6 Phyllotaxis in higher plants

Didier Reinhardt and Cris Kuhlemeier

6.1 Introduction

In plants, the arrangement of leaves and flowers around the stem is highly regular, resulting in opposite, alternate or spiral arrangements. The pattern of the lateral organs is called phyllotaxis, the Greek word for 'leaf arrangement'. The most widespread phyllotactic arrangements are spiral and distichous (alternate) if one organ is formed per node, or decussate (opposite) if two organs are formed per node. In flowers, the organs are frequently arranged in whorls of 3–5 organs per node. Interestingly, phyllotaxis can change during the course of development of a plant. Usually, such changes involve the transition from decussate to spiral phyllotaxis, where they are often associated with the transition from the vegetative to reproductive phase.

Since the first descriptions of phyllotaxis, the apparent regularity, especially of spiral phyllotaxis, has attracted the attention of scientists in various disciplines. Philosophers and natural scientists were among the first to consider phyllotaxis and to propose models for its regulation. Goethe (1830), for instance, postulated the existence of a general 'spiral tendency in plant vegetation'. Mathematicians have described the regularity of phyllotaxis (Jean, 1994), and developed computer models that can recreate phyllotactic patterns (Meinhardt, 1994; Green, 1996).

It was recognized early that phyllotactic patterns are laid down in the shoot apical meristem, the site of organ formation. Since scientists started to postulate mechanisms for the regulation of phyllotaxis, two main concepts have dominated the field. The first principle holds that the geometry of the apex, and biophysical forces in the meristem determine phyllotaxis (van Iterson, 1907; Schiiepp, 1938; Snow and Snow, 1962; Green, 1992, 1996). Alternatively, signal molecules have been proposed to regulate phyllotaxis (Schoute, 1913; Veen and Lindenmayer, 1977; Mitchison 1977; Schwabe, 1984). Recently, the latter view has gained support from studies that analyzed phyllotactic patterning under conditions of perturbed auxin transport (Reinhardt *et al.*, 2000; Vernoux *et al.*, 2000). Based on these and previous studies, we propose that auxin is the trigger of organ formation. Polar auxin transport determines the site of organ formation by regulating auxin distribution in the meristem. A model for the role of primordia in auxin distribution is proposed to account for the reiterative nature of phyllotaxis.

6.2 Phyllotactic patterns in plants

Spiral phyllotaxis is the most common arrangement of leaves in flowering plants and ferns. One leaf is formed per node, with the divergence angle between successive leaves approaching the Fibonacci angle of 137.5° (Steeves and Sussex, 1989; Jean 1994; Lyndon, 1998). Many important model plants in which phyllotaxis has been studied (for example, *Arabidopsis,* tomato, tobacco, lupin, sunflower, poplar) exhibit spiral phyllotaxis. More ancestral plants, such as gymnosperms (for example, *Araucaria, Pinus* and *Ginkgo),* as well as ferns *(e.g. Dryopteris* and *Osmunda)* also have spiral phyllotaxis. Given that most of the theories on the regulation of leaf arrangement have been developed in spiral systems, the present review focuses mainly on this form of phyllotaxis.

The sequence in which organs of spiral plants are formed is called the generative spiral. In addition, the arrangement of lateral organs can be described with sets of spirals, called contact parastichies, that run in opposing directions and intersect in the centers of the primordia (figure 6.1) (Steeves and Sussex, 1989; Jean, 1994; Lyndon, 1998). The numbers of spirals that can be observed in each direction in a given spiral system are represented by consecutive numbers in the Fibonacci series, in which each number is the sum of the two preceding numbers, i.e. 1, 1, 2, 3, 5, 8, 13, 21, etc. For example, in the spiral depicted in figure 6.1A, 13 spirals can be drawn from the center in clockwise direction, whereas 21 spirals can be observed in counterclockwise direction, therefore this spiral system exhibits a (13 + 21) phyllotactic pattern.

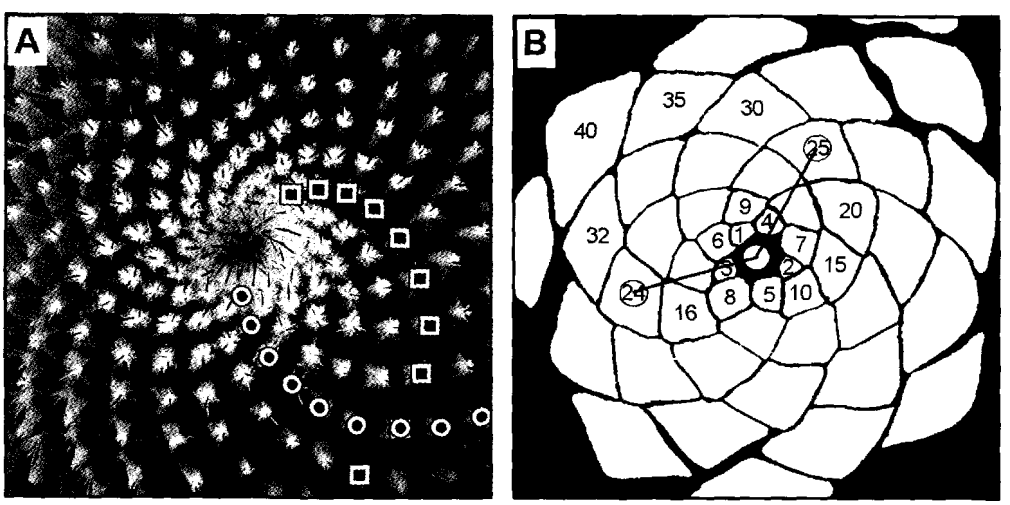

Figure 6.1 Spiral patterns in plants. **A: A** cactus exhibiting **a** (13 + 21) spiral pattern. One respective parastichy in each direction is represented by circles and squares. B: Schematic representation of a *(5*+8) spiral pattern. The series of leaf formation is shown with consecutive numbers, with 1 representing the youngest priniordium. The divergence angle is the angle between successive primordia (represented by black lines from primordia 24 and 25 to the center). The plastochron ratio is the ratio of the relative distances of consecutive primordia from the center (e.g. distance of 25 divided by distance of 24). (After Church, 1904.)

The principal parameters that determine the phyllotactic pattern are the divergence angle (DA) between successive leaves and the plastochron ratio (PR) (figure 6.1B) (for review, see Richards, 1951; Steeves and Sussex, 1989; Callos and Medford, 1994; Jean, 1994). The divergence angle is defined as the smallest angle between successive leaves (e.g. in figure 6.1B it is 134° between leaves 24 and 25). The PR is defined as the ratio between the distances of successive primordia from the meristem center (e.g. in figure 6.1B, the PR can be given as the distance of leaf 25 from the center divided by the distance of leaf 24 from the center). The PR is therefore a measure of the radial expansion of the shoot apex per plastochron. Since the divergence angle is more or less constant, phyllotactic patterns in spiral plants are mostly determined by the PR. The PR is strongly influenced by the shape and the size of primordia relative to the meristem. If the organs are small relative to their distance from the meristem center, the PR will be close to 1, and a spiral pattern with high numbers of parastichies will be generated (Williams, 1975; Jean, 1994). For example, in the sunflower capitulum which forms small floret primordia, a phyllotactic pattern of up to (55 + 89) can be observed (Jean, 1994). In contrast, a potato vegetative meristem forms relatively large leaf primordia, leading to a PR of approximately 1.3, and a spiral pattern of (2 + 3) (Steeves and Sussex, 1989).

Box 6.1 Mathematical models of phyllotaxis

The mathematical analysis of spiral phyllotaxis describes the arrangement and packing of the developing organs around the shoot axis and it emphasizes the regularity and precision of the patterns (Richards, 1951; Erickson and Michelini, 1957; Steeves and Sussex, 1989; Callos and Medford, 1994; Jean, 1994). However, if mathematical models focus on the geometry of the arrangement without considering the underlying cellular and molecular mechanisms, they remain descriptive, and their contribution to the elucidation of phyllotaxis can only be limited. In contrast, mathematical models that envisage chemical or physical principles in the regulation of phyllotaxis (Mitchison, 1977; Veen and Lindenmayer, 1977; Meinhardt, 1994; Green, 1996) are valuable tools, since they make predictions that can be tested experimentally (see sections 6.5 and 6.7).

In distichous or alternate phyllotaxis, one leaf is formed per node with a divergence angle of 180°, resulting in two rows of alternating leaves. Distichous phyllotaxis is characteristic of the grasses, among which the best-studied example is maize (Sharman, 1942, 1947; Veit *et al.*, 1998; Jackson and Hake, 1999). *Pisum sativum* is an example of a dicotyledonous plant with distichous phyllotaxis, in which leaf formation has been studied in detail (Lyndon, 1998).

In whorled phyllotaxis, two or more leaves are formed per node. The simplest case of whorled phyllotaxis is represented by decussate plants. In

decussate or opposite phyllotaxis, whorls of two leaves are formed per node, with each positioned at opposite sides of the stem. The divergence angle between successive leaf pairs is 90°. Decussate phyllotaxis is characteristic of the Lamiaceae (e.g. *Coleus, Mentha* and *Salvia).* Other examples of decussate genera are *Antirrhinum, Anagallis, Hypericum, Vinca* and *Acer.* Interestingly, some decussate plants, such as *Salvia,* retain the decussate pattern throughout their entire life span, whereas others such as *Antirrhinum* undergo a transition to spiral phyllotaxis after induction of flowering (see section 6.3).

After decussate, the next highest order whorled system is tricussate (trimerous) phyllotaxis, in which three leaves are formed per node (e.g. *Nerium oleander).* These leaves are positioned symmetrically at 120° from each other, and the divergence angle between whorls of successive nodes is 60°. Up to ten leaves per node are formed by plants of the genus *Galium* (e.g. *Galium verum).* As a general rule, the leaves in whorled systems are formed above the gaps between the leaves of the preceding whorl.

Flowers commonly exhibit whorled phyllotaxis. The standard flower consists of four concentric whorls, the first (outermost) whorl consisting of sepals, the second whorl consisting of petals, the third whorl consisting of stamens, and the fourth (innermost) whorl consisting of carpels (for a review of flower anatomy see Weberling, 1989). Interestingly, the sepals of angiosperm flowers usually have a different ontogeny from the petals. The sepals are formed succesively in a spiral sequence, but with little vertical growth between them, therefore resulting (secondarily) in a whorled arrangement. In contrast, the petals of most plants initiate more or less simultaneously, and thus constitute true whorls (Endress, 1994). Normally, the number of organs per whorl is strictly regulated, resulting, for example, in trimerous phyllotaxis in flowers of the Liliaceae, in quadrimerous phyllotaxis in the two outer whorls of crucifer flowers, such as *Arabidupsis,* and pentamerous phyllotaxis in the two outer whorls of flowers in Rosaceae and Solanaceae.

6.3 Changes in phyllotaxis

Although phyllotactic systems are remarkably stable, the phyllotactic patterns can change. Every dicotyledoneous plant starts as a bilateral symmetric embryo with a pair of cotyledons. Therefore, the initial phyllotaxis of dicotyledonous plants can be considered to be decussate. Many spiral plants, such as *Arabidupsis,* form a first pair of true leaves more or less simultaneously at opposite sides of the SAM with a divergence angle to the cotyledons of approximately 90° as in decussate phyllotaxis. *Arabidopsis* seedlings then gradually turn to spiral phyllotaxis (Medford *et al.,* 1992). When spiral phyllotaxis has been established, the plastochron ratio usually decreases during the course of development (Williams, 1975; Erickson and Meicenheimer, 1977). This is mainly due to the increasing

diameter of the apex. The decreasing plastochron ratio, then causes the switch to higher order spiral patterns. For example, in flax, the leaf arrangement changes from a (3+5) to a (5+8) spiral pattern (Williams, 1975).

Decussate plants maintain the initial pattern throughout vegetative growth, and sometimes during their entire life cycle (e.g. Lamiaceae). However, many decussate plants, such as snapdragon *Antirrhinum majus)* or *Epilobium* change to spiral phyllotaxis during the transition to flowering (Meicenheimer, 1982). Interestingly, the genus *Helianthus* exhibits both, with members that remain decussate during the entire vegetative phase (e.g. *Helianthus microcephalus* and *Helianthus tuberosus)* and only turn to spiral phyllotaxis at the onset of flowering, while other members establish spiral phyllotaxis after formation of a few leaf pairs (e.g. *Helianthus annuus).* Therefore, decussate and spiral phyllotaxis may in some cases share a common basis, and only the timing of transition may distinguish them.

Besides the developmental changes in phyllotaxis, spontaneous changes can occur in nature. Occasionally, decussate plants produce a supernumerary organ per whorl in several successive whorls, resulting in stable tricussate phyllotaxis (figure 6.2). This can involve only a branch (in woody plants, such as Acer, *Cornus, Ailanthus)* or a whole plant (e.g. *Helianthus tuberosus).* The tendency to form supernumerary organs is also found in flowers of some plant species, for example in tomato, which occasionally forms flowers with six petals instead of five, or in tulips, where flowers with eight (2 x 4) instead of six (2 x 3) perigon leaves can be found (Reinhardt and Kuhlemeier, unpublished).

Numerous mutations have been described that lead to changes in phyllotaxis (see section 6.6). Changes in phyllotaxis can also be evoked with chemical growth regulators and by physical interference with the meristem (see section 6.7).

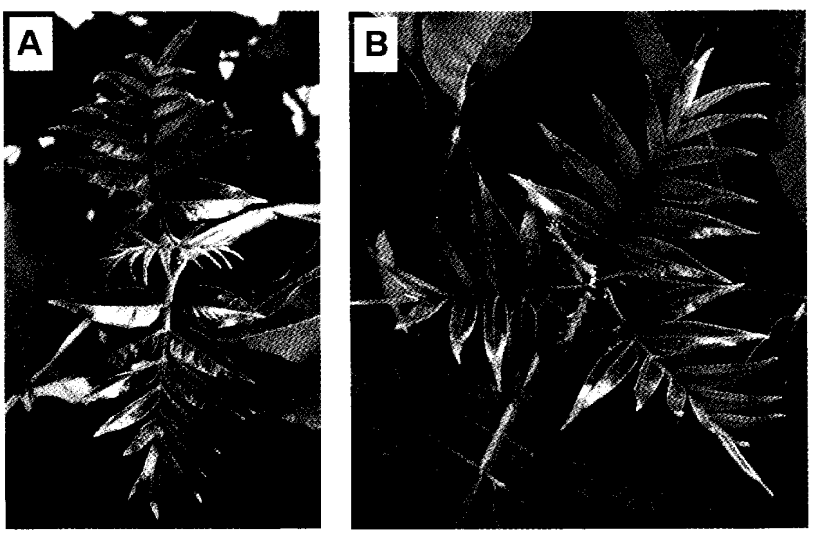

Figure 6.2 Spontaneous switch of phyllotaxis in a decussate plant. A: Decussate branch of an *Ailanthus altissima* tree. B: Tricussate branch of the plant shown in **(A).**

6.4 Anatomy and function of the shoot apical meristem

Leaves and flowers are initiated at the distal tip of the shoot in a specialized structure, the shoot apical meristem **(SAM)** (Steeves and Sussex, 1989; Medford, 1992; Lyndon, 1998; see also figure 6.3A). In addition to its function in lateral organ formation, the **SAM** continuously produces stem tissues and maintains a pool of undifferentiated cells for self-perpetuation. Because the patterns of leaves and flowers that are ultimately revealed on the developing plant are laid down in the meristem, in order to understand the basis of phyllotactic regulation, it is necessary to be familiar with the anatomy and function of the **SAM.** The anatomy of the meristems of higher plants exhibits some variability (Popham, 1951), but meristems of most plants share several common features that are summarized below. Based on functional, histological and cellular criteria, meristem organization has been described with models of cellular layers and with models of zonation, and these have been described in some detail in chapter 2 of this volume.

