CLAVATA3 signaling is an increase in the number of cells in the meristem that express the *WUSCHEL* gene. The *WUSCHEL* gene encodes a homeodomain transcription factor that is expressed in the L3 layer of the shoot apical meristem (Mayer et al., 1998) (Fig. 2). In *wuschel* mutants, the central zone is not maintained. Thus, *WUSCHEL* plays a role in the shoot apical meristem that is opposite to that of *CLAVATA3* — *WUSCHEL* promotes stem cell activity while *CLAVATA3* suppresses stem cell activity. In the experiment by Reddy and Meyerowitz (2005) in which *CLV3* was silenced using an inducible treatment, *WUSCHEL* expansion was seen 7 days post treatment. Earlier time points were not reported so it is not known how rapidly this expansion occurred and whether it was the result of respecification or increased division of *WUSCHEL*-expressing cells. However, an increase in *CLAVATA3* expression results in a decrease in the number of *WUSCHEL*-expressing cells within 3 h (Müller et al., 2006). This rapid downregulation is consistent with *CLAVATA3* modulating the activity of as yet unknown transcription factors to repress *WUSCHEL* transcription.

Because changes in WUSCHEL levels can affect the extent of *CLAVATA3* expression in the meristem (too little WUSCHEL activity causes a decrease, while too much WUSCHEL activity causes an increase in the number of *CLAVATA3* expressing cells), it has been proposed that the *CLAVATA3* signaling pathway and *WUSCHEL* make up a feedback loop (Fig. 2) (Fletcher et al., 1999; Brand et al., 2001; Schoof et al., 2001). In this model, *CLAVATA3* signals generated by the L1 and L2 layers are relayed to the L3 via the CLV1 receptor, decreasing the number of *WUSCHEL*-expressing cells. A decrease in *WUSCHEL*-expressing cells would result in a decrease in the number of

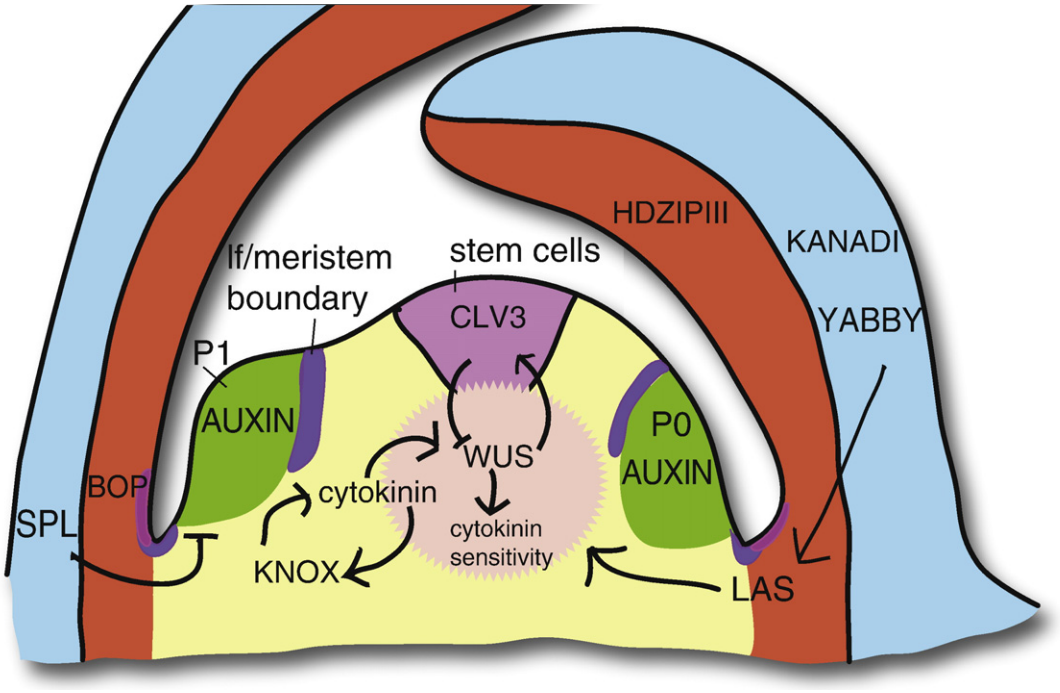

Fig. 2. The control of stem cell activity in the shoot apical meristem relies on signals originating from within the meristem as well as on signals from leaves. Drawing is a schematic of a meristem as seen in longitudinal section. Stem cell activity – specifically the size of the central zone – is repressed by the secreted CLAVATA3 peptide. CLAVATA3 acts to limit the size of the *WUSCHEL*-expressing zone, perhaps by limiting *WUSCHEL* transcription. *WUSCHEL* promotes stem cell activity and positively regulates CLAVATA3 activity, thus generating a feedback loop that stabilizes stem cell activity in the plant. The region of the pink "burst" is a region of high cytokinin sensitivity due to *WUSCHEL* action. WUSCHEL levels in turn are increased by cytokinin. Cytokinin biosynthesis is increased by the meristem expressed *KNOX* genes, the expression of which is in turn increased by high cytokinin levels. Note that the regions of high auxin activity (leaf primordia) and high cytokinin activity (central rib meristem) are clearly separated in the meristem. The boundaries between leaf primordia and the adjacent meristem (dark purple) become established sometime between P0 and P1 stages and are marked by expression of the *CUPSHAPED COTYLEDON* and *LATERAL SUPPRESSOR (LAS)* genes. As the leaves mature, the boundaries continue to express these genes and add other regulators such as *BLADE-ON PETIOLE* (*BOP*, light purple). *YABBY* is expressed in the abaxial domain of the leaf. It acts non-cell autonomously to promote adaxial fate and to promote stem cell activity in the meristem through the LATERAL SUPPRESSOR gene product. Inasmuch as *LATERAL SUPPRESSOR* is expressed at the adaxial boundary of the leaf and meristem, the effect of *YABBY* on *LATERAL SUPPRESSOR* expression could be through *YABBY*'s role in promoting adaxial development. *YABBY* is shown acting in a fairly mature leaf here but could act in a leaf that is much closer to the meristem, for instance a leaf at the P1 stage. *BOP* is expressed at the leaf–meristem boundary once the leaf is physically separate from the meristem, yet it also affects stem cell activity in the meristem. In yet another case of leaf to meristem signalling, *SQUAMOSA BINCING PROTEIN-LIKE (SPL)* gene products synthesized in the leaf decrease the rate of leaf production within the meristem.

CLAVATA3 expressing cells. Decreased CLAVATA3 production would result in upregulation of *WUSCHEL* and an increase in the number of *WUSCHEL*-expressing cells. Such a feedback loop could act to stabilize the number of stem cells in the shoot apical meristem.

Although the direct links between the CLAVATA signaling and WUSCHEL components of the feedback loop have remained elusive, several transcriptional regulators of *WUSCHEL* expression have been identified. These are the *STIMPY*, *SPLAYED*, *BARD1*, *OBERON1* and *OBERON2* genes. It is possible that one or more of these regulators are controlled by *CLAVATA3* signaling.

Mutations in the *STIMPY* gene cause phenotypes similar to *WUSCHEL* mutations (Wu et al., 2005). *STIMPY (WOX9)* encodes a member of the *WUSCHEL* family of homeodomain transcription factors. In weak *stimpy* loss-of-function mutants, cells in the vegetative meristem differentiate and growth at the meristem ceases. Based on marker gene expression, *STIMPY* is required to maintain *WUSCHEL* and *CLV3* expression. Like *wus* mutations, weak *stimpy* mutations are epistatic to mutations in the *CLAVATA* pathway indicating that *STIMPY* is downstream of *CLV* signaling. Most interestingly, weak *stimpy* mutants, but not *wuschel* mutants, could be rescued by adding sucrose to the growth medium. Because sucrose promotes cell division (through upregulation of CYCLIND), Wu et al. (2005) proposed that sucrose rescues *stimpy* mutants through its effect on cell division. Alternatively, it is possible that sucrose itself is a signal that has a role in meristem development. Strong *stimpy* mutant alleles cause embryo arrest at the early globular stage indicating that, unlike *WUSCHEL*, *STIMPY* plays roles in development outside the shoot apical meristem (Wu et al., 2007).

Two factors that interact directly with the *WUSCHEL* promoter are *SPLAYED* and *BARD1* (Kwon et al., 2005; Han et al., 2008). *SPLAYED* is an SNF2 chromatin remodeling ATPase. It binds within 500 bp of the transcriptional start site of the *WUSCHEL* gene and is required for the efficient transcription of the *WUSCHEL* locus (Kwon et al., 2005). In *splayed* loss-of-function mutants, meristems are reduced in size and WUSCHEL mRNA levels are greatly reduced. While *CLAVATA3* signaling is disabled and the number of *WUSCHEL*-expressing cells is increased in single *clavata1–4* mutants, levels of WUSCHEL mRNA as well as meristem size are normalized in a *clv1–4; splayed* double mutant. This is consistent with the *CLAVATA3* signal acting through *SPLAYED* to reduce *WUSCHEL* levels.

The BARD1 protein also binds to the *WUSCHEL* promoter and it interacts with *SPLAYED* in immunoprecipitation assays (Han et al., 2008). However, opposite to *SPLAYED*, BARD1 appears to be responsible for repression of *WUSCHEL* because in *bard1* mutants, *WUSCHEL* is highly upregulated and meristem size is substantially increased. *BARD1* encodes a protein with similarity to the human *BREAST CANCER ASSOCIATED GENE 1 (BRCA1)*. In humans, these proteins are implicated in DNA repair, transcriptional regulation, recombination, and cell cycle control (Irminger-Finger and Jefford, 2006). In *Arabidopsis*, *BARD1* is required for efficient DNA repair as well as for repression of *WUSCHEL* (Han et al., 2008; Reidt et al., 2006). The contrasting roles of BARD1 and SPLAYED proteins and their proximity on the *WUSCHEL* promoter suggest that they act antagonistically on the state of chromatin at the *WUSCHEL* locus.

Two additional transcriptional regulators have been implicated in maintaining a stem cell population in the shoot apical meristem—the

Arabidopsis OBERON1 and OBERON2 proteins. The *OBERON1* and *OBERON2* genes act redundantly with one another to maintain expression of the *CLAVATA3* and *WUSCHEL* genes in the meristem, and to maintain stem cell activity in the meristem (Saiga et al., 2008). When both genes are mutated, the result is arrest of the shoot apical meristem. OBERON mRNAs are found throughout the shoot apical meristem so the spatial localization of these products does not seem to be important to their function. *oberon1* and *2* mutations show similar epistasis patterns to *wuschel* mutations implying that the *OBERON* factors act in the same pathway as the *WUSCHEL* gene. The *OBERON1* and *2* genes encode proteins with plant homeodomain fingers. These fingers recognize histones in active chromatin in animals; it is unknown if they also do so in plants. While it is possible that the OBERON proteins are required to keep the chromatin at the *WUSCHEL* and/or *CLAVATA3* genes in an active state, it is not yet known whether the *WUSCHEL* or *CLAVATA3* genes are direct targets of *OBERON* regulation.

The WUSCHEL transcription factor contains both a likely activation domain as well as a carboxyterminal EAR domain predicted to act as a transcriptional repressor (Kieffer et al., 2006). Thus, *WUSCHEL* may act as either a transcriptional stimulator or repressor depending on the context in which it finds itself. A semi-dominant mutation in the *WUSCHEL* ortholog from *Antirrhinum*, *ROSULATA*, creates a truncated version of the ROSULATA protein that lacks the EAR domain (Kieffer et al., 2006). *Antirrhinum rosulata* mutants, similar to *Arabidopsis wuschel* mutants, have meristems that fail to maintain a population of stem cells in the shoot apical meristem. Transforming a similarly truncated version of the *WUSCHEL* gene into *Arabidopsis* also leads to a dominant negative phenotype (Kieffer et al., 2006). Screens for interacting proteins in yeast identified two proteins that interact with the carboxyterminal domain of *WUSCHEL*. These are two predicted corepressors, TOPLESS and TOPLESS4 (also called WUSCHEL INTERACTING PROTEINS 1 and 2) belonging to the larger family of Groucho/Tup1 corepressors (Long et al., 2006; Kieffer et al., 2006). Groucho/Tup1 corepressors lack the ability to bind DNA on their own. Instead they interact with, and rely on, sequence-specific DNA binding factors to recruit them to the appropriate regions of DNA (see review by Liu and Karmarkar, 2008). Gro/Tup1 corepressors cause transcriptional repression by altering nucleosome positioning, recruiting histone deacetylases and competing for limiting subunits of the mediator activator. The WUSCHEL protein, then, may exert its activity by altering and repressing the chromatin associated with its targets.

What then are the targets that *WUSCHEL* regulates? Liebfried et al. (2005) used an inducible version of the *WUSCHEL* gene to identify genes repressed in response to *WUSCHEL* overexpression. Among these are the A-type response regulators *ARR5*, *ARR6* and *ARR7*. Indeed, *ARR5* expression and *WUSCHEL* expression appear to be complementary in the shoot apical meristem (Gordon et al., 2009) consistent with a role for WUSCHEL in repressing *ARR5* transcription. WUSCHEL binds approximately 1000 bp upstream of *ARR7* indicating that transcription of the *ARR7* gene is likely to be directly regulated by *WUSCHEL*. The A-type response regulators are thought to inhibit cytokinin signaling (Müller and Sheen, 2007). Thus, one role of *WUSCHEL* may be to make the cells in which it is expressed more sensitive to cytokinin signals (Fig. 2). This is interesting in light of accumulating evidence that cytokinin plays an important role in controlling cell division and differentiation in the apical meristem. This evidence is discussed in the next section.

The role of cytokinin in promoting meristem activity

The plant hormone cytokinin has long been known to have a connection with shoot apical meristem function. Classically, varying the ratio of this hormone to auxin has been used to manipulate the outcome of tissue culture experiments. High cytokinin to auxin ratios yield shoots while low cytokinin to auxin ratios yield roots (Skoog and Miller, 1957).

Recent breakthroughs have led to an understanding of the mechanism of cytokinin signaling (reviewed in Müller and Sheen, 2007). Briefly, cytokinin is sensed at the plasma membrane by a transmembrane histidine kinase resulting in a transfer of phosphate to a histidine phosphotransfer (HPT) protein causing the HPT protein to move into the nucleus. This results in phosphorylation of B-type response regulators (RRs) which bind to DNA and transcriptionally activate target loci. The A-type RRs, such as the *WUSCHEL* targets above, lack DNA binding domains and appear to inhibit the action of the B-type RRs.

Kurakawa et al. (2007) identified an enzyme in rice, encoded by the *LONELY GUY* gene, that catalyzes the last step in the formation of bioactive cytokinin. *lonely guy-1* mutants have smaller vegetative meristems than normal and their floral meristems stop organ formation prematurely, resulting in fewer floral organs. The *LONELY GUY* transcript is expressed in the two to three outermost layers of the apical meristem dome, encompassing the entire stem cell containing region, and is not expressed in the rib meristem. *lonely guy-1* mutants show lower levels of cytokinin activity as measured by reporters responsive to cytokinin activation. Thus, localized cytokinin activation appears to be required for normal stem cell function in the rice shoot apical meristem.

Several lines of evidence suggest that cytokinin plays a role in meristem function in plants other than rice. First, the shoot apical meristem in *Arabidopsis* appears to be a site of cytokinin action. Müller and Sheen (2008) have developed a reporter for cytokinin activity, the Two Component output Sensor (TCS). TCS consists of concatemerized B-TYPE ARR binding sites fused to a minimal promoter and a luciferase or GFP reporter. The expression of this reporter is indeed elevated in *Arabidopsis* shoot apical meristems as expected if both cytokinin and functional B-TYPE ARR receptors are present. Gordon et al. (2009) analyzed TCS reporter expression in the *Arabidopsis* shoot apical meristem at higher resolution and found that it is expressed in a domain overlapping the domain where *WUSCHEL* transcript accumulates. An attractive hypothesis, then, is that *WUSCHEL* creates a domain of high cytokinin responsiveness by negatively regulating the ARR inhibitors of cytokinin response. Experiments to test whether this domain remains responsive to cytokinin in the absence of *WUSCHEL* function have yet to be conducted.

Second, Class 1 KNOX homeodomain containing transcription factors are required for meristem establishment and maintenance and also increase cytokinin levels in the shoot apical meristem (Endrizzi et al., 1996; Long et al., 1996; Kerstetter et al., 1997; Jasinski et al., 2005; Yanai et al., 2005). In maize, the *KNOTTED1* gene plays this role, while in *Arabidopsis*, the *SHOOTMERISTEMLESS* gene does so (Long et al., 1996; Kerstetter et al., 1997). *KNOTTED1* and *SHOOTMERISTEMLESS* are expressed throughout the shoot apical meristem except in incipient leaf primordia where they are downregulated (Fig. 1). When overexpressed, *KNOTTED1* and *SHOOTMERISTEMLESS* cause the formation of ectopic meristems (Sinha et al., 1993; Williams et al., 1997). Cytokinin levels are increased in plants overexpressing *KNOX* genes. The use of inducible systems to express the *Arabidopsis SHOOTMERISTEMLESS* gene shows that cytokinin biosynthetic genes increase their levels within 4 h of *SHOOTMERISTEMLESS* induction (Yanai et al., 2005; Jasinski et al., 2005). These studies showed a rapid activation of cytokinin responsive gene expression as well. Thus, increased cytokinin activity may be a direct consequence of *SHOOTMERISTEMLESS* transcriptional regulation. Further, application of cytokinin rescued *Arabidopsis shootmeristemless* loss-of-function mutants and expression of the *IPT* cytokinin biosynthetic gene from an *SHOOTMERISTEMLESS* promoter could suppress the *shootmeristemless* mutant phenotype (Jasinski et al., 2005). Thus, it appears that much of the effect of *KNOX* genes in

regulating meristem function operates through their effect on cytokinin biosynthesis (Fig. 2).

In an apparent positive feedback loop, cytokinin in turn may activate *KNOX* genes as Kurakawa et al. (2007) found that the cytokinin activating enzyme *LONELY GUY* is required for the accumulation of KNOX transcripts in rice (Fig. 2). In addition, Rupp et al. (1999) showed that overproduction of cytokinin results in increased steady state levels of KNOX gene transcripts. Thus, KNOX and cytokinin may act in a positive feedback loop with KNOX genes promoting cytokinin biosynthesis and cytokinins up-regulating KNOX biosynthesis. It is unlikely, however, that cytokinins play a role in initiating this feedback loop by setting up *KNOX* expression in the early embryo since the TCS reporter for cytokinin response is not active at the presumptive shoot apical meristem in the heart stage embryo where meristem specific *SHOOTMERISTEMLESS* gene expression is detectable (Müller and Sheen, 2008). Thus, some other cue beside cytokinin must activate the early steps of shoot apical meristem formation in the embryo.

In a third example of the importance of cytokinin to meristem function, the *WUSCHEL* locus also appears to participate in a positive feedback loop with cytokinin. As described above, *WUSCHEL* is thought to make cells more responsive to cytokinin by repressing the transcription of ARR genes. *WUSCHEL* levels in turn are increased by cytokinin (Lindsay et al., 2006; Gordon et al., 2009). Unlike the case for the *KNOX* genes, however, there is as yet no evidence that cytokinin can bypass the requirement for *WUSCHEL* gene function. How might the *KNOX* and *WUSCHEL* pathways interact? Since KNOX genes promote cytokinin biosynthesis, it seems likely that KNOX activity in the shoot apical meristem could indirectly increase *WUSCHEL* expression.

Fourth, study of mutations in a type ARR, encoded by the maize *ABPHYL* locus, has strengthened the case for cytokinin's role in promoting meristematic function in the shoot apical meristem (Giulini et al., 2004). *ABPHYL* encodes a type A response regulator and therefore is expected to play a role in inhibiting cytokinin responses. *ABPHYL* mRNA is found in the shoot apical meristem of the maize embryo. Loss-of-function mutations in *abphyl* cause enlarged embryonic meristems of similar size to those of cytokinin treated wild-type embryos. Consistent with a role of cytokinin in promoting proliferation of cells within the shoot apical meristem, wild-type maize embryos treated with cytokinin have enlarged shoot apical meristems. Thus, the role of *ABPHYL* in the embryonic meristem may be to antagonize cytokinin activity and limit meristematic activity.

Finally, a link between cytokinin levels and meristem size has been observed in mutants of the *Arabidopsis ALTERED MERISTEM PROGRAM1* locus. *amp1* mutants have increased meristem size, increased leaf initiation rates and increased levels of cytokinin. *ALTERED MERISTEM PROGRAM* encodes a predicted carboxypeptidase, the substrate of which is unknown (Helliwell et al., 2001). Understanding the precise role of *ALTERED MERISTEM PROGRAM* in development, however, has been difficult. Vidaurre et al. (2007) reported that *ALTERED MERISTEM PROGRAM* and *MONOPTEROS*, an activator of auxin regulated gene expression, act antagonistically to one another. Saibo et al. (2007) isolated a weak allele of *AMP1* in a screen for decreased response to simultaneous treatment with ethylene and gibberellin. Suzuki et al. (2008) identified the maize *viviparous8 (vp8)* mutant as an ortholog of *AMP1*. Unlike other viviparous mutants, *vp8* mutants show substantial alterations in vegetative development (Evans and Poethig, 1997). Like *Arabidopsis amp1* mutants, leaf initiation rate is increased in *vp8* mutants. Maize *vp8* mutants are also short-statured and display altered developmental timing. *viviparous8* mutants are defective in abscisic acid metabolism (Suzuki et al., 2008). Reduced levels of abscisic acid were measured in maize *vp8* mutants but auxin and cytokinin levels were unaffected. In summary, *AMP1* appears to affect several hormone signaling pathways indicating that it may carry out a regulatory step common to several signal transduction networks. Alternately, *AMP1* may affect a single signaling pathway that interacts with several plant hormone signaling pathways.

In summary, a growing body of evidence indicates that some threshold of cytokinin activity is required for stem cell maintenance in the shoot apical meristem. Moreover, the region of presumed maximal cytokinin responsiveness overlaps with the *WUSCHEL*-expressing region of the meristem. Whether the sole function of cytokinin in the shoot apical meristem is to maintain a small domain of *WUSHEL* expressing cells or whether cytokinin plays additional roles remains to be determined.