6.4.1 The layers the meristem

The meristem of most higher plants exhibits a layered organization (figure 6.3B) (Steeves and Sussex, 1989). The superficial layer, called L_1, represents the epidermis of the meristem. The cells in L_1 divide anticlinally, that is, the new cross walls are oriented perpendicularly to the surface of the meristem. The L_1 gives rise to the epidermis of the entire shoot, including the stomates and

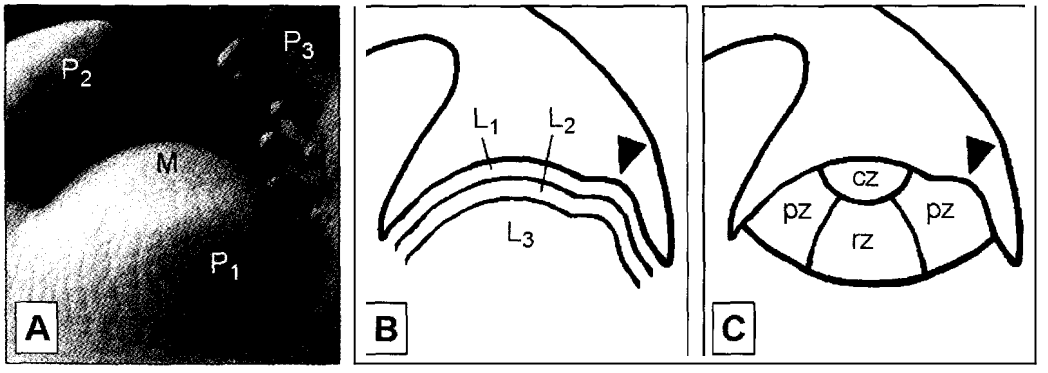

Figure 6.3 Organization of the shoot apical meristem **(SAM). A:** Tomato apex showing the shoot apical meristem (M) and the three youngest primordia (P1-P3) in clockwise spiral phyllotaxis. The youngest primordium is just being initiated at the flank (towards the viewer), the second youngest is visible on the left. B: Schematic representation of a tomato apex as in **(A),** showing the layers of the meristem (L_1, L_2 and L_3). All three layers contribute to the youngest primordium that bulges out at the right side of the flank (arrowhead). C: Schematic representation of a tomato apex as in (A), showing the zonal organization of the meristem. The central zone (cz) is located at the summit of the meristem. The peripheral zone (pz) surrounds the cz, forming a ring-shaped region adjacent to the cz. The rib zone (rz) is located immediately below the cz (and interior to the pz). A primordium is initiated from the right side of the peripheral zone (arrowhead).

trichomes. The cells in the subepidermal layer, L_2, divide mostly anticlinally like L_1, however, it has been observed that cells in L_2 divide periclinally early in the process of organ initiation (Lyndon, 1998). The L_2 layer produces the mesophyll, part of the ground tissues, and gives rise to the sporogenic tissues in the flowers. Thus, the products of meiosis are derived from L_2, that is, the genotype of L_2 cells determines the genotype of the gametes, whereas the genotype of L_1 and L_3 are not transmitted to the next generation. In the L_3 layer, the basal portion of the **SAM,** cell division has no preferred orientation. The tissues derived from L_3 give rise to the central tissues in stems and in lateral organs. Traditionally, the outer (superficial) cell layers (L_1 and L_2) that divide mostly anticlinally have been called the 'tunica' (the latin name for the roman shirt-like dress), whereas the core of the meristem is referred to as 'corpus' (the latin word for body) (Steeves and Sussex, 1989). For further discussion on meristem organization, refer to chapter 2 of this volume).

6.4.2 The zones of the meristem

Leaves are never initiated in the center of the meristem but always on its flank. Therefore, the meristem can be subdivided along its apical-basal axis into a central zone (cz), which remains undifferentiated, and a ring-shaped peripheral zone (pz), in which organ formation occurs (figure 6.3C) (Steeves and Sussex, 1989). Interior to the pz and below the cz, the rib zone (rz) is located; in this region, the central tissues of the stem are formed. The concept of a functional zonation in central and peripheral zone is also reflected by several cytological, histological and molecular properties of the meristematic regions.

6.4.2.1 Cell division activity and cytological features

Cell division frequency is usually lower in the center than in the periphery of the meristem (Steeves and Sussex, 1989; Lyndon, 1998; Laufs *et al.*, 1998b). Based on this finding, the concept of a 'méristème d'attente' has been developed (Buvat, 1952; reviewed in Steeves and Sussex, 1989). It assumes that the 'méristème d'attente', which roughly corresponds to the CZ does not give rise to the cells for leaf and stem growth, but that cell divisions in the 'anneau initial' (corresponding to the PZ) are the only source of new cells. The 'méristème d'attente' is pushed upward by the growing stem, and, only in flowers, it becomes activated to form the inner floral organs.

In an opposing view, the *CZ* is seen as a pool of undifferentiated cells harboring the apical initials. Due to the slow but incessant proliferation of these apical initials, their descendants are continuously displaced to the flank, where they become available for organ formation (Clark, 1997; Meyerowitz, 1997; Laufs *et al.,* 1998c).

The concept of a méristème d'attente' has been criticized for two main reasons. Firstly, although cells in the CZ divide less frequently than in the

periphery, mitoses still occur, and it has been estimated that the frequency of cell division is sufficient to replenish the periphery with cell material for organogenesis (Stewart and Dermen, 1970). Secondly, mutant sector analysis has shown that, in rare cases, a single somatic mutation event in the meristem can lead to a sector that comprises the entire shoot circumference (Furner and Pumfrey, 1992). In such an extreme case, the entire affected part of the shoot can be traced back to one single cell in the center of the meristem. Therefore, it is likely that all the mature plant tissues ultimately derive from cells in the meristem center. Although the cells in the CZ have been compared to stem cells in animals (Steeves and Sussex, 1989), there are also prominent functional differences. In animals, stem cells are set apart early in embryogenesis, and in the adult animal, their function is restricted to the replacement of tissues. In contrast, 'stem cells' in plants are involved in growth and morphogenesis during the entire life cycle of the plant.

In many cases, the cells in the meristem center are larger than the ones in the periphery. They are more vacuolated and exhibit various differences in subcellular organization, reflected in a difference in affinity to staining agents Nougarède, 1967; Steeves and Sussex, 1989). The differences in cellular organization may be associated with different metabolic activities or different cell division activity in the CZ and PZ.

6.4.2.2 Gene expression

Results from *in situ* hybridization studies have revealed that the expression of many genes is not uniform within the meristem. Genes involved in the cell cycle usually exhibit a punctate expression pattern, which corresponds with the distribution of dividing cells (Brandstatter *et al.,* 1994; Fobert *et al.,* 1994, 1996; Segers *et al.,* 1996). Genes for the lipid transfer protein (LTP) are expressed only in the L_1 layer (Fleming *et al.,* 1993; Sessions *et al.,* 1999). Other genes are expressed in a pattern consistent with the zonation model, that is, they are upregulated in the center or at the flank (Fleming *et al.,* 1993; Nishimura *et al.,* 1999). However, gene expression patterns in the meristem can also vary during the course of development (Fleming and Kuhlemeier, 1994).

In recent years, various genes with a role in meristem function have been identified (see also section 6.6). In many cases, the analysis of gene expression has revealed specific expression patterns within the meristem that reflected the zones of the meristem. *SHOOTMERISTEMLESS (STM),* which determines meristem identity, is expressed throughout the meristem except for the sites of incipient organ formation (figure 6.4A, G) (Long *et al.,* 1996; Long and Barton, 2000). *CLAVATA3 (CLV3)* and *CLAVATA1 (CLV1),* two genes that regulate the meristem size (Fletcher and Meyerowitz, 2000; see also section 6.6), are expressed in subdomains of the meristem center (figure 6.4B, C) (Fletcher *et al.,* 1999). Whereas *CLV3* is expressed mainly in the tunica, *CLV1* is expressed below *CLV3,* mainly in L_3. The *WUSCHEL (WUS)* gene which controls meristem

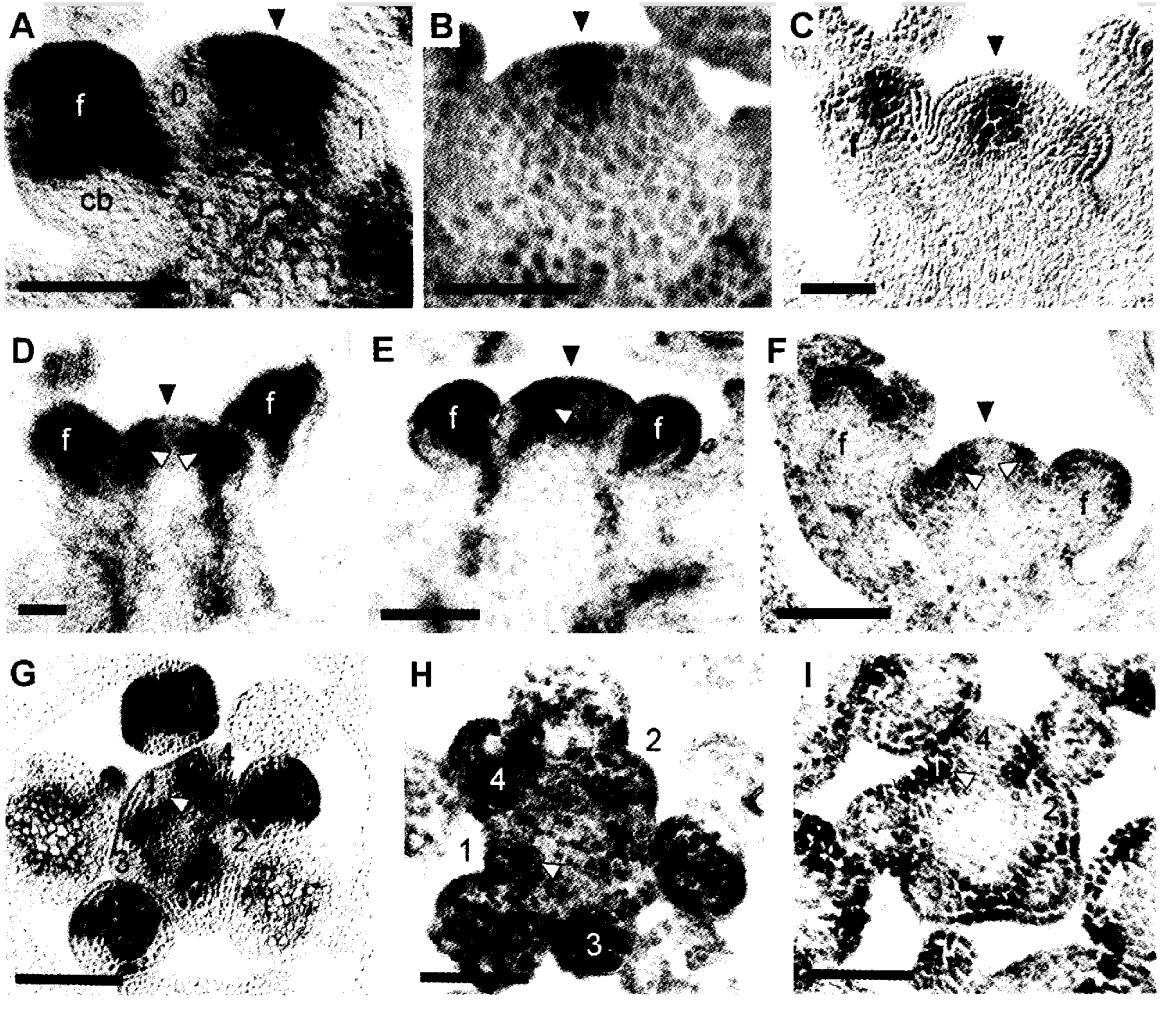

Figure 6.4 Expression patterns of meristem genes in floral apices of *Arabidopsis.* **A:** *SHOOT-MERISTEMLESS (STM)* is expressed throughout the inflorescence (arrowhead) and flower meristem **(f),** but excluded from young and incipient flower primordia (1 and 0, respectively). In the lower part of the flower primordium **(f),** *STM* is repressed in a patch that is interpreted as the cryptic bract (cb). (Reproduced with permission from Long and Barton, 2000.) B: *CLAVATA3 (CLV3)* is expressed in the meristem centre in L1 and L_2, and, to a lesser extent, in L_3. (Reproduced with permission from Fletcher *et al.,* 1999.) C: *CLAVATA1 (CLVZ)* is expressed in the meristem centre in L_2 and L_3, but not in L_1. In L3, the expression domain reaches deeper than *CLV3.* In flower meristems **(f),** *CLV1* expression is similar to that in the inflorescence meristem. (Reproduced with permission from Christensen *et al.,* 2000.) D: *MONOPTEROS (MP)* is expressed in the organogenic region at the flank of the inflorescence meristem (white arrowheads). In developing flowers (f), *MP* is expressed at the site of organogenesis in the inner whorls. (Reproduced with permission from Hardtke and Berleth, 1998.) E: *PIN-FORMED1 (PIN1)* is expressed at peripheral sites in the inflorescence meristem (white arrowhead), and in the center of developing flowers (f), in **a** similar pattern to expression of *MP.* (Reproduced with permission from Vernoux *et al.,* 2000.) F: *PINOID (PZD)* is expressed at the flank of inflorescence meristems (white arrowheads). In young flower primordia **(f),** *PZD* is also expressed at the flank, and at later stages in developing floral organs. (Reproduced with permission from Christensen *et al.,* 2000.) *G:STM* expression reflects phyllotactic pattern in the inflorescence. *STM* is downregulated (arrowhead) at the site of flower initiation (1), and in developing flower primordia (2 4). (Reproduced with permission from Long and Barton, 2000.) H: *PINZ* is induced (arrowhead) at the site of organ initiation and in developing flower primordia (2-4). (Reproduced with permission from Vernoux *et al.,* 2000.) **I:** *PZD* is induced (arrowhead) at the site of organ initiation. (Reproduced with permission from Christensen et *al.,* 2000.) **A** to F: longitudinal sections; G to I: transverse sections; **(A** to I: black arrowheads point to the center of the inflorescence meristem; **A** to I: Internal scale bars = 50 μm.)

maintenance is expressed, like *CLV1*,in the central region of L_3 (Mayer *et al.*, 1998; Schoof *et al.*, 2000j. Genes involved in organogenesis, such as *Monopteros, Pin-formedl* and *Pinoid* (see also section 6.6),are expressed in the periphery and in young primordia (figure 6.4D-F) (Hardtke and Berleth, 1998; Christensen *et al.*, 2000; Vernoux *et al.*, 2000). Thus, it appears that genes that control meristem perpetuation are expressed in the central zone, whereas genes involved in organogenesis are expressed in the peripheral zone. Interestingly, genes that are expressed in the pz respond to phyllotactic patterning information, for example, *STM* is repressed at the site of organ formation (figure 6.4G; Long *et al.*, 1996, Long and Barton, 2000). In contrast, genes that are required for organ initiation are induced at the site of organ formation (figure 6.4H and I) (Hardtke and Berleth, 1998; Christensen *et al.*, 2000; Vernoux *et al.*, 2000).