Terminating the floral meristem

Most floral meristems are determinate, producing a set number of floral organs in distinct whorls, terminating with a whorl of carpels. Floral meristems, then, differ from typical vegetative and inflorescence meristems in that the meristem loses its ability to maintain a population of pluripotent stem cells. They also differ in the pattern of initiation of primordia, which occurs in repeated whorls rather than a spiral. (Exceptions to this include members of the Magnoliaceae. Plants in this family produce flowers with numerous floral organs in a spiral pattern in what is thought to resemble a primitive pattern of flower development.)

In *Arabidopsis* floral meristems, the determinate pattern of growth requires the *AGAMOUS* MADS box transcription factor (Yanofsky et al., 1990). *AGAMOUS* is expressed in the inner two whorls of stage three floral meristems where it acts to terminate meristematic growth of the floral meristem. These inner two whorls give rise to the stamens and carpels respectively. The two carpels are made at the summit of the floral meristem and their production effectively consumes the stem cell containing region of the meristem, thus terminating growth. In the absence of *agamous* function, the floral meristem continues to produce organs instead of terminating. *AGAMOUS* is induced in stage 3 flowers by *WUSCHEL* activity and accomplishes meristem termination by negatively regulating the *WUSCHEL* gene, but repression of *WUSCHEL* is not observed until stage 6 of flower development, when carpels are specified (Lenhard et al., 2001; Lohman et al., 2001). This repression occurs a full 2 days after *AGAMOUS* is induced. Why does it take so long? Sun et al. (2009) used inducible variants of the AGAMOUS protein to answer this question. They show that *AGAMOUS* turns off *WUSCHEL* by activating, with a lag, transcription of the *KNUCKLES* gene, which in turn represses *WUSCHEL*.

Several pieces of evidence support a role for the *KNUCKLES* gene in turning off *WUSCHEL* expression in the floral meristem. First, *KNUCKLES* encodes a zinc finger transcription factor that, when mutant, causes extra whorls of organs to develop (Payne et al., 2004). Second, the KNUCKLES protein includes an EAR motif making it a likely transcriptional repressor. Third, *KNUCKLES* is expressed in the correct place (the center of the meristem) and time (stage 6 flowers) to repress *WUSCHEL* (Payne et al., 2004). Sun et al. (2009) showed that *AGAMOUS* binds to the promoter of the *KNUCKLES* gene. The *KNUCKLES* locus carries repressive chromatin marks (histone H3 Lys 27 trimethylation) in early floral primordia. Upon activation of a glucocorticoid inducible AGAMOUS protein, this mark decreases significantly by day 2. This decrease comes before increases in KNUCKLES mRNA levels. The authors speculated that a cycle of cell division and DNA replication is required to remove the repressive chromatin mark at the *KNUCKLES* locus and that this accounts for the lag in *KNUCKLES* activation. They suggested that this lag allows time for *WUSCHEL* to activate and establish *AGAMOUS* expression before *AGAMOUS* turns off *WUSCHEL*.

Several gene products are required for proper levels of *AGAMOUS* expression in the inner whorls of the *Arabidopsis* flower. Prunet et al. (2008) used enhancer screens to identify additional gene products required for floral meristem termination in *Arabidopsis*. In this way, they identified three genes: R—a protein of unknown function;

SQUINT—a cyclophilin (Berardini et al., 2001); and ULTRAPETALA1—a SAND domain containing protein (Carles et al., 2005). While these proteins are unrelated to one another, genetic experiments indicate that these proteins nevertheless act redundantly to one another to cause floral meristem termination. This suggests that there are multiple pathways acting to promote *AGAMOUS* transcript accumulation in the flower.

The ad/abaxial polarity system and its influence on meristem formation and activity

The ad/abaxial regulatory network has profound effects on meristem formation. This network is responsible for establishing polarity along the ad/abaxial axis of the leaf primordium (see Husbands et al., 2009 for review). Many components of this network are widely conserved across plant species. The network as understood today consists primarily of two sets of transcription factors. One set includes the class III HOMEODOMAIN LEUCINE ZIPPER (HDZIP) transcription factors (specifically the REVOLUTA, PHABULOSA and PHAVOLUTA proteins) and promotes adaxial leaf fates (the upper portion of the leaf). The other set includes the KANADI transcription factors and promotes abaxial leaf fates (the lower portion of the leaf). The spatial restriction of at least some ad/abaxial factors is mediated by microRNAs that either prevent translation of or degrade their respective mRNAs. The target genes regulated by these factors, and therefore how they realize polarity in the leaf, remain largely unknown.

Inspection of the expression patterns of the *Arabidopsis class III HDZIP* transcription factor genes, major positive regulators of adaxial polarity, clarifies why ad/abaxial patterning is so tightly linked to meristem formation (McConnell et al., 2001; Prigge et al., 2005). *Class III HDZIP* genes are expressed in a domain that encompasses the adaxial domains of leaf primordia and extends throughout the shoot apical meristem, forming a central "core" domain. Peripheral to and surrounding this is the domain that expresses abaxial transcription factors, such as KANADI. This arrangement is easiest to see in the embryo (Fig. 3). A central core of the embryo includes the adaxial domains of the cotyledons and the central portion of the hypocotyl. The developing shoot apical meristem, then, resides entirely within the adaxial domain at the juncture of the two cotyledon bases. Similarly, axillary meristems in the *Arabidopsis* rosette develop from the adaxial domain of the leaf base (Long and Barton, 2001). In summary, new meristems are formed entirely within the adaxial domain defined by *class III HDZIP* expression and are excluded from the abaxial domain.

If the specification of the adaxial domain of the embryo is required for the development of the shoot apical meristem, then mutants that cause a loss of adaxial domain specification should be defective in shoot apical meristem formation. Indeed, in mutants lacking class III HDZIP function (adaxial determinants), adaxial fates are transformed to abaxial fates and shoot apical meristems fail to form (Fig. 3; Talbert et al., 1995; Emery et al., 2003; Prigge et al., 2005). Similarly, mutants constitutively expressing the *KANADI* gene (an abaxial determinant) also exhibit ad to abaxial transformations and fail to form shoot apical meristems (Kerstetter et al., 2001).

The adaxial leaf environment is not only necessary but also sufficient for new meristem formation. Ectopic expression of class III HDZIP function (adaxial determinants) causes ectopic meristem function. Thus, in mutants ectopically expressing the PHABULOSA HDZIPIII protein, ectopic shoot apical meristems form not only from the adaxial side of the leaf base but also from around the entire circumference of the leaf base (McConnell and Barton, 1998). Ectopic expression of the shoot apical meristem markers *SHOOTMERISTEMLESS* and *WUSCHEL* is also seen when *PHABULOSA* expression is caused to expand basally in the embryo due to failure of microRNA mediated restriction of *PHABULOSA* expression (Griggs et al., 2009).

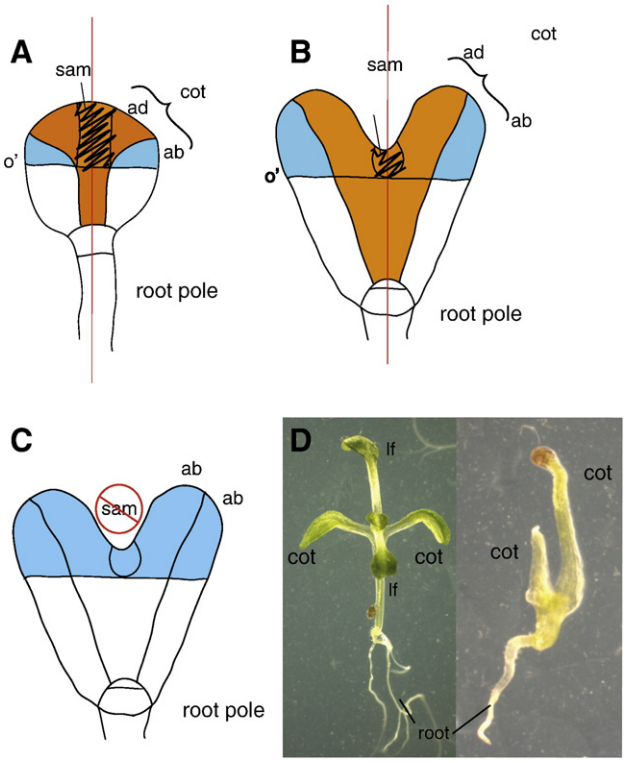

Fig. 3. Shoot apical meristem formation in the embryo depends on the genetic network that regulates ad/abaxial polarity. (A) Globular stage *Arabidopsis* embryo. Expression of adaxial polarity genes such as the HDZIPIII genes defines an adaxial, central core of cells in the embryo (orange). Expression of abaxializing genes such as KANADI define the abaxial domain (blue). The shoot apical meristem develops in a central, apical portion of the adaxial domain (black scribble). Note that the adaxial domain overlaps the cotyledon primordium domain. The junction of adaxial and abaxial domains in the cotyledon primordium marks the site of future cotyledon outgrowth. (B) Heart stage *Arabidopsis* embryo after cotyledon emergence. (C) In embryos that overexpress KANADI or that lack HDZIPIII function (*phabulosa, phavoluta, revoluta* triple mutants), adaxial fates are not established and no shoot apical meristem forms. (D) Wild-type (left) and *phb phv rev* triple mutant seedlings. The wild-type seedling has two ad/abaxially flattened cotyledons and has produced several leaves from an active shoot apical meristem. *phb phv rev* triple mutants produce two abaxialized, bladeless cotyledons (blade formation requires juxtaposition of ad and abaxial domains (Waites and Hudson, 1995)). There is no evidence of shoot apical meristem formation or activity.

The importance of restricting *PHABULOSA* to the apical domain of the embryo suggests that the HDZIPIII proteins play a role in the specification of apical/basal polarity as well as in the specification of ad/abaxial polarity.

Taken together, these observations indicate that adaxial domain specification is both necessary and sufficient to promote shoot apical meristem formation and that class III HDZIP function, in particular *REVOLUTA*, *PHABULOSA* and *PHAVOLUTA* activities, is involved in triggering new meristem formation.

There is evidence that the ad/abaxial polarity system affects the maintenance of shoot apical meristems as well as their establishment. The class III HDZIP *PHABULOSA*, *PHAVOLUTA* and *REVOLUTA* genes are expressed in the shoot apical meristem. LITTLE ZIPPER proteins form heterodimers with the larger HDZIPIII proteins and inhibit their activity (Wenkel et al., 2007; Kim et al., 2008). Failure to post-translationally repress class III HDZIP activity, as in *littlezipper3; littlezipper4* double mutants, causes formation of shoot apical meristems with expanded central zones as judged by an enlarged region of *CLAVATA3* expression (Kim et al., 2008). Similarly, mutants with ectopic *REVOLUTA* expression exhibit enlarged shoot apical meristems (Emery et al., 2003). Thus, increased class III HDZIP activity increases central zone size in the meristem. Consistent with this, reducing HDZIPIII activity by overexpressing the LITTLEZIPPER3 inhibitor causes the central zone of the meristem

to be consumed by the production of a central, radialized leaf (Wenkel et al., 2007).

The situation, however, is more complex as not all class III HDZIP proteins behave the same way. For instance, loss-of-function *corona* mutants cause a massively enlarged shoot apical meristem to form when combined with *clavata3* mutants indicating that *CORONA* is required to limit meristem size in a pathway that acts redundantly to the *CLAVATA* pathway (Green et al., 2005). Furthermore, in triple mutants lacking *phabulosa*, *phavoluta* and *corona* function or in lines overexpressing the microRNA166 small RNA and therefore similarly deficient for *PHABULOSA*, *PHAVOLUTA* and *CORONA* (but not *REVOLUTA*) the meristem is greatly enlarged (Green et al., 2005; Kim et al., 2005; Williams et al., 2005). This result suggests that the *PHABULOSA*, *PHAVOLUTA* and *CORONA* loci normally repress stem cell fates in the shoot apical meristem.

In summary, the shoot apical meristem develops within the adaxial domain of the embryo. The class III HDZIP proteins specify the adaxial domain and are necessary for shoot apical meristem formation in the embryo. The class III HDZIP proteins also play a role in maintaining the normal organization of the shoot apical meristem. Their role within the shoot apical meristem is complex and may involve both promoting and limiting the population of stem cells present in the shoot apical meristem.

Signaling from the leaf to the meristem

One of the more exciting discoveries of recent years has been the identification of gene products that mediate signaling between the leaf and the shoot apical meristem. The best understood of these is FT, an *Arabidopsis* protein that promotes flowering in response to changes in day length (see Turck et al., 2008 for review). Day length is sensed in the leaf, and long days stimulate the production of FT protein in the leaf. FT is made in phloem companion cells and is uploaded into the phloem through which it travels to the shoot apical meristem. Upon arrival in the shoot apical meristem FT partners with the bZIP protein FD to activate transcription of genes that mediate the transition from a vegetative shoot apical meristem to an inflorescence shoot apical meristem.

The petunia *HAIRY MERISTEM* gene and the *Arabidopsis SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)*, *YABBY* and *BLADE-ON-PETIOLE* genes all appear to act within the leaf to affect development at the shoot apical meristem. As yet, little is known about how this happens. It is still unclear if these gene products will share signaling strategies with FT or act through completely different mechanisms.

The petunia *HAIRY MERISTEM* gene encodes a predicted GRAS family transcription factor that is responsible for inhibiting differentiation at the shoot apical meristem (Stuurman et al., 2002). While some GRAS family members are involved in gibberellin signaling, *HAIRY MERISTEM* does not have the critical DELLA domain used in signaling and therefore likely does not operate in a gibberellin mediated process. In *hairy meristem* mutants the shoot apical meristem terminates and undergoes differentiation, forming hairs (normally found on the stem surface) on the surface of the meristem. *HAIRY MERISTEM* mRNA accumulates in the L3 layer of leaf primordia and in a ring beneath the vegetative meristem (corresponding to the stem provasculature). Because the *hairy meristem* mutant is caused by a revertable transposable element in the *HAIRY MERISTEM* gene, it was possible for Stuurman and coworkers to generate mosaic plants carrying both wild-type and *hairy meristem* mutant tissue. Analysis of these mosaics showed that the genotype of *HAIRY MERISTEM* in the L3 layer of the adjacent leaf was associated with normal meristem development. It is possible that the HAIRY MERISTEM gene product itself could move into the meristem. Alternatively, the *HAIRY MERISTEM* locus could control the synthesis of a mobile compound. Analysis of *hairy meristem*; *terminator* double mutants, where *TERMINATOR* is the petunia ortholog of the *WUSCHEL* locus, indicated that the double mutant phenotype was additive. This implicates *HAIRY MERISTEM* in a regulatory pathway parallel to the *CLAVATA WUSCHEL* pathway. *Arabidopsis* possesses a *HAIRY MERISTEM* homolog but there are not yet any reports on its function in the plant.

The *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (SPL9)* and *15 (SPL15)* transcription factor genes regulate the rate of leaf formation at the apical meristem (Schwartz et al., 2008; Wang et al., 2008). Wang et al. (2008) expressed microRNA156 under the control of a variety of region specific promoters to knock down SPL9 and SPL15 levels. Expression of mir156 in leaves but not in the meristem, increased leaf initiation rates from 0.6 leaves initiated per day to around 1 leaf initiated per day. The size and shape of the *SPL* deficient meristems were similar to wild-type. Overexpression of *SPL9* in the leaf reduced the rate of leaf initiation and made the shoot apical meristem smaller with fewer cells and a slower rate of cell division. Thus, *SPL9* and *SPL15* are required in the leaf to limit the rate of leaf initiation at the shoot apical meristem. It is not known whether the SPL proteins themselves constitute the mobile signal in this case or if the SPL proteins affect the production of a leaf to meristem signal.

YABBY mRNA is expressed in the abaxial (bottom) domain of the *Arabidopsis* leaf although initial expression may occur throughout the primordium (Goldschmidt et al., 2008). While *YABBY* expression is most prominent in the abaxial domain of the leaf, it (as well as its snapdragon homolog) is required for the development of adaxial leaf fates, i.e. fates on the opposite side of the leaf (Golz et al., 2004; Stahle et al., 2009). It is unknown how this occurs but Stahle et al. (2009) showed that the YABBY protein interacts with the LEUNIG and LUENIG-LIKE corepressors suggesting that YABBY exerts its function through transcriptional repression. Since the YABBY protein itself does not appear to move (Goldshmidt et al., 2008), it is likely that YABBY regulates the production of a cell non-autonomous signal.

Besides regulating polarity in the leaf non-autonomously, *YABBY* genes also regulate central zone size and primordium formation within the meristem non-autonomously (Goldshmidt et al., 2008). There are several members of the *YABBY* gene family, including the *FILAMENTOUS FLOWER* gene, and these have overlapping roles in meristem and primordium development (e.g. Kumaran et al., 2002). In *Arabidopsis fil yabby1* double mutants, the domains of both *CLAVATA3* and *WUSCHEL* genes are expanded outwardly and, in the case of *WUSCHEL*, upwardly (Goldshmidt et al., 2008). In addition, the placement of leaf primordia, or phyllotaxis, is altered such that new primordia do not develop in the normal spiral pattern. The action of *YABBY* on development within the shoot apical meristem requires the activity of the *LATERAL SUPPRESSOR* gene (Goldschmidt et al., 2008). *LATERAL SUPPRESSOR*, a member of the GRAS gene family of transcription factors, is expressed outside the domain of *YABBY* expression, at the adaxial boundary between the organ primordium and the shoot apical meristem (Fig. 2; Greb et al., 2003). *LATERAL SUPPRESSOR* remains expressed in a small region of the adaxial leaf base in leaf primordia throughout much of leaf development. *LATERAL SUPPRESSOR* mRNA is reduced in *yabby* mutants. Taken together, these observations suggest a "signal relay" model in which *YABBY* action in the primordium is required to stimulate *LATERAL SUPPRESSOR* synthesis at the primordium/meristem boundary (Fig. 2). *LATERAL* suppressor would then stimulate the production of an unknown signal sent from the adaxial leaf boundary to cells in the meristem. This signal would control central zone size and primordium placement. Note that *lateral suppressor* mutants on their own do not display defects in phyllotaxy or central zone size indicating that redundant gene activities likely mask this role of *LATERAL SUPPRESSOR*.

The *Arabidopsis BLADE-ON-PETIOLE1* and *2* genes share several features in common with the *YABBY* genes: they participate in the regulation of ad/abaxial polarity, they are required for normal gene expression at the meristem/primordium boundary, they affect phyllotaxy and they regulate meristematic activity (Ha et al., 2003; Ha et al., 2004; Hepworth et al., 2005; Norberg et al., 2005; Ha et al., 2007). *BLADE-ON-PETIOLE1* is expressed at the adaxial base of leaf

primordia after they have become morphologically distinct from the meristem (Ha et al., 2004). This is similar to the region in which *LATERAL SUPPRESSOR* is expressed (Greb et al., 2003). Thus, the effect of *BLADE-ON-PETIOLE* genes on phyllotaxis and meristematic activity is likely to be non-cell autonomous. The BLADE-ON-PETIOLE products are BTB-POZ domain containing proteins for which the molecular function is unknown.

The existence of a signal that is relayed by the leaf–meristem boundary to the meristem illustrates the importance of this boundary in development at the apical meristem. Based on *CUPSHAPED COTYLEDON* and *KNOX* gene expression patterns, boundaries between organ primordia and meristems, or between adjacent organ primordia, are set up while the leaf primordium is still contained within the shoot apical meristem and before the leaf primordium can be distinguished morphologically (Fig. 2; next section). As development proceeds, the primordium grows more rapidly than the meristem and the boundary becomes a groove separating the leaf primordium from the shoot apical meristem. In the case of interprimordial boundaries, the groove separates adjacent leaf primordia. These grooves are narrow bands of non-dividing, or slowly dividing, cells (Breuil-Breyer et al., 2004; Reddy et al., 2004). The position of the leaf primordium/meristem boundary at the outer edge of the shoot apical meristem along with its relatively low cell division rate would in principal make these boundaries well suited to act as sources of signals for spatial information. Indeed, the reversals in direction of auxin transport thought to be required for the normal pattern of leaf initiation are correlated with the position of these boundaries (Heisler et al., 2005). This is discussed in more detail in the section on phyllotaxis.

Morphogenesis in the apical domain of the embryo: The role of *cupshaped cotyledon* and *knox* genes

In dicotyledonous embryos, formation of the shoot apical meristem in the embryo is a stepwise process originating within the boundary between the two cotyledon primordia. The typical dicot embryo goes through a spherical or globular stage. The outgrowth of the cotyledon primordia from two lateral domains of the apical portion of this sphere, along with concurrent repression of growth at the presumptive shoot apical meristem, results in the formation of a heart stage embryo.

Molecular prepatterns distinguish several subdomains within the apical half of the globular stage embryo, well before cotyledon outgrowth (Fig. 4; Lu et al., 1996; Long and Barton, 1998; Mayer et al., 1998; McConnell et al., 2001; Takada et al., 2001; Belles-Boix et al., 2006). Distinct domains express unique combinations of genes responsible for distinguishing 1) the protodermal from the internal tissues (*ATML1*); 2) the adaxial tissues (*HDZIPIII)* from the abaxial tissues (*KANADI)*; and 3) the presumptive shoot apical meristem (*SHOOTMERISTEMLESS, KNAT6, CUPSHAPED COTYLEDON)* from the presumptive cotyledons (*AINTEGUMENTA)*. In addition, *WUSCHEL* mRNA is limited to a small number of subepidermal cells at the center of the apical domain of the embryo. Thus, the apical domain of the globular embryo consists of at least eight distinct molecular compartments (Fig. 4).

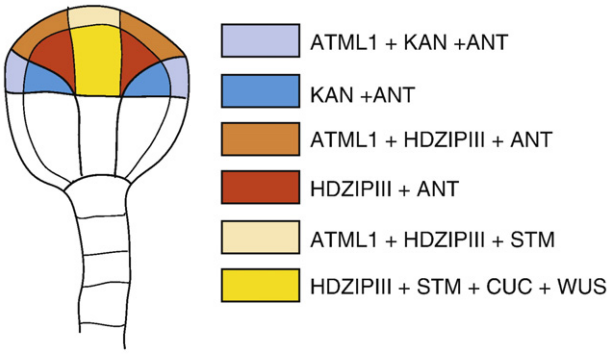

Fig. 4. Elements of pattern in the apical domain of the early *Arabidopsis* embryo. There are at least seven distinct regions that can be distinguished based on gene expression patterns in the late globular embryo. These are made up of combinations of markers for primordium/meristem identity (*AINTEGUMENTA* / *SHOOT MERISTEMLESS, CUPSHAPED COTYLEDON* and *WUSCHEL*), ad/abaxial regional identity (*HDZIPIII* / *KANADI*), and protodermal identity (*ATML1*) vs non-protodermal identity. Drawing shows a longitudinal, medial section through a late globular stage embryo. (Not shown here is a lateral domain that expresses *AINTEGUMENTA* and *CUPSHAPED COTYLEDON* but not *WUSCHEL*.)