6.5 Models for the regulation of phyllotaxis

Organs are always formed at the flank of the meristem at a characteristic distance from the summit, suggesting that some mechanism prevents organogenesis above or below this region. In the radial dimension, organs are formed at characteristic angles from each other, resulting in the phyllotactic arrangements discussed previously. The stunning regularity in phyllotactic arrangements has attracted the attention of many scientists, who have attempted to explain the phenomenon (Church, 1904; van Iterson, 1907; Richards, 1951; Cutter, 1959; Mitchison, 1977; Veen and Lindenmayer, 1977; Jean, 1994; Meinhardt, 1994, 1996; Green, 1996). The principal questions are: 1j How is the site of organ formation selected? 2) How are organs formed? **3)** How do pre-existing organs influence the position of new organs? The models for phyllotaxis fall broadly into two categories, namely biophysical and biochemical models, depending on whether the main principle of regulation is envisaged to be of a physical or chemical nature. Although both schools propose different mechanisms of regulation, they both agree with the widely accepted notion that pre-existing primordia influence the sites of future organ formation.

6.5.I Biophysical models

Various theories of phyllotaxis assume that organogenesis is regulated merely by the geometry of the apex and by tensile and compressive forces that act on the meristem surface. Since these theories attempt to explain phyllotaxis only within the physical parameters of the apex, they are referred to as biophysical models of phyllotaxis. Three of them are discussed here because they have received

particular attention, and are also relevant for the experiments discussed later.

- The 'theory of the first available space' states that the timing and positioning of organogenesis is regulated by the availability of a minimal free area on the meristem surface with a minimal distance from the summit and from pre-existing primordia. According to this model, which was first formulated by van Iterson (1907), the geometry of the apex is sufficient to explain phyllotaxis. The simplicity and elegance of this model has stimulated many experiments (Snow and Snow, 1931, 1933, 1962; Steeves and Sussex, 1989; see also section 6.7).
- Two further biophysical theories are based on the idea that differential growth between the tunica layer and the corpus drives organ formation. In the first of these models, originally proposed by Schuepp (1938), the tunica is assumed to grow faster than the corpus. The resulting accumulation of excess tunica surface leads to tangential compression of the tunica, which then responds, passively, with buckling, like a sheet of paper in which the ends are pushed together on a flat plane. The resulting bulge undergoes morphogenesis and develops into an organ. The site of organogenesis would be determined merely by the geometry of the apex. Green has forged this theory into a mathematical model that yields various phyllotactic arrangements found in nature (Green, 1992, 1996; Selker *et al.*, 1992).
- In the second model, growth of the tunica is envisaged to limit meristem growth, leading to compression of the subtending tissues and tangential tension of the tunica. Eventually, the tunica will yield, leading to the formation of a bulge that undergoes morphogenesis (Selker *et al.*, 1992). The site of bulging may be determined either directly by the geometry of the apex, or by local differences in cell wall extensibility or cellulose microfibril orientation (Green, 1985, 1986, 1994; Selker *et al.*, 1992).

6.5.2 Biochemical models

Biochemical models are based on the idea that diffusible signals of chemical nature regulate meristem activity and determine phyllotaxis. The origin of biochemical models can be traced to the 'field theory' of Schoute (1913). This model proposes that the primordia, as well as the summit of the meristem, represent centers that are surrounded by fields that may represent gradients of nutrients or signaling molecules.

Mathematical models for a biochemical regulation of phyllotaxis are based on the assumption that competing activities of short-range (autocatalytic) activators and long-range inhibitors determine the sites of local growth and differentiation (Meinhardt, 1994). Depending on the range of activation and inhibition, such models can create various patterns, such as distichous and decussate phyllotaxis

(Meinhardt, 1994), but the mechanism can also be adapted to create spiral phyllotaxis (Meinhardt, 1996).

6.6 Genetics of phyllotaxis

The regularity of phyllotactic patterns makes phyllotaxis an excellent subject for genetic analysis. Mutants affected in organ initiation or with altered organ arrangement can readily be identified in mutant screens. Indeed, mutants affected in most processes of organ formation and patterning have been isolated in mutant screens. Based on the phenotype, they fall broadly into five groups (table 6.1), although many of these are arranged in more than one group. That is, the mutations cause pleiotropic phenotypes. Some of the mutations listed **in** table 6.1 are discussed below.

6.6.1 Mutants with defects in meristem initiation or maintenance

Several mutants, such as *stm, wus, clvl* and *clv3,* are primarily affected in the establishment or maintenance of the meristem. In general, all mutations that lead to a loss of meristematic cells (e.g. *stm, wus, phd/zll),* or to an accumulation of meristematic cells (e.g. *clv, fas, fuf)* also indirectly affect organ initiation and arrangement. Such mutants have been reviewed extensively elsewhere (Barton, 1998; Laufs *et al.,* 1998c; Lenhard and Laux, 1999; Bowman and Eshed, 2000; Fletcher and Meyerowitz, 2000), and are described in more detail in chapter 2 of this volume.

6.6.2 Mutants with defects in meristem organization

In this class of mutants, the apical meristem is abnormally organized. For example, the layered organization may be lost or cell shape and size may be abnormal. All mutants with changes in meristem organization also exhibit abnormal phyllotaxis. However, in these cases, it is not clear whether the mutants are affected in the process of organ initiation per se, or whether the alterations in phyllotaxis are indirectly caused by the meristem defect. The *forever young (fey)* mutant in *Arabidopsis* may serve as an example of this type of mutation. In *fey* plants, phyllotaxis is highly irregular. Rosette leaves are initiated with divergence angles from 29° up to more than 180°, sometimes resulting in reversal of the generative spiral (Callos *et al.,* 1994). However, the meristems also exhibit histological defects, such as abnormally vacuolated cells, single necrotic cells and loss of the normal layered organization. These defects are evident from the earliest seedling stages, indicating that the meristem develops abnormally in the embryos. In most cases, the meristem aborts before flowering and the plant dies. The *FEY* gene is homologous to reductases, and is expressed throughout the plant, with the notable exception of meristematic tissues (Callos *et al.,* 1994).

Table 6.1 Mutants that are affected in organ formation and phyllotaxis

Mutant	Sp.	Phenotype	References
A Mutants with defects in meristem initiation or maintenance			
knotted1 (kn1)	Zm	loss of indeterminacy	Kerstetter *et al.*, 1997
no apical meristem (nam)	Ph	no meristem	Souer *et al.*, 1996
shoot meristemless (stm)	At	no meristem	Long *et al.*, 1996
cup-shaped cotyledon (cuc1/2)	At	no meristem	Aida *et al.*, 1997; Aida *et al.*, 1999
wuschel (wus)	At	meristem arrest	Laux *et al.*, 1996; Mayer *et al.*, 1998
pinhead (phd)	At	meristem arrest/ differentiation	McConnell and Barton, 1995; Lynn *et al.*, 1999
zwille (zll; identical to pinhead)	At	meristem arrest/ differentiation	Moussian *et al.*, 1998
interfascicular fiberless1 (ifl1)	At	meristem termination	Ratcliff *et al.*, 2000
revoluta (rev; identical to ifl1)	At	meristem termination	Talbert *et al.*, 1995
clavata1 (clv1)	At	meristem overproliferation	Clark *et al.*, 1993; Clark *et al.*, 1997
clavata3 (clv3)	At	meristem overproliferation	Clark *et al.*, 1995; Fletcher *et al.*, 1999
fasciata1 (fas1) and fasciata2 (fas2)	At	meristem overproliferation	Leyser and Furner, 1992
fully fasciated (fuf)	At	meristem overproliferation	Medford *et al.*, 1992
lanceolate (la)	Le	loss of indeterminacy	Mathan and Jenkins, 1962; Caruso, 1968
lateral suppressor (ls)	Le	no axillary meristems formed	Malayer and Guard, 1964; Schumacher *et al.*, 1999
defective embryo and meristem (dem)	Le	meristem missing/ defective	Keddie *et al.*, 1998
B Mutants with defects in meristem organization			
forever young (fey)	At	meristem disorganized	Medford *et al.*, 1992; Callos *et al.*, 1994
disrupted (dip)	At	meristem disorganized	Medford *et al.*, 1992
schizoid (shz)	At	meristem degeneration	Medford *et al.*, 1992
pasticcino1-3 (pas1-3)	At	meristem disorganized	Faure *et al.*, 1998
pinhead (phd)	At	meristem arrest/ differentiation	McConnell and Barton, 1995; Lynn *et al.*, 1999
zwille (zll; identical to pinhead)	At	meristem arrest/ differentiation	Moussian *et al.*, 1998
mgoun1 (mgo1) and mgoun2 (mgo2)	At	meristem oversized/ fasciation	Laufs *et al.*, 1998a
enhanced response to ABA (era)	At	increased meristem size	Bonetta *et al.*, 2000
wiggum (identical to era)	At	increased meristem size	Ziegelhoffer *et al.*, 2000

Table 6.1 (continued)

Mutant	Sp.	Phenotype	References
C Mutants with defects in organ initiation			
pin-formed1 (pin1)	At	no flowers	Okada *et al.*, 1991; Gälweiler *et al.*, 1998
pinoid (pid)	At	no flowers	Bennett *et al.*, 1995; Christensen *et al.*, 2000
monopteros (mp)	At	no flowers	Przemeck *et al.*, 1996; Hardtke and Berleth, 1998
arrested development1 (add1)	At	leaf formation blocked	Picket *et al.*, 1996
D Mutants with defects in organ separation			
no apical meristem (nam)	Ph	fused cotyledons	Souer *et al.*, 1996
cup-shaped cotyledon1/2 (cuc1/2)	At	fused cotyledons	Aida *et al.*, 1997; Aida *et al.*, 1999
pin-formed1 (pin1)	At	fused cotyledons and leaves	Okada *et al.*, 1991; Gälweiler *et al.*, 1998
E Mutants with altered organ number or organ position (phyllotaxis)			
terminal ear (te)	Zm	aberrant phyllotactic angles	Veit *et al.*, 1998
aberrant phyllotaxy (abphyl)	Zm	2 leaves per node	Jackson and Hake, 1999
perianthia (pan)	At	increased petal number	Running and Meyerowitz, 1996; Chuang *et al.*, 1999
pinoid (pid)	At	3 cotyledons	Bennet *et al.*, 1995; Christensen *et al.*, 2000
enhanced response to ABA (era)	At	increased organ number	Bonetta *et al.*, 2000
wiggum (identical to era)	At	increased organ number	Ziegelhoffer *et al.*, 2000
clavata1 (clv1)	At	increased organ number	Clark *et al.*, 1993; Clark *et al.*, 1997
clavata3 (clv3)	At	increased organ number	Clark *et al.*, 1995; Fletcher *et al.*, 1999
ettin (ett)	At	increased organ number	Sessions *et al.*, 1997
forever young (fey)	At	aberrant phyllotactic angles	Medford *et al.*, 1992; Callos *et al.*, 1994
aintegumenta (ant)	At	less floral organs	Elliott *et al.*, 1996; Klucher *et al.*, 1996
tousled (tsl)	At	less floral organs	Roe *et al.*, 1993
altered meristem program (amp-1)	At	1-4 cotyledons	Chaudhury *et al.*, 1993
no apical meristem (nam)	Ph	increased petal number	Souer *et al.*, 1996
leafy (lfy)	At	spiral instead of whorled phyllotaxis	Weigel *et al.*, 1992
floricaula (flo)	Am	spiral instead of whorled phyllotaxis	Carpenter *et al.*, 1995
squamosa (sqm)	Am	spiral instead of whorled phyllotaxis	Huijser *et al.*, 1992; Carpenter *et al.*, 1995

Abbreviations: Sp., Species; Am, *Antirrhinum majus*; At, *Arabidopsis thaliana*; Le, *Lycopersicon esculentum*; Ph, *Petunia hybrida*; Zm, *Zea mays*; ABA, abscissic acid.

From the available knowledge it cannot be deduced what the function of the FEY protein might be, and whether the defect in phyllotaxis is direct or indirect.

6.6.3 Mutants with defects in organ initiation

The *pin-formedl (pinl), pinoid (bid),* and *monopteros (mp)* mutants of *Arabidopsis* are defective in flower initiation, resulting in the formation of naked pin-like inflorescence stalks (Okada *et al.,* 1991; Bennett *et al.,* 1995; Przemeck *et al.,* 1996). In the inflorescence meristem, the mutation specifically blocks organ initiation but not other meristem functions, since stem growth and meristem self-perpetuation are unaffected. This is in contrast to mutants like *add1* and *add2* (Picket *et al.,* 1996), *shz* (Parsons *et al.,* 2000) or *wus* (Mayer *et al.,* 1998), in which all meristem functions are arrested. The mutated genes in *pinl, pid,* and *mp* encode a putative auxin efflux carrier *(PIN1,* Galweiler *et al.,* 1998), a protein kinase involved in auxin responses *(PID;* Christensen *et al.,* 2000), and an auxin-response transcription factor *(MP;* Hardtke and Berleth, 1998), respectively. Moreover, *pinl* and *mp* have reduced auxin transport capacities in the inflorescence stem (Okada *et al.,* 1991; Przemeck *et al.,* 1996). Thus, the mutant phenotypes and the analysis of the cloned genes implicate auxin as a major player in flower initiation. The inflorescence meristem of *pinl* mutant plants is special for two reasons: firstly, it does not exhibit a deregulated phyllotactic pattern but has no pattern at all; and secondly, the defect in organogenesis is separated from basic meristem function. For these reasons, *pinl* has been very useful for the analysis of flower initiation and meristem patterning (see section 6.8).