As the cotyledons grow out, cells in the presumptive shoot apical meristem divide, albeit at a much slower rate than in the cotyledon primordia. As the shoot apical meristem increases in size, *CUPSHAPED COTYLEDON* expression is limited to the boundary between cotyledon and shoot apical meristem tissues while SHOOTMERISTEMLESS mRNA is expressed throughout the entire developing shoot apical meristem. Other pattern elements within the developing shoot apical meristem are added during this phase: *UNUSUAL FLORAL ORGANS* expression appears in a peripheral region within the developing shoot apical meristem and the central zone is established as detected by the appearance of *CLAVATA3* expression (Long and Barton, 1998). The *WUSCHEL* mutant phenotype, a seedling with a small shoot apical meristem that terminates prematurely, is consistent with a block at this last step of meristem formation.

The boundary between the cotyledons, then, takes on special importance as the site where a shoot apical meristem first develops. Genes expressed at this boundary are required for both shoot apical meristem formation and cotyledon separation. Among these are the petunia *NO APICAL MERISTEM* gene and the *Arabidopsis CUPSHAPED COTYLEDON* genes. Petunia *nam* mutants make a single, goblet-shaped cotyledon and lack a shoot apical meristem (Souer et al., 1996). When mutant, the *Arabidopsis CUPSHAPED COTYLEDON 1*, *2*, and *3* genes cause a similar phenotype (Aida et al., 1997; Takada et al., 2001; Vroemen et al., 2003; Hibara et al., 2006). The *CUPSHAPED COTYLEDON* genes act redundantly to one another and it is necessary to make higher order mutants to observe a mutant phenotype. Here I will refer to them as a single activity for simplicity's sake. The *CUPSHAPED COTYLEDON* genes, like the petunia *NO APICAL MERISTEM* gene, encode a plant-specific transcription factor with a conserved NAC domain at their aminoterminus (Olson et al., 2005).

Patterns of gene expression in the globular embryo readily explain the goblet shape of *cupshaped cotyledon* mutant seedlings (Long and Barton, 1998; Takada et al., 2001; Vroemen et al., 2003; Hibara et al., 2006; Jurkuta et al., 2009). A ring of *AINTEGUMENTA* gene expression distinguishes the peripheral domain, from which the cotyledons arise, from the central domain, where the meristem will form. *CUPSHAPED COTYLEDON*, *SHOOTMERISTEMLESS* and *KNAT6* are expressed in a stripe across the middle of the embryo between the cotyledon primordia (Fig. 5). *CUPSHAPED COTYLEDON* expression is observed first throughout the entire apical portion of the embryo and subsequently becomes limited to a stripe between the two cotyledon primordia in the embryo. The lateral portions of this boundary give rise to the separations between cotyledons while the central portion of the boundary gives rise to the shoot apical meristem. The lateral regions cease growing while the adjacent cells in the cotyledon lobes grow more rapidly, thus generating morphological separations. In *cupshaped cotyledon* mutants, all cells around the periphery of the globular stage embryo grow out, generating a ring of cotyledon tissue. Thus, wild-type *CUPSHAPED COTYLEDON* activity is required to prevent growth of the intercotyledonary regions. Recent experiments in which *CUPSHAPED COTYLEDON* is ectopically expressed in the stem and leaf show that the presence of *CUPSHAPED COTYLEDON* activity may cause a more general role in growth inhibition. Ectopic *CUPSHAPED COTYLEDON* activity causes a reduction of growth both by decreasing cell division rates and decreasing cell size (Peaucelle et al., 2007; Sieber et al., 2007).

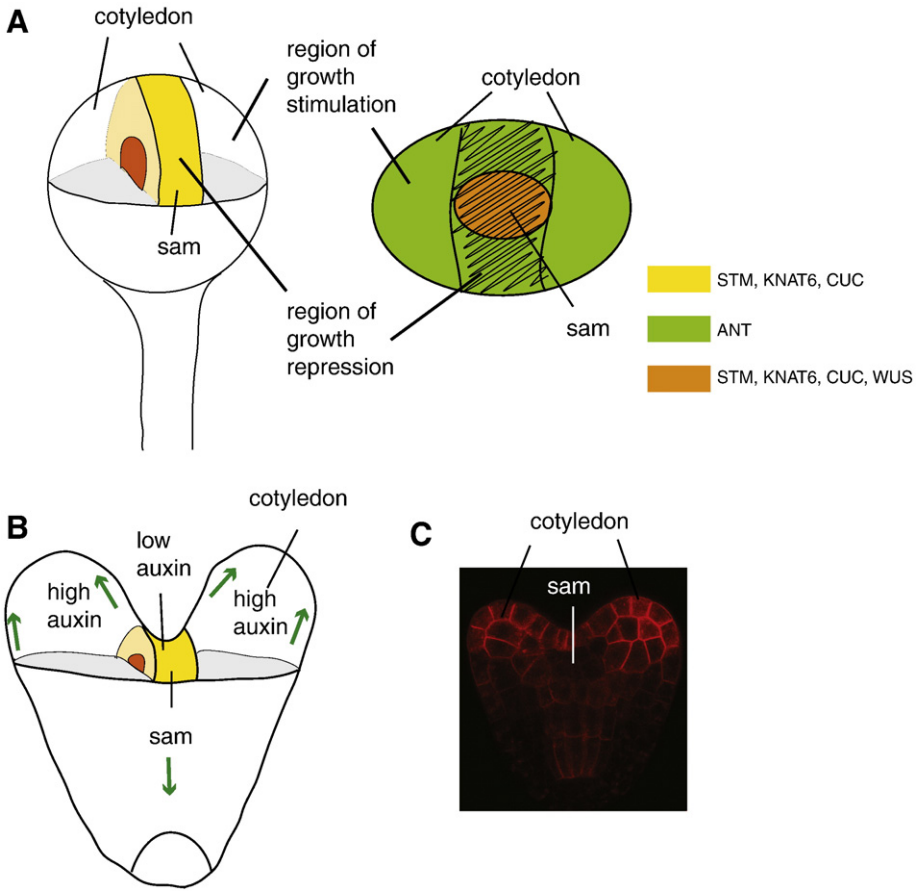

Fig. 5. The role of *CUPSHAPED COTYLEDON* and *KNOX* genes and auxin in establishing apical pattern in the *Arabidopsis* embryo. (A) Schematic of globular embryo (left) showing formation of a boundary between the two cotyledons. The green oval (right) shows organization of gene expression patterns as viewed from above. This boundary will give rise to the shoot apical meristem (sam). The boundary expresses the *SHOOTMERISTEMLESS (STM)*, *KNAT6* and *CUPSHAPED COTYLEDON (CUC)* genes. The boundary (black scribble) is laid across a ring-shaped pattern of *AINTEGUMENTA* expression (green). Growth is repressed in the boundary region while it is stimulated in the presumptive cotyledons, giving rise to the characteristic heart shape of the embryo. When *CUPSHAPED COTYLEDON* or *SHOOTMERISTEMLESS* and *KNAT6* are defective, all parts of this ring grow out, forming a single, cupshaped cotyledon. In contrast, ectopic expression of *CUPSHAPED COTYLEDON* results in failure of cotyledons to form. (B) Heart stage embryo showing outgrowth of cotyledons. High levels of auxin promote cotyledon formation while low levels promote shoot apical meristem formation. Green arrows show direction of auxin transport. (C) Confocal microscope image of GFP-labelled PIN reporter showing expression of PIN in heart stage embryo. PIN expression is high in cotyledon primordia where auxin levels are elevated. The subprotodermal regions of the shoot apical meristem are devoid of PIN protein expression suggesting that these tissues experience lower levels of auxin and/or are unable to respond to auxin. Gray plane across embryos in A and B is the O′ boundary. The O′ boundary is formed when the embryo proper transitions from the four to the eight cell stage. It separates the top half of the globular embryo from the bottom half.

A key question is how the position of the intercotyledonary boundary is specified in the embryo. Understanding the spatial regulation of the *CUPSHAPED COTYLEDON* genes should provide important information toward an answer to this question. As was shown for the *WUSCHEL* gene, the *Arabidopsis CUPSHAPED COTYLEDON* genes require the action of SWI/SNF domain containing genes for their expression (Kwon et al., 2006). There are four SWI/SNF domain containing proteins in *Arabidopsis* and two of these, *SPLAYED* and *AtBRM*, are required. Interestingly, while *AtBRM* is involved in increasing transcript levels of all three *CUPSHAPED COTYLEDON* genes, *SPLAYED* is predominantly required for upregulation of *CUPSHAPED COTYLEDON2*. It is not known if this regulation occurs through a direct action of *SPLAYED* and *ATBRM* on the *CUPSHAPED COTYLEDON* genes or through action on other, still unknown, upstream actors.

The accumulation of *CUPSHAPED COTYLEDON* mRNA is determined in part by the activity of the *CUPSHAPED COTYLEDON* promoters and in part by the activity of microRNA 164 (encoded by the microRNA 164a, b and c genes) which restricts *CUPSHAPED COTYLEDON1* and *2* expression to a narrower domain and lower levels than specified by their promoters alone (Laufs et al., 2004; Mallory et al., 2004; Sieber et al., 2007). In mutants where the *CUPSHAPED COTYLEDON1* or *2* mRNAs are resistant to mir164, or in mutants defective for all three mir164 genes, no dramatic effects on the embryo were reported. Thus, microRNA regulation of the *CUPSHAPED COTYLEDON* genes does not seem to be important for patterning the embryo. Postembryonic effects of loss of microRNA regulation were observed, however. These included altered phyllotaxy in the inflorescence but not the vegetative meristem, altered floral organ numbers, and an increase in the width of boundary regions.

The *KNOX* genes *KNAT6* and *SHOOTMERISTEMLESS* also participate in forming the boundary between the two cotyledons in the embryo. *SHOOTMERISTEMLESS* is expressed in a similar stripe as the *CUPSHAPED COTYLEDON* genes and acts downstream of them (Aida et al., 1999; Long and Barton, 1998; Takada et al., 2001). Loss-of-function *shootmeristemless* mutations result in failure to form a shoot apical meristem and cotyledon fusion, but the fusion is less severe than that of *cupshaped cotyledon* mutants. More recently, the *KNAT6* gene, also a *KNOX* gene, has been shown to be required for cotyledon separation (Belles-Boix et al., 2006). Its role in the early embryo is redundant to that of *SHOOTMERISTEMLESS* and can only be detected when *shootmeristemless* is also mutant. Together, *shootmeristemless knat6* mutations cause a severe cotyledon fusion phenotype similar to that seen in *cupshaped cotyledon* mutants.

KNOX proteins require formation of heterodimers with BEL-LIKE HOMEODOMAIN (BLH) proteins in order to regulate transcription (see for instance Cole et al., 2006.). The BLH genes *ARABIDOPSIS*

HOMEOBOX1 (ATH1), PENNYWISE and *POUNDFOOLISH* are likely to perform this function redundantly since triple *ath1; pennywise; poundfoolish* mutants lack a shoot apical meristem (Rutjens et al., 2009). However, only slight cotyledon fusion is observed in the triple mutants so there are likely other, as yet unidentified, BLH proteins that cooperate with KNOX proteins to achieve cotyledon separation. The ATH1 gene is required for the correct separation between the abaxial leaf and the stem (Gomez-Mena and Sablowski, 2008). In its absence, the leaf and stem appear nearly continuous. This is the first example of a gene affecting the boundary between stem and organ.

Like *shootmeristemless* and *cupshaped cotyledon* mutants, *wuschel* mutants also fail to develop a shoot apical meristem (Laux et al., 1996). However, there are important differences in their phenotypes. *WUSCHEL* mutants show no indications of cotyledon fusion. The shoot apical meristem develops farther in *wuschel* mutants than in *shoot-meristemless* mutants with more cell divisions occurring at the developing shoot apical meristem in *wuschel* mutants than in *shoot-meristemless* or *cupshaped cotyledon* mutants. Double mutant studies indicate that *shootmeristemless* and *wuschel* mutations act additively suggesting that they affect two distinct pathways (Lenhard et al., 2002). Perhaps a bit surprising given the later manifestation of its phenotype, *WUS* is expressed earlier than the *SHOOTMERISTEMLESS* and *KNAT6* genes. *WUS* is expressed at the 16-cell stage in a group of subepidermal cells in the apical domain of the embryo (Mayer et al., 1998). There are several *WUSCHEL*-like genes (so called *WOX* genes) so it is possible that *WUSCHEL* plays an earlier role in the embryo that is masked by the action of redundantly encoded genes. The establishment of the domain of *WUSCHEL* expression in the embryo does not seem to involve cytokinin activity as the cytokinin response TCS reporter is not expressed in this part of the embryo (Müller and Sheen, 2008).

Patterning the apical domain of the embryo: the role of auxin

An early clue that auxin levels are responsible for promoting cotyledon separation came from experiments on Indian mustard embryos. When globular stage, but not heart stage, embryos were cultured in the presence of polar auxin transport inhibitors, the resulting seedlings exhibit a cup-shaped cotyledon phenotype (Liu et al., 1993). Analysis of mutants deficient in auxin biosynthesis or auxin transport provides additional support for this notion. This work has led to a model in which the cotyledons develop at regions of high auxin concentration while the boundary between them develops at a site of low auxin concentration (Fig. 5).

Three families of proteins have been implicated in auxin transport: the PINFORMED (PIN), P-glycoprotein ABC transporter (PGP) and AUX families. PIN and PGP proteins are thought to be auxin efflux mediators, transporting auxin out of the cell while AUX proteins transport auxin into the cell (see Petrasek and Friml, 2009 for review of auxin transport.). Auxin is transported out of the cell in a polar direction allowing it to accumulate in specific regions of the meristem or the embryo. PIN and PGP-type transporters are redundantly encoded by gene families. Higher order combinations of *pin* and *pgp* mutants show defects in cotyledon development (Vieten et al., 2005; Mravec et al., 2008). A proportion of embryos develop with cup-shaped cotyledons while others develop as large globular shaped embryos and show no evidence of cotyledon emergence. Thus, polar auxin transport is required for proper cotyledon formation.

The detection of fluorescently tagged PIN proteins on one face of the cell allows one to infer the direction of auxin transport. Based on this type of observation, auxin is thought to be transported away from the presumptive shoot apical meristem in the globular stage embryo and, by the heart stage, transported through the protoderm into the developing cotyledons (Fig. 5; Benkova et al., 2003; Petrasek and Friml, 2009). This is consistent with the detection of high levels of auxin in the developing cotyledon primordia based on marker genes such as the DR5 reporter or PIN protein levels (known to increase in response to auxin). It is however difficult to determine the polarity of PIN localization in cells of the globular and early heart stages of embryogenesis (see, for instance, Fig. 5D) making inferences regarding direction of auxin transport direction less robust.

If directional transport of auxin by polarly localized PIN proteins is critical to cotyledon formation, a natural question is "What determines PIN protein localization in the cell?" PIN protein localization is regulated by the AGC kinase PINOID (Christensen et al., 2001; Friml et al., 2004). In the shoot apical meristem, PIN protein localizes to the apical face of the cell and auxin transport is deduced to be upward. In *pinoid* mutants, PIN localization switches from apical to basal in the inflorescence meristem. Thus, PINOID kinase is thought to direct PIN to the apical pole of the cell and therefore determines the polarity of auxin transport. Additional members of this gene family include the PINOID2, WAG1 and WAG2 kinases. Notably, triple mutant *pid1 wag1 wag2* embryos do not make cotyledons (Cheng et al., 2008). The location of PIN protein was not determined in these triple mutants. However, the prediction is that PIN protein is basally located in cells of the triple mutants embryos resulting in a failure to develop high auxin concentrations in the positions of the cotyledon primordia, with the consequence that cotyledons do not develop.

Strikingly, removing *CUPSHAPED COTYLEDON* or *SHOOTMERISTEMLESS* function from embryos defective in auxin transport rescues cotyledon development and outgrowth. Furutani et al. (2004) showed this in *pin1 pinoid* double mutants. These mutants are defective in one of the pin mutant auxin transport facilitator genes (PIN1) as well as in one of the kinases (PINOID) responsible for properly localizing the PIN proteins. In *pin1 pinoid* double mutants, auxin levels are not elevated at the sites of the developing cotyledons and the *CUPSHAPED COTYLEDON* and *SHOOTMERISTEMLESS* transcripts, instead of being restricted to the intercotyledonary stripe, are expanded throughout the top of the embryo. Thus, the entire peripheral region of the apical dome of the globular embryo behaves like the intercotyledonary space in *pin pinoid* double mutants and no cotyledons form. This cotyledonless phenotype can be restored to normal by removing *CUPSHAPED COTYLEDON* or *SHOOT MERISTEMLESS* activity genetically. This indicates that a major role for auxin in cotyledon development is the restriction of *CUPSHAPED COTYLEDON* and *SHOOTMERISTEMLESS* gene products.

Members of the *MACCHI-BOU4/ENHANCER OF PINOID/NAKED PINS IN YUCCA MUTANTS* (*MAB4/ENP/NPY*—this locus will be referred to as *MAB4* here) family of genes interact genetically with mutations in the *PINOID* gene (Treml et al., 2005; Cheng et al., 2007; Furutani et al., 2007). While *mab4* and *pid* single mutant embryos display only mild defects, *mab4 pid* double mutants lack cotyledons and look a bit like match heads. (The name *MACCHI-BOU* means match head in Japanese.). These mutants also display decreased PIN protein in the protodermal layer of the presumptive cotyledons and show reversals in polar localization of PIN proteins in apical regions of the embryo. Thus, *MAB4* and *PIINOID* are both involved in determining the proper localization of PIN proteins.

Interestingly, the PINOID and MAB4 proteins show similarities to the PHOTOTROPIN and NON PHOTOTROPIC HYPOCOTYL3 (NPH3) proteins respectively. PHOTOTROPIN is a blue light responsive kinase that phosphorylates NPH3 (Pedmale and Liscum, 2007). This kinase action is required for the relocalization of PIN proteins responsible for auxin-mediated bending toward light. If the *PINOID MAB4* system works similarly, we would expect PINOID to phosphorylate MAB4 which should then mediate relocalization of PIN protein. Whereas light stimulates the interaction between PHOTOTROPIN and NPH3, the PINOID MAB4 interaction would presumably be stimulated by spatial cues. PINOID lacks the domain that PHOT1 uses to interact with NPH3 so PINOID and MAB4 would have to interact through a different mechanism, perhaps via a third protein.

How are increased auxin levels sensed by the cell? Auxin signaling is mediated through the action of ARF transcription factors. ARF transcription factors bind to IAA repressor proteins which prevent ARF proteins from activating transcription. In response to auxin, the IAA protein is degraded and ARF is released and can activate gene expression from auxin regulated promoters (Dharmasiri and Estelle, 2002; Liscum and Reed, 2002).

Because both ARF and IAA proteins are members of very large gene families it has been difficult to identify the complete set of ARF and IAA proteins involved in organ formation and while a few have come to light, there are likely others still undiscovered.

The *IAA18* gene has recently been implicated in patterning the apical domain of the embryo and specifically in cotyledon development (Ploense et al., 2009). A dominant mutation in *IAA18* that presumably prevents it from being degraded in response to auxin, causes embryos to develop with cotyledon defects. These include fewer cotyledons, extra cotyledons and fused cotyledons. Mutations in the *ARF5* gene, *MONOPTEROS*, cause seedlings to develop with a single cotyledon. In double mutants, the *iaa18 monopteros* mutations cause a cotyledonless phenotype. The mutual enhancement suggests that IAA18 interacts with additional ARF targets and that *MONOPTEROS*/*ARF5* may be targeted by additional IAA proteins. It is known that the ARF7 protein NONPHOTOTROPICHYPOCOTYL4 (NPH4) acts redundantly with MONOPTEROS in cotyledon formation. Mutants homozygous for both *mp* and *nph4* fail to generate cotyledons (Hardtke et al., 2004).

The MONOPTEROS ARF transcription factor binds to the *DORNRÖSCHEN* promoter and is required for *DORNRÖSCHEN* mRNA accumulation in the cotyledon primordia (Cole et al., 2009). *DORNRÖSCHEN* encodes an AP2-type transcription factor. Cole et al. did not observe any additivity or novel phenotypes in *dörnroschen monopteros* double mutants and concluded that the two genes interact in a linear pathway with *MONOPTEROS* acting upstream of *DORNRÖSCHEN*.

Mutations in the TOPLESS corepressor affect apical patterning (Long et al., 2002; Long et al., 2006). Seedlings either completely lack cotyledons and an apical meristem or they exhibit cup-shaped cotyledons. *topless* mutant seedlings express *AINTEGUMENTA* throughout the entire apical domain – in central as well as peripheral regions – and fail to express the *CUPSHAPED COTYLEDON* and *SHOOTMERISTEMLESS* genes. This is consistent with a failure to restrict the auxin response to peripheral regions of the embryo. IAA proteins contain an EAR domain that allows binding to the TOPLESS family of corepressors (Szemenyei et al., 2008). Thus, the observed cotyledonless phenotype of *topless* mutants is likely due to failure to degrade one or more EAR domain containing IAA proteins. *IAA18* and the presumed redundantly acting IAA proteins are good candidates for interactors with the *TOPLESS* family of corepressors.