6.6.4 Mutants with defects in organ separation

The *no apical meristem (nam)* mutation in *Petunia* results in the formation of fused cotyledons and the failure to initiate a functional SAM during embryogenesis (Souer *et al.,* 1996). However, occasionally, escape shoots are formed. These develop normally during the vegetative phase, but flowers exhibit various defects in organ number, identity and position, resulting in sterility.

The *NAM* gene is expressed at the outer meristem boundary of the embryonic **SAM,** and at the boundary between floral organ primordia. From the phenotype and expression pattern of the *NAM* gene, it appears that *NAM* defines the boundary between organs and the SAM, and between incipient organs within the meristem, possibly by negatively regulating growth. In the absence of such boundaries, the embryonic SAM cannot be established, although later in vegetative growth *NAM* appears to be dispensable. Although *NAM* itself may not be the earliest determinant of meristem patterning, it certainly responds to patterning information early in the process of organ initiation.

Double mutants in the redundant genes *Cup-shaped cotyledon1* and *2 (cuc1* and *cuc2)* in *Arabidopsis* exhibit a dramatic seedling phenotype, in that the

cotyledons are completely fused and the SAM is missing (Aida *et al.*, 1997). The *CUC2* gene, which is homologous to ***NAM***, is expressed at the boundary between cotyledon primordia in early embryogenesis, and later marks the boundary between the cotyledons and the SAM, a similar expression pattern to *NAM* (Aida *et al.*, 1999). Therefore, *CUC2* (and probably also *CUC1*) appears to serve a similar function to *NAM* in restricting growth between organs. The role of the CUC-like genes is described in more detail in chapter 3 of this volume.

In addition to its inflorescence phenotype, which leads to its name (see section 6.6.3.), *pin1* has a severe vegetative phenotype. Leaves are often oversized, fused or cup-shaped, and sometimes even circular, trumpet-shaped leaves are initiated (Okada *et al.*, 1991; Okada and Shimura, 1994; Reinhardt *et al.*, 2000; Reinhardt and Kuhlemeier, unpublished results). Hence, auxin transport is necessary, not only for organ initiation, but also, directly or indirectly, for determination of organ size and organ boundaries. Interestingly, several genes that are normally expressed in a phyllotactic pattern in the meristem are deregulated in *pin1* mutants (Vernoux *et al.*, 2000). For example, *CUC2*, whose normal expression pattern defines organ boundaries, is expressed in the entire peripheral zone in the meristem of *pin1* mutants, indicating that not only organ initiation but also patterning of the meristem requires PIN1 function.

6.6.5 Mutants with altered organ number or organ position (phyllotaxis)

This group of mutants is particularly heterogeneous, since it reflects the complex nature of factors that influence phyllotaxis. For example, the *fey* mutation (see section 6.6.2), conditions a strong phyllotaxis phenotype. However, this effect may well be indirectly caused by the disorganization of the meristem.

Many mutants with enlarged meristems form supernumerary organs, and examples include *clv1* and *clv3*, and *wiggum/era* (Clark *et al.*, 1993, 1995; Bonetta *et al.*, 2000; Ziegelhoffer *et al.*, 2000). In these cases, it is likely that it is not the patterning mechanism itself that is affected by the mutation, but that the patterning mechanism operates on a larger surface, allowing for more organs to be initiated per whorl. An interesting case is represented by the *abphyl* mutant in maize. In *abphyl* plants, the meristem forms two leaves per node instead of one, resulting in a change from distichous to decussate phyllotaxis (Jackson and Hake, 1999). However, this change was only observed in about 50% of the mutant plants, with the rest having normal phyllotaxis. Although the switch from distichous to decussate appears to be a 'clean' transformation from one phyllotactic pattern to another, it is associated with an increase in meristem size. Significantly, in *abphyl* mutant plants with normal phyllotaxis, meristem size is also normal. Moreover, in decussate *abphyl* plants, the SAM sometimes splits, resulting in twin shoots that now exhibit normal distichous phyllotaxis, possibly because after the split, meristem size is decreased (Kerstetter and Hake, 1997). These results indicate that increased meristem

size is at least associated with, if not causal for, altered phyllotaxis in *abphyl* mutants.

Several homeotic mutations in flower meristem identity genes have been reported to cause changes in phyllotaxis (Coen *et al.,* 1990; Huijser *et al.,* 1992; Weigel *et al.,* 1992). For example, in the *leafy (lfy)* mutant of *Arabidopsis,* inflorescence shoots are formed in place of flowers. Together with inflorescence identity, these meristems exhibit a spiral instead of the whorled phyllotaxis of normal flowers (Weigel *et al.,* 1992). Similarly, in *the floricaula (flo)* and *squamosa (squa)* mutants of *Antirrhinum,* flowers are converted into inflorescence shoots that exhibit spiral phyllotaxis (Coen *et al.,* 1990; Huijser *et al.,* 1992; Carpenter *et al.,* 1995). In all these cases, not only phyllotaxis but also determinacy and meristem identity are changed as a consequence of the homeotic transformation. In fact, these meristems exhibit the appropriate phyllotaxis for the new identity. Therefore, the change in phyllotaxis is likely to be the indirect consequence of altered meristem identity due to the homeotic transformation, and not caused by direct interference with the patterning mechanism.

There are at least two mutants, *terminal ear* and *perianthia,* which exhibit altered phyllotaxis without other effects on meristem size or organization. Thus, these mutants may be affected directly in the patterning mechanism that determines phyllotaxis. The *terminal ear (tel)* mutation in maize causes several deviations in the development of internodes and leaves (Veit *et al.,* 1998). Leaf number is increased and the internodes are dramatically reduced, resulting in the ear-like appearance of the terminal tassel. In addition, the radial position of leaves deviates from the normal 180°. The *TEI* gene, which encodes a putative RNA-binding protein, is expressed in the apex in semicircular bands marking the tissues between successive leaves in the two rows of leaves. Considering the phenotype with excess leaves, and the fact that leaves in wild-type plants are initiated at the site of lowest *TE1* expression, *TE1* has been proposed to function by inhibiting leaf formation. Consistent with this model, leaves of *tel* mutants appear to initiate higher on the apical dome than those of wild-type plants (Veit, B.E., personal communication).

Plants of *Arabidopsis* with a mutation in the *PERIANTHIA (PAN)* gene form pentamerous flowers instead of the tetramerous flowers characteristic of crucifers (Running and Meyerowitz, 1996). Among the patterning mutants, *pan* is significant for several reasons: firstly, *pan* flowers have virtually no defect in organ formation and organ differentiation, and they are fully fertile, that is, the genetic lesion specifically affects the patterning mechanism, not general development; secondly, *pan* floral meristems exhibit normal size and structure, indicating that the change in organ number is not the indirect consequence of altered meristem organization; and thirdly, the mutation results in the conversion to a floral pattern which is commonly found in nature, for example in ancestral plants of the Capparaceae family (Chuang *et al.,* 1999). Therefore, the PAN gene may have played a role in the evolution of crucifer flower pattern by reducing

organ number from five to four. The ***PAN*** gene encodes a putative transcription factor of the bZIP family, however, the function of the PAN protein is still elusive (Chuang *et al.,* 1999).

Box 6.2 Do specific phyllotaxis genes exist?

This survey of the meristem and phyllotaxis mutants shows that most mutants exhibit highly pleiotropic phenotypes. Notably, specific transitions from one phyllotactic pattern to another (e.g. spiral to decussate) are never observed. In many cases, effects on phyllotaxis (organ number or position) are associated with altered meristem size or organization, and thus such effects may well be indirect. The pleiotropic phenotypes of many phyllotaxis mutants, and the rare occurrence of specific patterning mutants, may be due to one or more of the following: 1) mutations in specific patterning genes may be lethal; 2) phyllotaxis may be regulated by genes with redundant functions; **3)** the mechanisms that regulate phyllotaxis may be intimately linked with other (unrelated) functions, leading to pleiotropic phenotypes in the mutants; and 4) genes might determine phyllotactic patterns not directly, but indirectly by controlling the parameters of the system that generates the patterns (Douady and Couder, 1996). Thus, phyllotaxis may be a complicated multigenic trait.

In conclusion, genetics is providing an increasingly detailed picture of the regulation of meristem establishment and maintenance (Barton, 1998; Laufs *et al.,* 1998c; Lenhard and Laux, 1999; Bowman and Eshed, 2000; Fletcher and Meyerowitz, 2000; refer also to chapter 2 of this volume). However, genetic studies have not provided a coherent picture of phyllotactic regulation, including an answers to the following principal questions: (i) What mechanism determines the site of organ formation? (ii) How is organ initiation regulated at the cellular level (cell division, cell expansion)? (iii) Which regulatory molecules (growth factors, hormones, morphogens, etc.) are involved in the regulation of phyllotaxis? The analysis of several mutants *(pin1,* ***pid, mp,*** *ett)* has hinted at a central role of auxin in phyllotaxis. However, mutant analysis has not revealed how auxin is involved and what role the corresponding genes may play in organ initiation and spatial patterning.

6.7 Experimental evidence for models of phyllotaxis

Proponents of both biophysical and biochemical models of phyllotaxis have employed mathematical modelling to support their notions (Richards, 1951; Williams, 1975; Mitchison, 1977; Veen and Lindenmayer, 1977; Schwabe, 1984; Meinhardt, 1994; Green, 1996). However, it has been more difficult to obtain direct experimental evidence for the basis of phyllotactic patterning

mechanisms, mainly due to the minute size and the delicacy of the meristem, which is only approximately 100–200 μm in diameter. Nevertheless, microsurgery and pharmacological approaches have been successfully used to gain insight into meristem function.

The most frequently applied experimental approach has been microsurgery. Meristems have been dissected and isolated, and incisions have been made to isolate primordia or parts of the meristem from the remaining meristem (reviewed in Steeves and Sussex, 1989; Callos and Medford, 1994). The rationale for such experiments was to test to what extent meristem development is dependent on the subtending tissues (Ball, 1948; Smith and Murashige, 1970), and how the pre-existing primordia affect subsequent organ formation and the patterning of the apex (Snow and Snow, 1931, 1933, 1962; Wardlaw, 1949).

Ball (1948) isolated the meristem from primordia and vasculature by four deep vertical incisions around the meristem. The meristems that remained on a plug of central pith tissue continued to develop almost normally, although phyllotactic patterning was not analyzed in detail. It was concluded that the meristem is an independent pattern-generating unit that relies on the more mature apical tissues only for basic nutrients. In contrast, complete isolation of meristems disrupted organ formation completely (Smith and Murashige, 1970). Only after about 10 days of culture on medium, such meristems regained the capacity to form leaves, and later, normal spiral phyllotaxis was re-established. Thus, the meristem seems not to be autonomous from the more mature tissues. Although the meristems of Ball (1948) appeared to contain only the apical non-patterned dome, we now know that even in the absence of any morphological or histological signs, the meristem contains a pre-pattern. This is shown by specific gene expression patterns in the meristem (see section 6.4.2.2). Also, it has been concluded, based both on theoretical considerations and on experimentation, that the determination of leaf position is influenced by pre-existing primordia (Steeves and Sussex, 1989). Clearly, regular patterns can only be established if new leaves are positioned relative to pre-existing leaves. Therefore, some form of positional information needs to be provided to the meristem from the primordia.

Experiments analogous to Balls have been carried out with 1-naphthylphthalamic acid (NPA), an inhibitor of polar auxin transport (Reinhardt *et al.*, 2000; see also section 6.8). NPA suppresses leaf formation, whereas meristem self-perpetuation and stem growth are not affected. Apices that were cultured on NPA-containing medium for prolonged times consisted only of a stem with the meristem at the tip, thus lacking any pre-pattern conferred by pre-existing primordia. When such meristems were transferred to medium without NPA, leaf formation was reinitiated but phyllotaxis was abnormal and spiral phyllotaxis was re-established only gradually (Reinhardt *et al.*, 2000). Thus, although pre-existing leaves are not necessary to form new primordia, they do provide spatial information to determine the position of new primordia.

Snow and Snow (1931, 1933) surgically isolated primordia or the site of incipient primordium formation (I_1) from adjacent tissue by incisions in order to understand the role of these tissues in further organ positioning. For instance, if I_1 was isolated, I_2 was initiated at the expected position, however, I_3 was formed closer to I_1 than normal (figure 6.5). Snow and Snow interpreted their findings as evidence for a 'first available space' mechanism (Snow and Snow, 1931, 1933, 1962). They argued that, since I_1 was prevented from occupying its 'normal space' on the meristem, more space was available for I_3 which is initiated in proximity to I_1. Therefore, I_3 'moved' towards the resulting gap, and was initiated closer to I_1 and almost opposite to I_2.

However, the results could also be interpreted as evidence for negative signaling from pre-existing primordia. This points to the fact that first-available-space and negative-field models are difficult to distinguish experimentally because they make similar predictions. In both models, the probability of leaf formation increases with the distance from pre-existing primordia.

Wardlaw (1949) performed similar surgical experiments on apices of the fern, *Dryopteris*. Although, in these experiments, the effect of surgical treatments on the site of primordium initiation was similar to the results of the experiments performed by Snow and Snow, the author came to different conclusions. Firstly, the availability of free space seemed not to be limiting, since the primordia of

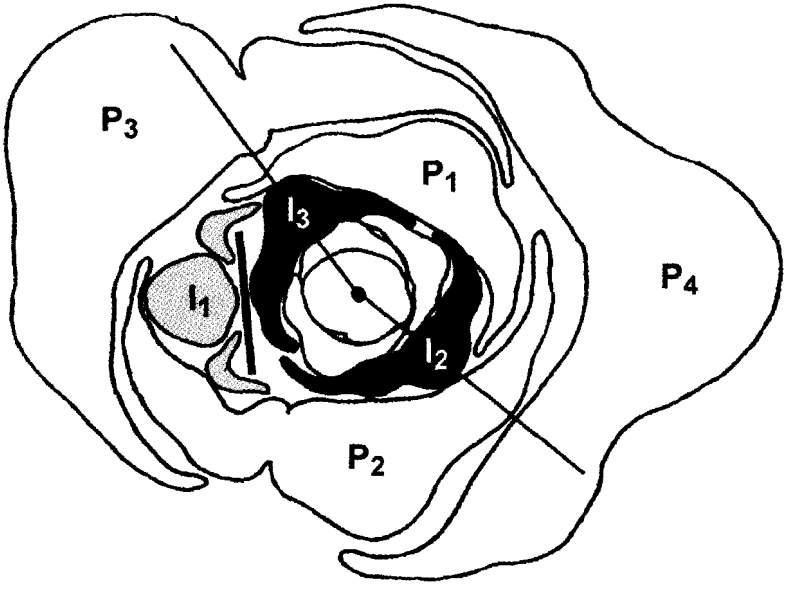

Figure *6.5* Isolation of an incipient leaf primordium (I_1) leads to the displacement of the second next primordium (I_3). In a lupin apex, the site of incipient leaf formation (I_1) was isolated from the rest of the meristem by a vertical incision (represented by a black bar). After **3** weeks, the apex was fixed and sectioned. In addition to I_1, which grew out after isolation, five new primordia were formed (interior to the black bar). Whereas I_2 is at the expected site, I_3 is closer to I_1, and almost opposite to I_2. The divergence angle between I_2 and I_3 is approximately 165°, whereas normally the divergence angle is approximately 136°. (After Snow and Snow, 1931.)