Arabidopsis *bobber* mutants were isolated in a screen for embryo arrest mutants that showed misexpression of the meristem marker *SHOOTMERISTEMLESS* (Jurkuta et al., 2009). The *bobber* mutation causes expansion of meristem fate across the top of the embryo at the apparent expense of cotyledon fate. *bobber* mutants arrest as globular embryos. The *bobber* mutation causes loss of expression of the primordium-specific markers *AINTEGUMENTA* and causes ectopic expression of the *SHOOTMERISTEMLESS* gene. Unlike the *pin pinoid* double mutants described above, *bobber* mutants cannot be rescued by eliminating *SHOOTMERISTEMLESS* activity. Surprisingly, the bobber phenotype is not accompanied by decreased auxin at the apex but rather by apparently uniform and high levels throughout the globular embryo as determined using the DR5 auxin response reporter gene. The observation that embryos with low levels of auxin and embryos with high levels of auxin both exhibit similar phenotypes suggests that cells read a gradient of auxin concentration rather than an absolute value. The *BOBBER* gene encodes a small protein homologous to the NUDC protein of *Aspergillus* and exhibits chaperone activity in vitro (Jurkuta et al., 2009; Perez et al., 2009). One possible role for the BOBBER protein is to act a chaperone for one or more proteins involved in auxin transport.

Much of auxin transport occurs in the protoderm. Consistent with a role for auxin in cotyledon formation, mutations that disrupt development of the protoderm show cotyledonless phenotypes. For instance, two redundantly acting receptor-like kinases act to promote cotyledon development. These are the *RECEPTOR-LIKE PROTEIN KINASE1 (RPK1)* and *TOADSTOOL2 (TOAD2)* receptor kinases (Nodine et al., 2007; Nodine and Tax, 2008). *rpk1 toad2* double mutants show failure to develop cotyledons, failure to express the organ marker *AINTEGUMENTA* in the presumptive cotyledons, and ectopically express the *SHOOTMERISTEMLESS* marker in the presumptive cotyledons. The double mutants fail to develop high concentrations of auxin in the cotyledon primordia. The *rpk1 toad2* double mutants also show disrupted development of the protoderm as determined by the failure to express the protoderm specific marker *ATML1*. It is possible that this explains the failure to accumulate auxin in the presumptive cotyledon primordia as auxin transport into the developing cotyledons is thought to occur in the protodermal layer. Other genes that regulate the formation of protoderm are also required for the formation of cotyledons. These include the class IV HDZIP genes *ATML1* and *PROTODERMAL FACTOR* (Abe, 2003).

Formation and outgrowth of axillary meristems

Axillary meristems are formed in the axils of leaves, at the junction of leaf and stem (Fig. 1). Many of the same genes involved in development of the primary shoot apical meristem in the embryo are also involved in the development of axillary meristems. These include the class III *HDZIP* genes, the *CUPSHAPED COTYLEDON2* and *3* genes and the *KNOX* genes (Talbert et al., 1995; Hibara et al., 2006; Raman et al., 2008). Other genes, however, appear to be involved specifically in the formation of axillary meristems. These include the *BLIND*/*REGULATOR OF AXILLARY MERISTEM (RAX)* genes and *LATERAL SUPPRESSOR (LAS)* genes (Schumacher et al., 1999; Schmitz et al., 2002; Greb et al., 2003; Keller et al., 2006; Mueller et al., 2006). Like *CUPSHAPED COTYLEDON* genes, *RAX* and *LAS* genes are expressed at the boundary between leaf and meristem where the axillary meristem will form. *RAX* genes are R2R3 myb transcription factor encoding genes that function in both vegetative and floral meristem formation. *RAX* genes are broadly conserved and promote axillary meristem formation in tomato and *Arabidopsis*. *LAS* encodes a GRAS domain transcription factor required to promote axillary meristem formation during vegetative growth. This function is conserved in tomato, where the *LAS* gene was first isolated, in *Arabidopsis* and in rice (Li et al., 2003). *RAX* genes appear to act upstream of the *CUPSHAPED COTYLEDON* genes while the LAS gene acts downstream of *CUPSHAPED COTYLEDON*. The *LATERAL ORGAN FUSION1* and *2* genes are two additional myb transcription factors that are required to promote axillary meristem formation (Lee et al., 2009). These genes are also involved in formation of the intercotyledon boundary during embryogenesis.

While it is generally true that lateral meristems form in the axils of leaves, *Arabidopsis* floral meristems appear to be an exception to this as there are no obvious leaves that subtend the flowers. Closer inspection provides evidence that highly reduced leaves, sometimes called cryptic bracts, subtend and are associated with axillary floral meristems (Long and Barton, 2001; Dinneny et al., 2003). These can be observed histologically by the presence of organ specific markers such as AINTEGUMENTA mRNA. Outgrowth of the cryptic bracts can occur in certain experimental situations. For instance, experiments in which the floral meristem is chemically ablated cause the cryptic bract to develop into a visible bract (Nilsson et al., 1998). This suggests that the floral meristem is responsible for repressing the bract. The PUCHI AP2/ERF transcription factor is expressed in the floral meristem and is required for bract suppression to occur (Karim et al., 2009). Thus,

identification of genes transcriptionally regulated by PUCHI may help to understand the mechanism of bract suppression. The *BLADE-ON-PETIOLE* genes (Norberg et al., 2005) also play a role in suppressing the cryptic bract. The *BLADE-ON-PETIOLE1* and *2* genes are expressed in floral meristems. It is less clear if they are expressed in the cryptic bract and therefore it is unknown whether they act cell non-autonomously or within the bract itself.

Both vegetative and inflorescence meristems of *Arabidopsis* and maize are indeterminate. In tomato and petunia, by contrast, the inflorescence meristem is consumed in the production of a terminal flower (Quinet et al., 2006). Axillary meristem development then becomes critical for the production of additional flowers. The uppermost axillary meristem grows out, producing a few leaves and another terminal flower and the cycle repeats. This type of growth is called sympodial.

Several loci have been identified in *Arabidopsis* that are necessary for the maintenance of inflorescence meristem indeterminacy. One of these is the *TERMINAL FLOWER* gene product (Shannon and Meeks-Wagner, 1991; Ohshima et al., 1997; Conti and Bradley, 2007). The TERMINAL FLOWER gene product is related to the mobile flowering signal, FT and indeed, the TERMINAL FLOWER protein appears to move from a site within the L3 domain of the inflorescence meristem to the upper layers of the meristem. It is likely that it is within these layers that it interacts with an as yet unknown partner to prevent differentiation of the inflorescence meristem central zone. Interestingly, *TERMINAL FLOWER* is also highly expressed in the axillary meristems of plants growing vegetatively under short days. This suggests that *TERMINAL FLOWER* may play a role in axillary meristem development. Grbic and Bleecker (2001) reported an increase in axillary meristems in *terminal flower* mutants suggesting that *TERMINAL FLOWER* may play a role in repressing the development of axillary meristems.

Once formed, the outgrowth of lateral meristems is controlled by the interplay of auxin and the newly discovered strigolactones (reviewed in Dun et al., 2009). Components of the strigolactone system are conserved across several families of plants (McSteen, 2009). Auxin made by the shoot apical meristem travels down the shoot and suppresses outgrowth of meristems. This suppression occurs in a gradient, such that axillary meristems farthest from the apex are released from inhibition first. Strigolactones move in the opposite direction. They are made by the roots and travel upward to repress bud outgrowth. Auxin and strigolactone act outside of the bud to mediate growth suppression. It is not known how this action is transmitted to the bud itself. However, the net effect is that the auxin and strigolactone signals are necessary for repression of bud growth by TCP family transcription factors. The TCP family of transcription factors include the maize *teosinte branched* gene and the *Arabidopsis BRANCHED1* and *2* genes (Doebley et al., 1997; Aguilar-Martinez et al., 2007). The *Arabidopsis BRANCHED* loci repress axillary meristem initiation as well as outgrowth. In the absence of strigolactone signals, *BRANCHED1* and *2* levels are decreased. *BRANCHED1* and *2* are expressed in the bud and act downstream of the *RAX* loci. Experiments in which *TCP* genes were overexpressed or converted into dominant negative forms indicate that TCP transcription factors may act to repress *CUPSHAPED COTYLEDON* gene expression (Koyama et al., 2007).

Phyllotaxy—placement of leaf primordia

The role of auxin in the formation of leaf and floral primordia in the vegetative and inflorescence meristems parallels the role of auxin in cotyledon formation in the embryo. Importantly, high concentrations of auxin within the shoot apical meristem are associated with the formation of leaf primordia. Some of the first clues to this came from experiments in which auxin was applied to the surface of the tomato shoot apical meristem (Reinhardt et al., 2001). This caused outgrowth of primordia at the site of application. However, this was only true for application to positions in the peripheral zone; auxin application to the central zone did not cause leaf primordia to develop. Thus, the peripheral zone but not the central zone is competent to form leaf primordia in response to auxin. It is not known what makes the central zone refractory to auxin action. However, given the recent finding that cytokinin biosynthesis is localized to the apical meristem and, given that cytokinin and auxin act antagonistically to one another, it is possible that high levels of cytokinin in the meristem are in part responsible for this phenomenon.

Reinhardt et al. (2003) observed PIN protein location immunologically in shoot apical meristems and inferred that PIN proteins transport auxin toward the incipient leaf primordium within the shoot apical meristem. Based in part on these experiments, Reinhart et al. proposed a model in which high local auxin concentrations trigger the formation of a new leaf primordium (Fig. 6). Examination of the auxin response reporter DR5 confirms that newly specified leaves occur at positions of higher concentration (Bainbridge et al., 2008).

Increased concentrations of auxin in presumptive leaf primordia depend on polar auxin transport by PIN proteins. In the absence of PIN gene function, the inflorescence meristem is barren, producing no floral meristems (Okada et al., 1991). Similarly, *pinoid* mutants,

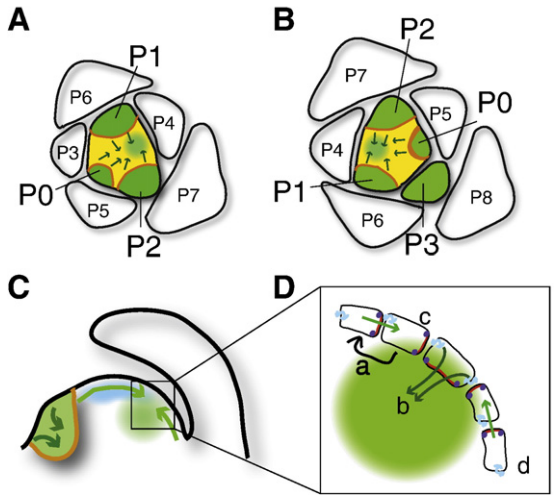

Fig. 6. Model for the control of phyllotaxy in the shoot apical meristem by auxin. (A) Diagram of a transverse section through a vegetative shoot apical meristem. Primordia are labeled from youngest (P0) to oldest (P7). Auxin transport (green arrows) is directed toward the next primordium that will form (fuzzy green circle), causing high local auxin levels to build up. Establishment of the new leaf primordium is associated with changes in gene expression within the primordium (decreased *KNOX* expression, increased *PIN*, *AINTEGUMENTA* and *MONOPTEROS* expression) and the establishment of a boundary separating the adaxial domain of the primordium from the rest of the apical meristem. (B) With the establishment of a new primordium, auxin transport is shifted in the direction of the next primordium. It is not known what causes this shift. It is possible that temporal and spatial fluctuations in PINOID kinase activity may be involved. These fluctuations may occur in response to high and/or depleted levels of auxin. (C) Longitudinal drawing of a vegetative shoot apical meristem showing the flow of auxin in the L1 layer of the shoot apical meristem toward the incipient leaf primordium. Blue area shows central zone, which is refractory to the generation of new primordia in response to auxin. (D) Blowup of boxed region in C. PIN auxin efflux transporters (red) are localized to one face of the cell and direct the flow of auxin in that direction. High auxin in an adjacent cell is thought to feed back on neighboring cells to cause localization of the PIN protein at the "high auxin" end of the cell (a). At the site of convergence of the two counter-oriented auxin flows, auxin changes direction and flows into the primordium (b). This site is congruent with the ad/abaxial boundary of the primordium and with the location of the presumptive midvein. The PINOID KINASE and associated MACCHI BOU4 protein (shown as purple dots) are critical in determining what pole of the cell the PIN protein recycles to (c). (The actual location of the PINOID KINASE and MACCHI BOU proteins is not known. Neither is it known what signals these proteins might respond to.) Auxin influx carriers (light blue) transport auxin into the cell, increasing the efficiency of the flow and strengthening the gradient (d).

produce barren pin-shaped inflorescences (Bennett et al., 1995). Primordium formation on both of these mutants was rescued by application of auxin on the meristem thus bypassing the requirement for PIN and PINOID proteins to achieve high local concentrations of auxin (Reinhardt et al., 2003). In contrast, auxin application could not rescue primordium formation on *monopteros* mutant inflorescences. Monopteros encodes the auxin activated transcription factor ARF5. *MONOPTEROS* is expressed in newly specified leaf primordia in the apical meristem. In its absence, the inflorescence meristem, just as in *pin* and *pinoid* mutants, is barren and devoid of floral primordia. The inability to rescue monopteros mutants is consistent with *MONOPTEROS*/*ARF5* acting downstream of the auxin signal.

The major effect of *pin*, *pinoid* and *monopteros* mutations is on the inflorescence meristem and not on the vegetative meristem. As all of these genes are members of large gene families, it is likely that redundant action of related proteins is responsible for the more normal growth of the vegetative rosettes in these mutants. The inflorescence meristem may be especially sensitive to perturbation in auxin transport since it produces floral primordia at a far faster pace than the vegetative meristem produces leaf primordia (3 flowers per day compared with less than one leaf per day). Consistent with a role for these factors in leaf formation on the vegetative meristem, Schuetz et al. (2008) generated *pin monopteros* double mutants. These produce large leafless domes in the vegetative stage.

The targets of ARF activation and/or repression in the developing leaf primordium are not known. Production of the PIN protein and transcription of the organ specific gene *AINTEGUMENTA* increase in the incipient primordium while *SHOOTMERISTEMLESS* expression decreases (Heisler et al., 2005; Bainbridge et al., 2008). However a direct connection between changes in expression of these genes and ARF gene action has not been made.

Because PIN transporters are continuously endocytosed and recycled back to the plasma membrane, it is possible for these proteins to be reapportioned from one cell face to another and thus change the direction of auxin flow (Petrasek and Friml, 2009). Heisler et al. (2005) observed living inflorescence meristems carrying fluorescently tagged PIN1 proteins and inferred the direction of auxin transport over several plastochrons. In this case, live imaging is made easier since the PIN transporters are principally found in the L1 layer of the meristem (Fig. 6). Auxin flow is in the direction of the incipient primordium. Once this primordium reaches a critical stage, PIN proteins relocalize and auxin is directed toward the site where the next primordium will form.

What, then, determines where PIN proteins are localized? Classical theories of “canalization” hold that auxin feeds back on its own transport such that regions with high auxin flux attract additional auxin flux, thus reinforcing these pathways of auxin transport (Rolland-Lagan and Prusinkiewicz, 2005). Feedback by auxin on its own transport can be seen as increased auxin induces synthesis of PIN transporters and also increases the proportion of PIN protein found at the cell surface (Petrasek and Friml, 2009). To date, the best candidates for controlling PIN polarity are *PINOID* type kinases and the associated MAB4-like proteins (Furutani et al., 2007). Cell specific transcription and/or activation of these could have the potential to shift the location of PIN proteins within the cell and thus reverse the gradient. Such cell specific changes could occur in response to critically high thresholds of auxin (in the new primordium) or critically low thresholds of auxin (in the position furthest from the new primordium).

Heisler et al. (2005) noted that shifts in the direction of auxin transport are temporally and spatially correlated with the establishment of the boundary between the adaxial domain of the new primordium and the meristem. Thus, substances made at the leaf-meristem boundary might play a role in reversing auxin transport. For this reason, misexpression of boundary-expressed genes might be expected to alter phyllotaxis.

Misexpression of *CUPSHAPED COTYLEDON* genes does indeed alter phyllotaxis but, surprisingly, this is not due to changes in the pattern of primordium initiation. Dominant *CUPSHAPED COTYLEDON2* mutations that prevent *CUC2* mRNA from being recognized by mir164 show phyllotactic abnormalities in inflorescences (Peaucelle et al., 2007). Triple mutants defective in the genes for microRNA164a, microRNA164b and microRNA 164c show similar phyllotactic abnormalities (Sieber et al., 2007). These mutants cause increased and broadened expression of *CUPSHAPED COTYLEDON* mRNAs. Surprisingly, when the inflorescence meristems in these mutants are examined, there is no evidence of primordia forming in the wrong positions. However, there are significant differences in growth of the primordia after they emerge from the meristem. This occurs both in the vertical dimension, resulting in shortened internodes, and in the radial dimension, resulting in primordia that show a smaller angle of divergence. Consistent with this interpretation, the ectopic expression of *CUPSHAPED COTYLEDON* mRNA is closely correlated with growth retardation resulting in fewer and smaller cells. These studies emphasize that apparent alterations in phyllotaxy need not act at the stage of primordium initiation.

Misexpression of the boundary-expressed *JAGGED LATERAL ORGANS (JLO)* gene has consequences that suggest that it could play a role in shifting the direction of auxin transport (Borghi et al., 2007). *JLO* encodes a putative transcription factor of the *LATERAL ORGAN BOUNDARY* family. When an inducible form is activated, *SHOOTMERISTEMLESS* mRNA is increased and PIN auxin transporter mRNA is decreased. A dominant negative form of *JLO* disrupts the formation of interorgan boundaries causing inappropriate outgrowth of cotyledonary lobes. Thus, *JLO* is a boundary-expressed gene that is responsible for lowering PIN activity and maintaining *SHOOTMERISTEMLESS* levels high. By lowering PIN activity, *JLO* could potentially contribute to auxin flow away from the boundary. Null *jlo* mutants arrest as globular stage embryos; no cotyledon primordia emerge. Because arrest at the globular stage is a common phenotype and therefore has many causes, it is still not known if the failure of cotyledon primordia emergence is due to alterations in auxin or *KNOX* activity.

Until recently, auxin influx carriers were not thought to play a critical role in contributing to polar auxin transport. In part, this is because auxin is thought to be able to enter cells passively. However, when Bainbridge et al. (2008) analyzed a quadruple mutant defective for four members of the AUX family of influx carriers, they found that these mutants showed substantial defects in phyllotaxis. These defects were accompanied by a loss of sharp peaks of auxin accumulation in the shoot apical meristem. The authors suggested that the AUX carriers are necessary for efficient uptake of auxin into cells, resulting in higher auxin concentrations in adjacent cells that can feedback to adjacent cells to increase the amount of polarly localized PIN protein (Fig. 6).

Mutations in orthologs of genes in the auxin transport pathway have been identified in the grasses and these cause similar phenotypes indicating that much of this pathway is conserved (McSteen, 2009). However, it is also true that patterns of phyllotaxis vary and that a given pattern is characteristic of a species and therefore genetically determined. What the genetic factors are that contribute to the stabilization of particular phyllotactic patterns remains to be determined.

Environmental control of meristem growth and development

Most research on development of the shoot apical meristem assumes an endogenous pathway of regulation with little input from the environment. It seems highly likely, though, that development at the shoot apical meristem is influenced by the overall nutritional status of the plant. Changes in growth rates would presumably require coordinated changes between the various zones of the meristem. If

this is true, we would expect to find mutations in genes responsible for modulating meristem growth and development in response to the environment. Such mutations should cause mutant phenotypes that are dependent on environmental conditions.

Interestingly, the phenotype of *clavata* mutants depends on day length, indicating that the *CLV* signaling pathway is required to mediate growth control under distinct environmental conditions (Müller et al., 2008). Loss-of-function mutations in the *CLAVATA1*, *CLAVATA2*, *CLAVATA3* and *CORYNE* genes all show more severe phenotypes in long days than in short days. Mutant floral meristems all made more carpels under continuous light (24 h light) or long days (16 h light) than under short days (8 h light). An increase in carpel number reflects an increase in central zone size. Thus, the requirement for *CLAVATA* signaling is more stringent the longer the day. It is not clear whether this is due to the length of the day *per se* or to the amount of total light the plants receive. If it is the latter, the plants exposed to more light may produce more photosynthate which could allow for more rapid growth. The *CLAVATA* signaling pathway then might play a role in curbing growth in the floral meristem under energy rich conditions so that it is compatible with the formation of a flower with a specific number of floral organs.

The identification of several genes that act in the leaf to influence meristem activity (i.e. the *Arabidopsis YABBY* genes and the petunia *HAIRY MERISTEM* gene) provides evidence for the existence of as yet unknown signals made in the leaf and sensed by the meristem. It is likely that similar routes are taken by signals sent from the leaf to the meristem that report on the local environment and status of the leaf.

The TCP genes that repress axillary meristem development in *Arabidopsis* and maize are thought to be sensitive to environmental signals (Hubbard et al., 2002; Aguilar-Martinez et al., 2007). This could allow the plant to branch more extensively in response to a rich environment. Much remains to be learned about the stimuli and responses that activate the meristems. With the advances that have occurred both in the area of environmental signaling and in plant development, this is an area that seems poised to make substantial progress in the coming years.

The next 20 years

The last two decades have seen an explosion in our knowledge of shoot apical meristem structure and function, especially in a select few model organisms. Molecular domains expressing unique combinations of genes within the meristem have been coarsely mapped. Major pathways – CLV/WUS; KNOX/cytokinin; auxin flux/phyllotaxy; the ad/abaxial network – regulating stem cell activity, leaf initiation and meristem formation have been identified. We have in our possession a burgeoning basket of transcription factors and chromatin remodelers that regulate meristem development. The roles of old friends from classical plant physiology – cytokinin and auxin – are being exposed, revealing sophisticated mechanisms for signal distribution and transduction in the shoot apical meristem.

What will the next two decades bring? Developments in recent years certainly provide hints of exciting days to come. New signaling molecules and mechanisms of signal transduction will be discovered. Live, real-time imaging will continue to improve allowing us to visualize molecules in their native environment and to determine the immediate consequences of experimental manipulations. High throughput approaches using next generation DNA sequencing will allow us to exhaustively enumerate transcriptional targets; to profile transcriptomes and their changes at the single cell level; and to establish new plant species as experimental models. The continued development of powerful *in situ* proteomic techniques will allow us to understand the biochemistry of the spatially complex meristem. We will achieve a complete understanding of the regulatory networks operating in the meristem, and how these interact with other networks in the plant. We will construct networks in a spectrum of plants to understand the diversity and evolution of regulatory networks. We will understand how environmental variables are sensed by and influence the growth and development of the shoot apical meristem. The challenge will be to use this knowledge to improve our management of croplands and wildlands. These are critical obligations for scientists living on a small and increasingly crowded planet and an urgent charge for the next generation of researchers.

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