Dryopteris are widely spaced. Secondly, aberrant organ positioning induced by surgical manipulation did not always occur at the site of 'first available space'. Therefore, Wardlaw concluded that signals emanating from primordia were the basis of organ positioning rather than the availability of free space.

One general pitfall of surgical experiments has to be taken into account when interpreting such results. That is, isolating very young primordia from meristems creates wounds, particularly as isolation of the site of incipient leaf formation requires tangential incisions into the meristem. However, the meristem is likely to respond to such insults with wound development and alterations in growth dynamics (Pilkington, 1929; Steeves and Sussex, 1989), effects that would be expected to alter meristem function and phyllotaxis, making conclusions difficult.

Schwabe (1971) used a pharmacological approach to interfere with phyllotaxis. He treated *Chrysanthemum* plants with the auxin transport inhibitor, TIBA. This treatment resulted in a stable transformation from spiral to distichous phyllotaxis. However, not only leaf position, but the whole geometry of the apex was altered. In particular, the vertical distance of leaf primordia was increased. Based on these experiments, Schwabe favored a 'field model' of phyllotaxis, according to which organ positioning is regulated by negative influences from the two youngest primordia (figure 6.6). The author assumed that under natural

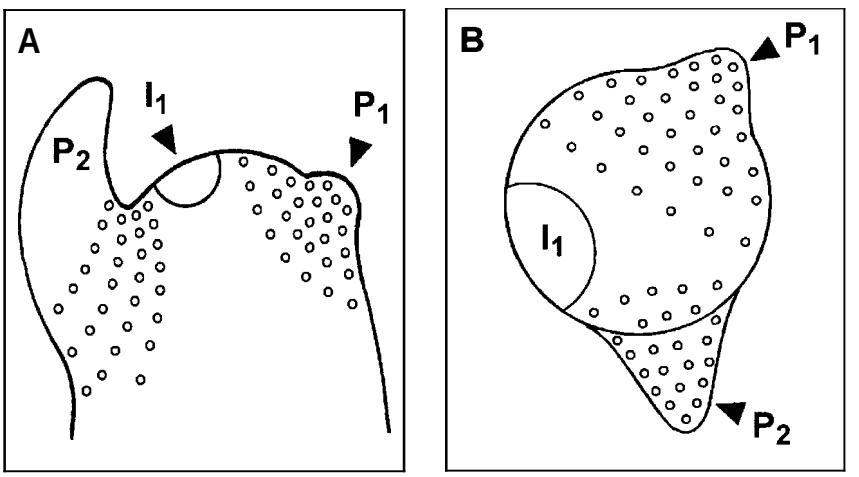

Figure 6.6 A model for phyllotaxis based on inhibitory fields emanating from leaf primordia. A: Shoot apex in a longitudinal representation with two preformed primordia (P_1 and P_2). The primordia produce an inhibitor of leaf formation that diffuses into the meristem (inhibitor represented by small circles). At a certain distance from the primordia, the concentration of inhibitor is low enough to allow leaf formation (I_1). P_2 is lower than P_1 and more remote from the meristem flank, thus the inhibitory influence is weaker than that from P_1. Consequently, the next primordium will be formed closer to P_2 than P_1. (After Schwabe, 1971, 1984.) B: The situation is similar to that in **(A)** but represented as a transverse section at the level of P_1. Due to its proximity to the meristem, the gradient emanating from P_1 covers a larger surface than the gradient from P_2, which is lower (indicated by a line between P_2 and the meristem). Therefore, I_1 is closer to P_2 than P_1, leading to a divergence angle of approximately 137°, characteristic of spiral phyllotaxis.

conditions, the youngest primordium (P_1) had a stronger 'repellent' effect than the second youngest (P_2), so that the incipient primordium (I_1) would be placed closer to P_2 than P_1 (as observed in spiral phyllotaxis). Since TIBA caused an increase in the vertical distance between primordia, he concluded that P_2 became too remote from the meristem to have any inhibitory effect. Consequently, I_1 would be placed as far as possible from P_1, and after a few plastochrons, stable distichous phyllotaxis would be established. Thus, the effect of TIBA on phyllotaxis was thought to be indirect.

Meicenheimer (1981) studied the effect of inhibitors of auxin transport and action on the decussate (bijugate) plant, *Epilobium*. In these experiments, decussate phyllotaxis was transformed into spiral phyllotaxis by local application of inhibitors to young primordia. Although Meicenheimner concluded, in agreement with Schwabe, that gradients of inhibitors emanating from primordia determine phyllotaxis, the role of auxin was interpreted differently. Whereas Schwabe suggested that auxin played only an indirect role by regulating growth parameters in the apex and by affecting the range of inhibitors in the meristem, Meicenheimer proposed that auxin itself could be the inhibitor.

In addition to auxin transport inhibitors, auxin itself has been applied to shoot apices (Snow and Snow, 1937). In general, application of exogenous auxin has been shown to induce increased primordium size and fusions of primordia (see following section).

6.8 Auxin regulates initiation and radial position of leaves and flowers

Hormones, especially auxin, gibberellin and cytokinin, have long been thought to play a role in meristem development and organ formation (Snow and Snow, 1937; Bedesem, 1958; Kiermayer, 1959, 1960; Schwabe, 1971; Maksymowych and Erickson, 1977; Meicenheimer, 1981; Marc and Hackett, 1992; Chaudhury *et al.*, 1993; Dewitte *et al.*, 1999). However, the specific role of hormones in phyllotaxis has not been established. In many of the earlier studies, the experimental treatments were not sufficiently restricted in time and space, or the time between treatment and sampling was rather long (e.g. a week or more). Therefore, it has been difficult to separate direct and indirect effects of the growth regulators.

Recently, the role of auxin in organ initiation and phyllotaxis was studied using both the auxin transport mutant, *pin-formed1*, and specific inhibitors of polar auxin transport (Reinhardt *et al.*, 2000). In this study, it was shown that the auxin transport inhibitor, NPA, specifically inhibited leaf initiation in tomato meristems. However, meristem perpetuation and stem growth were not affected, since the apices continued to grow, forming pin-like shoots (further referred to as NPA pins). Thus, auxin transport is specifically required for

leaf initiation, but not for general meristem growth. The separation of organ formation from general meristem growth makes it possible to analyze these processes independently. To establish the role of auxin in leaf formation, small droplets of lanolin containing indole-3-acetic acid (IAA) were administered to the flank of NPA pins, at a distance from the summit that corresponds to the distance in natural leaf formation. This treatment induced leaf formation after one day (figure 6.7A; Reinhardt *et al.*, 2000).

Mutations in the *Arabidopsis pin-formed1 (pin-1)* gene lead to a defect in organ formation in the inflorescence, resulting in pin-shaped stalks resembling tomato apices cultured on auxin transport inhibitors (Okada *et al.*, 1991; see also section 6.6.3). This mutation was recently traced to a putative auxin efflux carrier (Galweiler *et al.*, 1998),indicating that, as with leaf formation in tomato, flower initiation in the *Arabidopsis* inflorescence requires polar auxin transport. Administration of droplets containing IAA to the flank of *pin-I* meristems induced flower primordia (figure 6.7B; Reinhardt *et al.*, 2000). In both tomato and *Arabidopsis* pins, organs were only induced at the flank of the meristems, as with untreated meristems, but never on the summit or below the flank. Even if IAA was applied to the summit of the meristem, organ formation could not

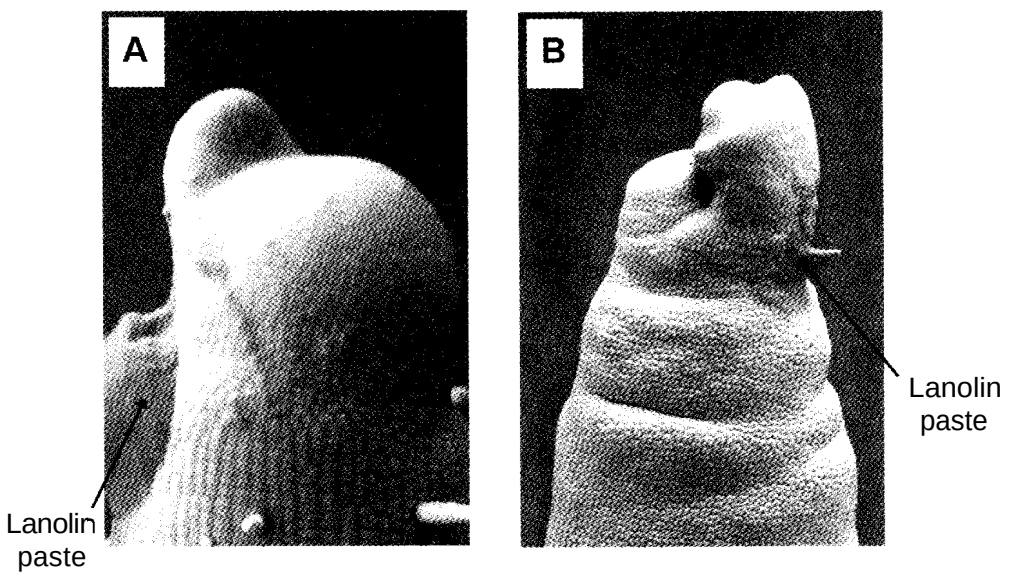

Figure 6.7 Auxin induces organ formation on tomato 1-naphthylphthalamic acid (NPA) pins and *Arabidopsis pin-formedl* apices. **A:** Tomato apices were cultured on medium containing the auxin transport inhibitor NPA. This resulted in the inhibition of leaf formation, whereas stem growth continued, resulting in the formation of a naked pin-like shoot. Local administration of lanolin paste containing 1 mM indole-3-acetic acid (IAA) to the flank of such NPA pins induced leaf primordia at the site of treatment. (Reproduced with permission from Reinhardt *et al.*, 2000.) B: The inflorescence meristem of *Arabidopsis pin1* mutant plants is inhibited in flower formation, resulting in the formation of pin-shaped stalks. Local administration of lanolin paste containing 1 mM IAA to the flank **of** such inflorescence meristems induced flowers at the site of treatment. (Reproduced with permission from Reinhardt ***et*** *al.*, 2000.)

be induced at this position, but only at the flank, indicating that auxin was able to diffuse over this short distance in the absence of polar transport. In the radial dimension, organogenesis always coincided strictly with the site of auxin application. From these observations, a number of conclusions can be drawn:

- Auxin is necessary and sufficient to induce organs at the SAM.
- Since auxin only induced organs at the flank but not in the meristem center, an auxin independent pre-pattern appears to be maintained in pin-meristems functionally equivalent to the zonation observed in untreated meristems, comprising a central zone of undifferentiated stem cells and a peripheral zone that is capable of organogenesis if supplied with auxin. Within the ring-shaped tissue at the flank, auxin could induce organs at any position.
- Auxin was able to induce both leaves on vegetative tomato pins, and flowers on *pin1-1* inflorescence pins. Therefore, auxin appears to be a universal inducer of organogenesis in plants, and other factors in the meristem appear to determine organ identity.

In this context, it is interesting to note that although flowers are formed as lateral structures in phyllotactic patterns like leaves, they are not organs in the strict sense, but they represent determinate lateral meristems (Coen and Nugent, 1994). The results discussed above show that the initiation of leaves and flowers share a common mechanism involving auxin, and therefore they may not represent fundamentally different processes. In most angiosperm families, flowers initiate in the axils of bracts (Coen and Nugent, 1994). In crucifers, such as *Arabidopsis*, however, flowers are not subtended by bracts. It has been proposed that this is a derived condition, and that remnants of cryptic bracts may still be involved in flower formation (Coen and Nugent, 1994; Long and Barton, 2000). Interestingly, in the *leafy* mutant of *Arabidopsis* in which flowers exhibit inflorescence shoot characteristics, the abnormal flowers are occasionally subtended by bracts (Weigel *et al.*, 1992). Therefore, flower formation in *Arabidopsis* may have evolved from a process that is homologous to lateral shoot formation, and in *leavy* mutants, this ancestral pathway of flower formation (including bracts) is revealed by the partial loss of flower identity (Weigel *et al.*, 1992; Coen and Nugent, 1994). According to this idea, it is possible that in the *Arabidopsis* inflorescence meristem, auxin induces primordia that consist of both an abaxial portion that corresponds to a cryptic bract (that is inhibited to grow by the *LEAFY* gene), and an adaxial portion that represents the actual flower primordium. Indeed, the *STM* gene, a marker for meristem tissues, is downregulated on the abaxial side of flower primordia, in a similar way to leaf initiation (Long *et al.*, 1996; Long and Barton, 2000; see also section 6.4.2.2). This region has therefore been interpreted as the cryptic bract. In this context, it would be interesting to see whether auxin could induce leaves on *pin1/leafy* double mutant meristems.

Polar auxin transport is required for leaf and flower initiation. But where is auxin produced, and where is it transported to in order to promote organ initiation? Auxin is thought to be produced in young developing tissues of the shoot, particularly in young leaves (Davies, 1995). However, it is not clear whether auxin is produced in the meristem proper. Therefore, inhibition of auxin transport could lead to either accumulation or depletion of auxin in the meristem, depending on whether or not the meristem is a source of auxin. The fact that exogenous auxin can restore organ formation in pin meristems suggests that these meristems experience auxin depletion rather than accumulation, and that auxin depletion is the reason for inhibited organogenesis. If the inhibition of polar auxin transport leads to depletion of auxin in the **SAM,** we have to assume that, under normal conditions, acropetal polar auxin transport from subtending tissues regulates organ formation in the meristem. Alternatively, polar auxin transport could lead to reallocation of auxin within the meristem to build up gradients that determine phyllotaxis.

Based on the conclusions above, we proposed that dynamic gradients of auxin in the meristem determine the site of organ formation, and that pre-existing leaves influence these auxin gradients by modulating acropetal auxin transport or auxin distribution within the meristem. If gradients of auxin determine phyllotaxis, the pattern of organ formation would be expected to be sensitive to

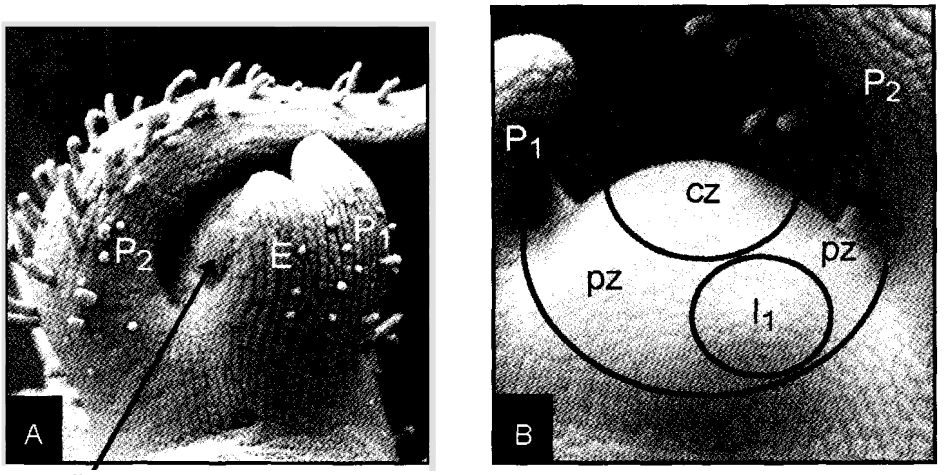

Figure 6.8 Regulation of phyllotaxis by auxin. **A: A** tomato apex with two preformed leaf primordia (P_1 and P2) was treated with lanolin paste containing 10 mM indole-3-acetic acid (IAA). The site of application was at I_2, i.e. the site where the second next primordium would normally be formed (between P_1 and P_2). The next primordium (I_1) was expected to form in the back (not visible). Instead, an ectopic primordium was induced at the site of **IAA** application (E). (Reproduced with permission from Reinhardt *etal.,* 2000.) B: A model for the role of auxin in organ formation. **A** vegetative apex with two leafprimordia (P_1 and P2) and the meristem, consisting of the peripheral zone pz and the central zone cz. This apical-basal prepattern is auxin-independent. Auxin accumulation at the site of incipient leaf formation (I_1) induces the cells in the peripheral zone to form a leaf. The pre-existing leaf primordia (P_1 and P_2) determine the site of auxin accumulation.

exogenous application of auxin. This is indeed the case, since exogenous auxin is able to induce ectopic primordia on the flank of otherwise untreated meristems (figure 6.8A; Reinhardt *et al.*, 2000). This indicates that in normal meristems, as in tomato and *Arabidopsis* pins, various positions around the meristem flank are competent of organogenesis if supplied with auxin. This suggests that it is the availability of auxin that determines organ position. This observation is in agreement with the idea that gradients of auxin determine phyllotaxis (figure 6.8B). However, the existence of auxin gradients remains to be confirmed either by direct auxin measurements or by using auxin-inducible markers.

6.9 A model for the role of auxin transport in phyllotaxis

How could dynamic auxin gradients be generated in a pattern consistent with phyllotaxis? Auxin is known to induce the formation of vascular tissues. The vasculature, in turn, is the route of auxin transport. These findings led Sachs (1991a and b) to propose the 'canalization hypothesis', which assumes that in a tissue in which auxin is initially uniform, small random differences in auxin synthesis, transport or metabolism lead to uneven distribution of auxin (figure 6.9). In cells that experience elevated levels of auxin, its capacity to

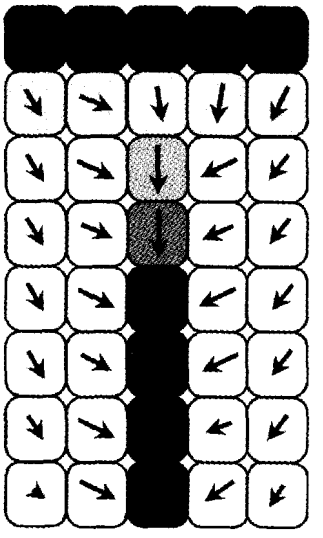

Figure 6.9 Determination of vascular strands by auxin according to the 'canalization hypothesis' Auxin producing cells (top row of cells) deliver auxin to neighboring cells, which initially experience equal auxin levels (represented by the second row of cells from top). Small random differences in auxin synthesis, transport or metabolism lead to uneven distribution of auxin. In cells which experience elevated levels of auxin, the capacity to absorb auxin and to transport it along the direction of pre-existing polarization is increased (cell in the center of the third row of cells). This leads to further accumulation of auxin in these cells. The resulting positive feedback mechanism leads to the confinement of the transport routes to narrow files. Auxin is accumulated in these cells (dark cells in the lower half of the central column of cells) and determines that they differentiate into vascular tissues. (Drawn according to models from Sachs, 1991a and b.)

absorb auxin and to transport it along the direction of pre-existing polarization is increased. This leads to further accumulation of auxin in these cells. The resulting positive feedback mechanism, referred to as canalization, leads to the confinement of the transport routes to narrow files, which become determined by auxin to differentiate into vascular tissues. This mechanism is thought to determine the vascular patterning in leaves (Sachs, 1991a and b; Berleth and Mattsson, 2000; Berleth *et al.*, 2000). If vascular differentiation starts from a pre-patterned situation due to the existence of a source or a sink of auxin, then the direction of the developing vasculature is dictated by the pre-pattern. In general, auxin sinks 'attract' vascular strands, and similarly, auxin sources induce the differentiation of vasculature in their vicinity (Sachs, 1991a and b).

Auxin transport has been studied extensively in roots. Auxin is thought to be transported to the root tip through the central stele. In the root tip, auxin is redistributed to the flanks of the root, and then transported back (towards the elongation zone) in the epidermal and cortical cell files (Jones, 1998). Normally, auxin redistribution in the root tip is equally efficient in all radial directions. However, if the root is gravistimulated, auxin flux is preferentially redirected to the lower side of the root with respect to the gravity vector. The higher accumulation of auxin in the lower half of the root inhibits root elongation on this side, leading to curvature and gravitropic reorientation of root growth (Davies, 1995).

One can speculate that acropetal transport in the shoot apex and lateral redistribution of auxin within the meristem could be the basis of phyllotaxis. Auxin produced in maturing leaves could be delivered to the stem and acropetally transported to the meristem in a way analogous to transport in the stele of the root. In the meristem, it becomes laterally redistributed by a PIN-dependent pathway that involves the youngest pre-existing primordia. These primordia could influence the distribution of auxin by absorbing auxin from the adjacent meristem tissues in a way analogous to canalization. Thus, the young primordia would act as sinks of auxin, and in the meristem, sufficient auxin to promote new organ formation could only be accumulated at some distance from the preformed primordia (figure 6.10). As soon as a certain level of auxin accumulation is reached at this position (I_1), organ formation would be induced, and at the same time the induced cells would gain sink capacity. This provides the opportunity for them to participate in the determination of the position of the next primordium. Such a reiterative mechanism could create the phyllotactic patterns found in nature. If only the youngest primordium (P_1) absorbs auxin, leaves would always be initiated at 180° from each other, as is the case in distichous phyllotaxis (figure 6.10A). If the two youngest primordia (P_1 and P2) compete for auxin, but the ability to absorb auxin declines with increasing age of the primordia, and P_1 has the strongest effect, then new primordia would be placed between P_1 and P_2, but closer to the latter, as is the case in spiral phyllotaxis (figure 6.10B). If the range of efficient auxin withdrawal by primordia is smaller than half the meristem diameter, two leaves could be

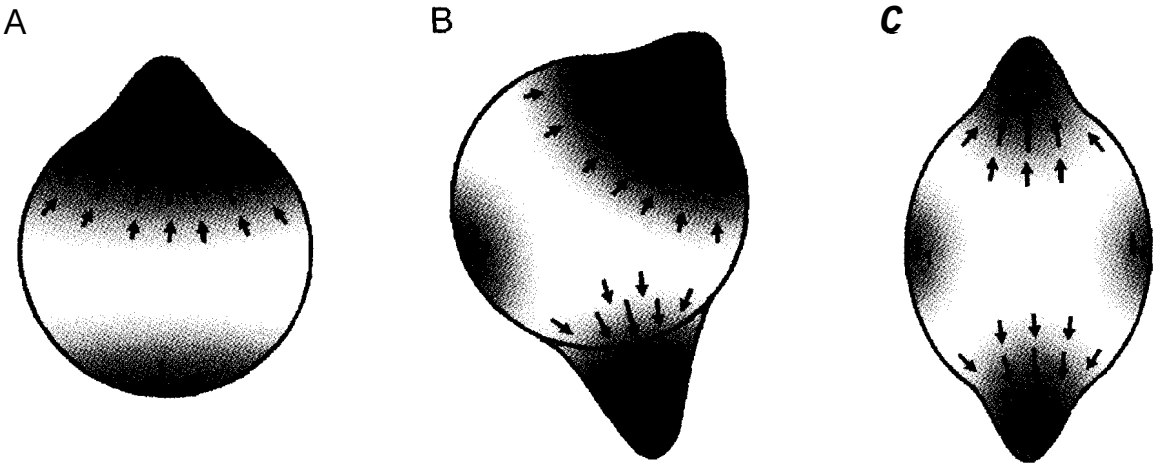

Figure 6.10 A model for the role of polar auxin transport in phyllotaxis. The figure shows three schematic transverse sections through meristems of a distichous (A), a spiral **(B)** and a decussate plant **(C),** respectively. The sections are at the level of the youngest primordia (P_1). Primordia are envisaged to absorb auxin (represented by darker shades) from the meristem by a mechanism involving polar auxin transport, thus acting as sinks for auxin. They determine the distribution of auxin, which is acropetally transported to the meristem from the stem. Only at a certain distance from absorbing primordia, can auxin accumulate to levels sufficient to promote leaf formation (I_1). A: Distichous phyllotaxis—in this system, only the youngest primordium (P_1) absorbs auxin, whereas P_2 has either lost the capacity to absorb auxin or it is too remote from the meristem. Therefore, I_1 is always initiated opposite to P_1. B: Spiral phyllotaxis—if the two youngest primordia (P_1 and P_2) compete for auxin, but the ability to absorb auxin declines with increasing age of the primordia (and with increasing distance from the meristem), then P_1 has the strongest effect. Thus, the next primordium will be placed between P_1 and P2, but closer to the latter. C: Decussate phyllotaxis—here, the range of efficient auxin withdrawal by primordia is smaller than half the meristem diameter. Hence, two leaves can be initiated at a time (I_1). Due to mutual competition for auxin, they will be placed at opposite positions, and the resulting leaf pairs will determine future leaf pairs to be initiated with a divergence angle of 90°.

initiated at a time. Due to a mutual competition for auxin, these leaves would be placed at opposite sites, and the resulting leaf pairs would determine future leaf pairs to be initiated with a divergence angle of 90°, as is the case in decussate phyllotaxis (figure 6.10C).

In this context, it is interesting to note that both the auxin transport protein, *PIN1*, and the auxin response transcription factor, *Monopteros*, are upregulated early in organ initiation, before a bulge is visible (figure 6.4D, E, H; Hardtke and Berleth, 1998; Vernoux *et al.*, 2000). Therefore, auxin, as well as auxin transport capacity might be present very early in incipient primordia. Although the youngest primordia (P_1 and P_2) are probably incapable of auxin biosynthesis, and may function as auxin sinks, they will, at some point, start to produce auxin and deliver it to the stem, thus acting as auxin sources. Hence, phyllotaxis could be influenced both by the range of auxin absorption around the youngest primordia and by the timing of the sink-to-source transition of older primordia.

To determine how pre-existing primordia affect auxin distribution within the apex, it will be important to measure auxin levels in the different apical tissues. Furthermore, it has to be established at which stage primordia start to produce

auxin, and to determine the direction of auxin fluxes between primordia of different developmental stages and the meristem, and within the apical stem just below the meristem.

6.10 How does auxin regulate growth in the meristem?

First, it should be noted that, by definition, to grow means to increase in volume. This cannot be achieved in the absence of cell expansion. Cell division alone only leads to smaller and smaller cells with no net growth, as in early embryogenesis of amphibians (Slack, 1991). In contrast, growth could principally take place in the absence of cell division, simply by expansion of existing cells, as in the development of the unicellular trichomes in *Arabidopsis* (Hulskamp *et al.*, 1994). It is obvious that the formation of a primordium requires the coordination of cell division and cell expansion. However, mechanistically, cell division and cell expansion can be linked in different ways. The primary event in organ initiation could be the activation of the cell cycle, followed by cell expansion and differentiation of the proliferating cells. Alternatively, growth could be induced by increased tissue expansion followed by insertion of cross walls to subdivide the increasing cell volumes. While both mechanisms of growth occur in plants (Kaplan and Hagemann, 1991; Jacobs, 1997), the degree to which both operate in the SAM is not clear.

How could auxin induce organ initiation? That is, what are the downstream processes that are initiated by auxin? Auxin is known to regulate various cellular responses, including cell division and cell expansion (Davies, 1995). In the induction of lateral roots, auxin induces cell division, presumably by direct activation of the cell cycle in pericycle cells (De Veylder *et al.*, 1999). In the gravitropic response, auxin is thought to act by regulating differential tissue expansion in the absence of cell division (Davies, 1995). Thus, it appears that the cellular responses to auxin depend on the tissue context and on the developmental stage. Most tissues in which auxin effects have been studied are differentiated tissues. In contrast, little is known about the regulation of cell growth in the meristem and the cellular responses of meristematic tissues to auxin.

Cell division activity in the meristem and in developing primordia can be determined quantitatively by counting the number of dividing cells (using mitotic figures or diagnostic cell cycle genes) and recording the orientation of newly formed cross walls (Laufs *et al.*, 1998b, Lyndon, 1998). Quantitative analysis of cell division activity in *Arabidopsis* floral apices has shown that division rate was increased at the site of incipient flower formation, indicating that induction of the cell cycle may be one of the first signs of growth (Laufs *et al.*, 1998a).

Similarly, in vegetative meristems of *Pisum*, the cell cycle was accelerated at the site of incipient leaf formation (Lyndon, 1998). However, not only the

rate but also the orientation of cell division changed. An increased proportion of cells divided periclinally, that is, the new cell walls were aligned parallel to the meristem surface. Since in plants, new cell walls are usually orientated perpendicular to the direction of growth (Lyndon, 1990), this could indicate that cell division responds to increased outward expansion, instead of being the cause of outgrowth. Indeed, Lyndon (1998) pointed out that 'the occurrence of periclinal divisions does not, and cannot, cause outward growth'.

Although the role of cell division and expansion at the earliest stages of organ formation is not clear, a number of studies provide indirect evidence that organ formation may be regulated at a supercellular level, possibly by differential regulation of tissue expansion rather than at the level of cell division. These include:

- In wheat seedlings that were y-irradiated to block cell division, the meristem produced bulges that resembled leaf primordia. These bulges were induced by directional cell expansion of the tunica cells (Foard, 1971).
- Leaf formation in transgenic tobacco plants with reduced cell cycle activity occurred with similar rates as in control plants, and the final size of the leaves was normal. However, cell number was decreased and cell size was increased (Hemerly *et al.*, 1995).
- In maize plants with the *tangled* mutation, the orientation of cell division is deregulated, resulting in an uneven leaf surface. However, mutant plants are similar to wild-type siblings, especially in terms of phyllotaxis and overall leaf shape (Smith *et al.*, 1996).
- In the *Arabidopsis tonneau/fass* mutant, cell division is completely irregular. Although the dimensions of the plants are abnormal, they contain all the tissues at normal positions (Torres-Ruiz and Jurgens, 1994; Traas *et al.*, 1995).

6.11 A role for expansin and the cytoskeleton in organ initiation

If local tissue expansion drives organ initiation, one of the earliest stages in organogenesis should be the induction of agents that induce cell expansion. Expansins are cell wall proteins that are implicated in cell expansion due to their ability to increase cell wall extensibility *in vitro* (McQueen-Mason and Cosgrove, 1994, 1995) and in cell cultures (Link and Cosgrove, 1998). Therefore, they are thought to control cell growth *in planta* (Cosgrove, 1997, 2000). In *Arabidopsis*, tomato and rice, expansins are encoded by gene families comprising several dozen members that are differentially expressed. It is thought that differential expression in the tissues allows precise fine-tuning of cell wall extensibility in space and time. In general, expansin expression coincides with growing tissues, with their induction occurring under conditions that promote

growth (Keller and Cosgrove, 1995; Cho and Kende, 1997; Brummel *et al.*, 1999a; Caderas *et al.*, 2000; Catala *et al.*, 2000). In transgenic *Arabidopsis* plants overexpressing an expansin gene, leaf growth was enhanced (Cho and Gosgrove, 2000). However, in some cases, expansin expression was not correlated with growth (Rose *et al.*, 1997; Caderas *et al.*, 2000), indicating that expansins may also function as modulators of cell wall properties in the absence of cell wall extension (Cosgrove, 2000). It has been shown that an expansin which is specifically expressed in tomato fruits, contributes to tissue softening in ripening fruits (Brummel *et al.*, 1999b).

Since expansin regulates growth of developing tissues, it was of interest to see whether applied expansin was also able to control growth in the undifferentiated cells of the **SAM.** Expansin protein was applied locally to the flank of tomato meristems at a radial position at which primordium formation was not expected (I2). This treatment induced ectopic organogenesis, indicating that promoting tissue expansion is sufficient to drive morphogenesis (figure 6.11a; Fleming *et al.*, 1997, 1999). It was therefore postulated that local cell wall softening could be the critical step in the initiation of primordia. Although expansin-induced primordia exhibited dorsoventrality and expressed markers for leaf identity (Fleming *et al.*, 1997, 1999), they never developed a vascular system and, therefore, could not grow beyond the stage of a young primordium. This is in contrast to leaves induced by auxin (see-section 6.8), which did grow to

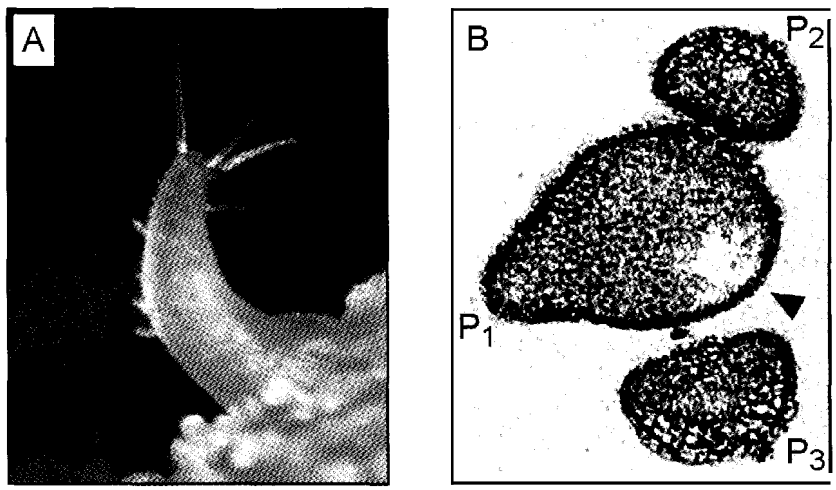

Figure 6.11 A role for expansin in primordium formation at the shoot apical meristem **(SAM). A:** Expansin protein was applied to the flank of a tomato meristem. This treatment induced the formation of a leaf-like structure with trichomes. Although such primordia exhibit dorsoventrality and express markers for leaf identity, they never develop into mature leaves. (Reproduced with permission from Fleming *et al.*, 1997.) B: *In situ* hybridization of a tomato apex at the level of the youngest leaf primordium (P_1). The next oldest primordia (P_2 and P_3) are visible in clockwise phyllotaxis. The section was hybridized with a probe for *LeExp18*, a tomato expansin gene. At the site of incipient leaf fromation (arrowhead), *LeExp18* is upregulated (indicated as a bright signal). (Reproduced with permission from Reinhardt *et al.*, 1998.)

normal sizes and had all the tissues of normal leaves (Reinhardt *et al.,* 2000). This suggests that expansin induces only a subset of events required for proper leaf formation, whereas auxin induces the whole program.

The ability of exogenous expansin to induce primodium formation prompted the authors to look for endogenous expansin genes that could be involved in leaf formation. Indeed, a tomato expansin gene, *LeExp18,* was identified that is expressed in meristematic tissues. *LeExp18* is upregulated at the site of incipient leaf formation (figure 6.11B; Reinhardt *et al.,* 1998), and induction of the gene occurs very early in the induction of leaf primordia before any sign of organ formation can be observed histologically. Interestingly, *LeExp18* expression in the meristem is induced by exogenous auxin (Reinhardt and Kuhlemeier, unpublished results). Thus, *LeExp18* may be a primary target of auxin in organ initiation, and its expression pattern may reflect elevated auxin levels at I_1. Together, these results suggest that expansin is likely to regulate organogenesis at a very early stage, possibly by acting as the initial trigger of local growth.

Cell expansion is intimately linked with the cytoskeleton (Gunning and Hardham, 1982; Cyr, 1994). The orientation of cortical microtubules predicts the orientation of cellulose microfibrils, and this determines the direction of cell expansion. Although direct proof is missing, the dynamic cortical microtubules are thought to determine the orientation of cellulose microfibrils by guiding the cellulose synthase complex along the plasmalemma (Cyr, 1994). The orientation of microfibrils then dictates the direction of expansion, since the cell wall can most easily expand in the direction perpendicular to the microfibrils. This scenario appears to be true for unidirectional expansion in cylindrical cells with predominant hoop-reinforcement (Green, 1984). If localized tissue expansion is the primary event in organ initiation, then changes in the cortical microtubule arrangement may be useful markers to study the earliest steps in organogenesis.

The orientation of microfibrils in the meristem L_1-layer has been studied extensively by Green and co-workers (Green, 1986; Green, 1988; Jesuthasan and Green, 1989), who observed that the pattern of microfibrils in the meristem L_1 layer corresponds with the patterns of local tissue expansion during leaf initiation (figure 6.12). Green concluded that undirected internal pressure in the meristem could be translated into directional expansion merely by spatial and temporal fine-regulation of cellulose deposition in the L_1 layer (Green, 1994). Although this theory offers an elegant mechanism to explain the regulation of differential growth, it does not explain the patterning mechanism that regulates microfibril orientation. Interestingly, auxin has been reported to induce reorientation of cortical microtubules (Nick *et al.,* 1992; Shibaoka, 1994). Thus auxin could act both by increasing cell wall extensibility through expansin induction, and influence the direction of expansion through reorientation of microtubules.

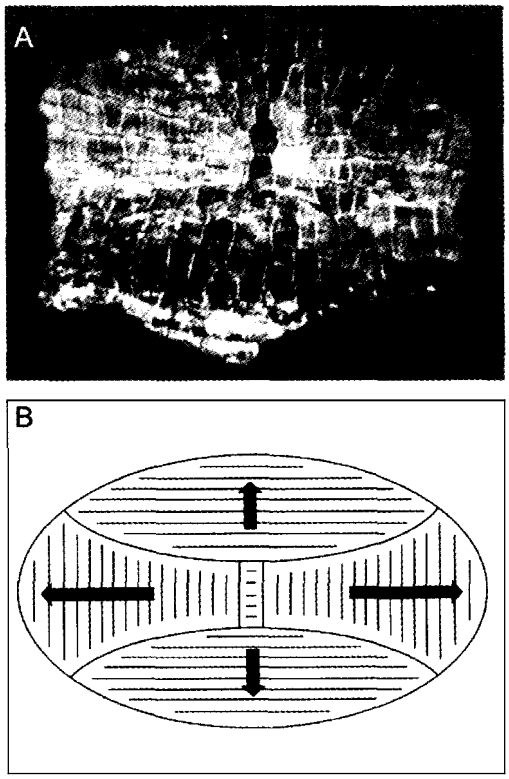

Figure 6.12 The orientation of cell wall microfibrils predicts the pattern of growth in the meristem of *Vinca major*. A: In **a** superficial (paradermal) section of a meristem of *Vinca major*, the orientation of microfibrils in the L_1 layer can be seen in polarized light. Areas in which the microfibrils are arranged in N-S orientation appear bright. In darker areas, the microfibrils are arranged in W-E orientation. *Vinca major* is a decussate plant. In the specimen analysed, the next pair of leaves would have been formed to the left and to the right. (Reproduced with permission from Green, 1985.) B: Schematic representation of the reinforcement patterns seen in **(A).** Horizontal and vertical lines represent the orientation of the microfibrils. Arrows indicate the direction of growth, as determined by the reinforcement pattern. Growth will preferentially be directed towards the sites of leaf formation (long arrows).

6.12 Conclusions

The regularity of the spatial arrangement of leaves, flowers and floral organs, has amazed people since the first descriptions of phyllotactic patterns. Phyllotaxis has evoked numerous models to explain the mechanisms that regulate pattern formation in the meristem. During past decades, tremendous progress has been made in understanding meristem establishment and maintenance, as well as the process of organ initiation. However, the molecular and cellular mechanisms that regulate phyllotaxis are only now beginning to unfold.

Whereas originally, biophysical and biochemical models of phyllotaxis have been considered to represent opposing mechanisms, it now appears that they may converge to a coherent model of phyllotaxis with auxin at the center. Evidence is accumulating that patterning mechanisms involve molecular signaling between cells within the meristem. Examples include the signaling system involving

the **CLAVATA** proteins in the regulation of the stem cell population (Fletcher and Meyerowitz, 2000), or auxin in the patterning of the meristem and organ initiation (Kuhlemeier and Reinhardt, 2001). On the other hand, downstream mechanisms that realize the patterns may well be based on biophysical principles, e.g. modulation of the cytoskeleton arrangement and of cell wall properties. In the future, the combination of genetics with pharmacological approaches and micromanipulation is expected to allow deeper insights into the determinants of phyllotaxis.

Acknowledgements

The authors would like to thank Peter Endress, Daniel Moser and Jeroen Stuurman for helpful discussions. They also thank Elliot M. Meyerowitz, Detlef Weigel, Jan Traas, Kathryn Barton and Thomas Berleth for providing *in situ* images.

References

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997) Genes involved in organ separation in *Arubidopsis:* an analysis of the *cup-shaped cotyledon* mutants. *Plant Cell,* **9** 841-857.

Aida, M., Ishida, T. and Tasaka, M. (1999) Shoot apical meristem and cotyledon formation during *Arubidopsis* embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development,* **126** 1563-1570.

Ball, E. (1948) Differentiation in the primary shoots of *Lupinus albus* L. and of *Tropaeolum majus* **L.** *Symp Soc Exp Biol,* **2** 246-262.

Barton, K.M. (1998) Cell type specification and self renewal in the vegetative shoot apical meristem. *Curr Opin Plant Biol,* **1** 37-42.

Bedesem, P.P. (1958) Histogenetic effects of 2,3,5-triiodobenzoic acid on the shoot apices and leaf primordia of tomato. *Bull **Torr** Bot Club,* **85** 434-472.

Bennett, S.R.M., Alvarez, J., Bossinger, G. and Smyth, D.R. (1995) Morphogenesis in pinoid mutants of *Arabidopsis thaliana. Plant J,* **8** 505-520.

Berleth, T. and Mattsson, J. (2000) Vascular development: tracing signals along veins. *Curr Opin Plant Biol,* **3** 406-411.

Berleth, T., Mattson, J. and Hardtke, C.S. (2000) Vascular continuity and auxin signals. *Trends Plant Sci, 5* 387-393.

Bonetta, D., Bayliss, P., Sun, S., Sage,T. and McCourt, P. (2000) Farnesylation is involved in meristem organization in *Arabidopsis. Planta,* **211** 182-190.

Bowman, J.L. and Eshed, Y. (2000) Formation and maintenance of the shoot apical meristem. *Trends Plant Sci,* ***5*** 110-115.

Brandstatter, J., Rossbach, ***C.*** and Theres, K. (1994) The pattern of *histone H4* expression in the tomato shoot apex changes during development. *Planta,* **192** 69-74.

Brummel, D.A., Harpster, M.H. and Dunsmuir, P. (1999a) Differential expression of expansin gene family members during growth and ripening of tomato. *Plant Mol Biol,* **39** 161-169.

Brummel, D.A., Harpster, M.H., Civello, P.M., Palys, J.M., Bennett, A.B. and Dunsmuir, P. (1999b) Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell,* **11** 2203-2216.

Buvat, R. (1952) Structure, kvolution et fonctionnement du méristème apical de quelques dicotylkdones. *Ann Sci Nat Bot Ser,* **11** 199-300.

Caderas, D., Muster, M., Vogler, H., Mandel, T., Rose, J.K.C., McQueen-Mason, S. and Kuhlemeier, C. (2000) Limited correlation between expansin gene expression and elongation growth rate. *Plant Physiol,* **123** 1399-1413.

Callos, J.D. and Medford, J.I. (1994) Organ positions and pattern formation in the shoot apex. *Plant J,* **6** 1-7.

Callos, D.J., DiRado, M., Xu, B., Behringer, F.J., Link, B.M. and Medford, J.I. (1994) The *Forever Young* gene encodes an oxidoreductase required for proper development of the *Arabidopsis* vegetative shoot apex. *Plant J,* **6** 835-847.

Carpenter, R., Copsey, L., Vincent, C., Doyle, S., Magrath, R. and Coen, E. (1995) Control of flower development and phyllotaxy by meristem identity genes in *Antirrhinum. Plant Cell,* **7** 2001-2011.

Caruso, J.L. (1968) Morphogenetic aspects of a leafless mutant in tomato. I. General patterns in development. *Am J Bot,* 55 1169-1176.

Catala, C., Rose, J.K.C. and Bennett, A.B. (2000) Auxin-regulated genes encoding cell-wall-modifying proteins are expressed during early tomato fruit growth. *Plant Physiol,* **122** 527-534.

Chaudhury, **A.M.,** Letham, S., Craig, S. and Dennis, E.S. (1993) *amp1-a* mutant with high cytokinin levels and altered embryonic pattern, faster in vegetative growth, constitutive photomorphogenesis and precocious flowering. *Plant J,* **4** 907-916.

Christensen, S.K., Dagenais, N., Chory, J. and Weigel, D. (2000) Regulation of the auxin response by the protein kinase PINOID. *Cell,* **100** 469-478.

Cho, H.-T. and Cosgrove, D.J. (2000) Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana. Proc Natl Acad Sci USA,* **97** 9783-9788.

Cho, H.-T. and Kende, H. (1997) Expression of expansin genes is correlated with growth in deepwater rice. *Plant Cell,* **9** 1661-1671.

Chuang, C.-F., Running, M.P., Williams, R.W. and Meyerowitz, E.M. (1999) The *PERIANTHIA* gene encodes a bZIP protein involved in the determination of floral organ number in *Arabidopsis thaliana. Genes Dev,* **13** 334-344.

Church, A.H. (1904) *On the Relation of Phyllotaxis on Mechanical Laws.* Williams and Norgate, London, UK.

Clark, S.E. (1997) Organ formation at the vegetative shoot meristem. *Plant Cell,* **9** 1067-1076.

Clark, S.E., Running, M.P. and Meyerowitz, E.M. (1993) *CLAVATA1,* a regulator of meristem and flower development in *Arabidopsis. Development,* **119** 397-418.

Clark, S.E., Running, M.P. and Meyerowitz, E.M. (1995) *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same process as *CLAVATA1. Development,* 121 2057-2067.

Clark, S.E., Williams, R.W. and Meyerowitz, E.M. (1997) The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis. Cell,* **89** 575-585.

Coen, E.S. and Nugent, J.M. (1994) Evolution of flowers and inflorescences. *Development* (Suppl.), 107-116.

Coen, E.S., Romero, J.M., Doyle, S., Elliot, R., Murray, G. and Carpenter, R. (1990) *Floricaula:* a homeotic gene required for flower development in *Antirrhinum majus. Cell,* **63** 1311-1322.

Cosgrove, D.J. (1997) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *Plant Cell,* **9** 1031-1041.

Cosgrove, D.J. (2000) Loosening of plant cell walls by expansin. *Nature,* **407** 321-326.

Cutter, E.G. (1959) On a theory of phyllotaxis and histogenesis. *Biol Rev,* **34** 243-263.

Cyr, R.J. (1994) Microtubules in plant morphogenesis: role of the cortical array. *Annu Rev Cell Biol,* **10** 153-180.

Davies, P.J. (1995) *Plant Hormones—Physiology, Biochemistry and Molecular Biology,* Kluwer Academic Publishers, Dordrecht, The Netherlands.

De Veylder, L., de Almeida Engler, J., Burssens, S., Manevski, A., Lescure, B., Van Montagu, M., Engler, G. and Inzé, D. (1999) A new D-type cyclin of *Arabidopsis thaliana* expressed during lateral root primordia formation. *Planta,* **208** 453-462.

Dewitte, W., Chiappetta, A., Azmi, A., Witters, E., Strnad, M., Rembur, J., Noin, M., Chriqui, D. and Van Onkelen, H. (1999) Dynamics of cytokinins in apical shoot meristems of a day-neutral tobacco during floral transition and flower formation. *Plant Physiol,* **119** 111-121.

Douady, S. and Couder, Y. (1996) Phyllotaxis as a dynamic self organizing process. Part III: The simulation of the transient regimes of ontogeny. *J Theor Biol,* **178** 295-312.

Endress, P.K. (1994) *Diversity and Evolutionary Biology of Tropical Flowers.* Cambridge University Press, Cambridge.

Elliott, R.C., Betzner, A.S., Huttner, E., Oakes, M.P., Tucker, W.Q., Gerentes, D., Perez, P. and Smyth, D.R. (1996) ***AINTEGUMENTA,*** an APETALA2-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. *Plunt Cell,* **8** 155-168.

Erickson, R.O. and Meicenheimer, R.D. (1977) Photoperiod induced change in phyllotaxis in *Xanthium. Am J Bot,* **64** 981-988.

Erickson, R.O. and Michelini, F.J. (1957) The plastochron index. *Am J Bot,* **44** 297-305.

Faure, J.-D., Vittorioso, P., Santoni, V., Fraisier, V., Prinsen, E., Barlier, **I.,** Van Onckelen, H., Caboche, M. and Bellini, C. (1998) The *PASTICCINO* genes of *Arubidopsis thaliana* are involved in the control of cell division and differentiation. *Development.* **125** 909-918.

Fleming, A.J. and Kuhlemeier, C. (1994) Activation of basal cells of the apical meristem during sepal formation in tomato. *Plant Cell,* **6** 789-798.

Fleming, A.J., Mandel, T., Roth, I. and Kuhlemeier, C. (1993) The patterns of gene expression in the tomato shoot apical meristem. *Plant Cell,* **5** 297-309.

Fleming, A.J., McQueen-Mason, **S.,** Mandel, T. and Kuhlemeier, C. (1997) Induction of leaf primordia by the cell wall protein expansin. *Science,* **276** 1415-1418.

Fleming, A.J., Caderas, D., Wehrli, E., McQueen-Mason, S. and Kuhlemeier, C. (1999) Analysis of expansin-induced morphogenesis on the apical meristem of tomato. *Planta,* **208** 166-174.

Fletcher, J.C. and Meyerowitz, E.M. (2000) Cell signaling within the shoot meristem. *Curr Opin Plant Biol,* **3** 23-30.

Fletcher, J.C., Brand, U., Running, M.P., Simon, R. and Meyerowitz, E.M. (1999) Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. *Science,* **283** 1911-1914.

Foard, D.E. (1971) The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. *Can J Bot,* **49** 694-702.

Fobert, P.R., Coen, E.S., Murphy, G.J.P. and Doonan, J.H. (1994) Patterns of cell division revealed by transcriptional regulation of genes during the cell cycle in plants. *EMBO J,* **13** 616-624.

Fobert. P.R., Gaudin, V., Lunness, P., Coen, E.S. and Doonan, J.H. (1996) Distinct classes of *cdc2*-related genes are differentially expressed during the cell division cycle in plants. *Plant Cell,* **8** 1465-1476.

Furner. I.J. and Pumfrey, J.E. (1992) Cell fate in the shoot apical meristem of *Arabidopsis thaliana. Development,* **115** 755-764.

Gälweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, **K.,** Yephremov, A. and Palme, K. (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science,* **282** 2226-2230.

Goethe, J.W. (1830) Ueber die Spiraltendenz der Vegetation, in *Schriften zur Botanik und Wissenschaftslehre,* dtv Gesamtausgabe 1963, München, Germany.

Green, P.B. (1984) Analysis of axis extension, in *Positional Controls in Plant Development* (eds. P.W. Barlow and D.J. Carr), Cambridge University Press, Cambridge, **UK.** pp. 1-32.

Green, P.B. (1985) Surface of the shoot apex: a reinforcement-field theory for phyllotaxis. *J Cell Sci,* (Suppl.) **2** 181-201.

Green, P.B. (1986) Plasticity in shoot development: a biophysical view, in *Plasticity in Plants* (eds. D.H. Jennings and A.J. Trewavas), Symposia of the Society for Experimental Biology, 40, Company of Biologists, Cambridge, pp. 211-232.

Green, P.B. (1988) A theory for inflorescence development and flower formation based on morphological and biophysical analysis of *Echeveria*. *Planta*, **175** 153-169.

Green, P.B. (1992) Pattern formation in shoots: a likely role for minimal energy configurations of the tunica. *Int J Plant Sci*, **153** S59-S75.

Green, P.B. (1994) Connecting gene and hormone action to form, pattern and organogenesis: biophysical transductions. *J Exp Bot*, **45** 1775-1788.

Green, P.B. (1996) Expression of form and pattern in plants—a role for biophysical fields. *Cell Dev Biol*, **7** 903-911.

Gunning, B.E.S. and Hardham, A.R. (1982) Microtubules. *Annu Rev Plant Physiol*, **33** 651-698.

Hantke, **S.S.,** Carpenter, R. and Coen, **E.S.** (1995) Expression of *Floricaula* in single cell layers of periclinal chimeras activates downstream homeotic genes in all layers of floral meristems. *Development*, **121** 27-35.

Hardtke, C.S. and Berleth, T. (1998) The *Arubidopsis* gene, *MONOPTEROS*, encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J*, **17** 1405-1411.

Hemerly, A., de Almeida Engler, J., Bergounioux, C., Van Montagu, M., Engler, G., Inzé, D. and Ferreira, P. (1995) Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. *EMBO J*, **14** 3925-3936.

Huijser, P., Klein, J., Lonnig, W.-E., Meijer, H., Saedler, H. and Sommer, H. (1992) Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene, *squamosa*, in *Antirrhinum majus*. *EMBO J*, **11** 1239-1249.

Hülskamp, M., Miséra, S. and Jurgens, G. (1994) Genetic dissection of trichome cell development in *Arabidopsis*. *Cell*, **76** 555-566.

Jackson, D. and Hake, **S.** (1999) Control of phyllotaxis in maize by the *abphyll* gene. *Development*, **126** 315-323.

Jacobs, T. (1997) Why do plant cells divide? *Plant Cell*, **9** 1021-1029.

Jean, R.V. (1994) Phyllotaxis: a systematic study in plant morphogenesis. Cambridge University Press, Cambridge, UK.

Jesuthasan, S. and Green, P.B. (1989) On the mechanism of decussate phyllotaxis: biophysical studies on the tunica layer of *Vinca major*. *Am J Bot*, **76** 1152-1166.

Jones, A.M. (1998) Auxin transport: down and out and up again. *Science*, **282** 2201-2202.

Kaplan, D.R. and Hagemann, W. (1991) The relationship of cell and organism in vascular plants. *BioScience*, **41** 693-703.

Keddie, J.S., Carroll, B.J., Thomas, C.M., Reyes, M.E.C., Klimyuk, V., Holtan, H., Gruissem, W. and Jones, D.G.J. (1998) Transposon tagging of the *Defective embryo and meristems* gene of tomato. *Plant Cell*, **10** 877-887.

Keller, E. and Cosgrove, D.J. (1995) Expansins in growing tomato leaves. *Plant J*, **8** 795-802.

Kerstetter, R.A. and Hake, **S.** (1997) Shoot meristem formation in vegetative development. *Plant Cell*, **9** 1001-1010.

Kerstetter, R.A., Laudencia-Chingcuanco, D., Smith, L.G. and Hake, **S.** (1997) Loss-of-function mutations in the maize homeobox gene, *knotted1*, are defective in shoot meristem maintenance. *Development*, **124** 3045-3054.

Kiermayer, **O.** (1959) Zur Beeinflussung der morphoregulatorischen Wirksamkeit von 2,3,5-Trijodobenzoesaure (TIBA) durch α-Naphthylessigsäure. *Physiol Plant*, **12** 841-853.

Kiermayer, **O.** (1960) Die Formative Wirksamkeit der 2,3,5-Trijodobenzoesäure (TIBA) in Gegenwart von Gibberellinsaure **(GA).** *Planta*, **55** 153-168.

Klucher, K.M., Chow, H., Reiser, L. and Fischer, R.L. (1996) The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene, *APETALA2*. *Plant Cell*, **8** 137-153.

Kuhlemeier, C. and Reinhardt, D. (2001) Auxin and phyllotaxis. *Trends Plant Sci*, **6** 187-189.

Laufs, P., Dockx, J., Kronenberger, J. and Traas, J. (1998a) *MGOUN1* and *MGOUN2*: **two** genes required for primordium initiation at the shoot apical and floral meristems in *Arabidopsis thaliana*. *Development*, **125** 1253-1260.

Laufs, P., Grandjean, O., Jonak, C., Kiêu, K. and Traas, J. (1998b) Cellular parameters of the shoot apical meristem in *Arahidopsis*. *Plant Cell,* **10** 1375-1389.

Laufs, P., Jonak, C. and Traas, J. (1998c) Cells and domains: two views of the shoot meristem in *Arabidopsis. Plant Physiol Biochem,* **36** 33-45.

Laux, T., Mayer, K.F.X., Berger, J. and Jurgens, G. (1996) The *wuschel* gene is required for shoot and floral meristem integrity in *Arahidopsis*. *Development,* **122** 87-96.

Lenhard, M. and Laux, T. (1999) Shoot meristem formation and maintenance. *Curr Opin Plant Biol,* **2** 44-50.

Leyser, H.M.O. and Furner, I.J. (1992) Characterization of three shoot apical meristem mutants of *Arabidopsis thaliana*. *Development,* **116** 397-403.

Link, B.M. and Cosgrove, D.J. (1998) Acid-growth response and α-expansins in suspension cultures of Bright Yellow 2 tobacco. *Plant Physiol,* **118** 907-916.

Long, J. and Barton, M.K. (2000) Initiation of axillary and floral meristems in *Arabidopsis. Dev Biol*, **218** 341-353.

Long, J.A., Moan, E.I., Medford, J.I. and Barton, M.K. (1996) A member of the *KNOTTED* class of homeodomain proteins encoded by the STM gene of *Arubidopsis*. *Nature,* **379** 66-69.

Lyndon, R.F. (1990) *Plant Development: The Cellular Basis,* Unwin Hyman, London, UK.

Lyndon, R.F. (1998) *The Shoot Apical Meristem—Its Growth and Development,* Cambridge University Press, Cambridge, UK.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M.K. (1999) The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. *Development,* **126** 469-481.

Maksymowych, R. and Erickson, R.O. (1977) Phyllotactic change induced by gibberellic acid in *Xanthium* shoot apices. *Am J Bot,* **64** 33-44.

Malayer, J.C. and Guard, A.T. (1964) A comparative developmental study of the mutant *sideshootless* and normal tomato plants. *Am J But,* **51** 140-143.

Marc, J. and Hackett, W.P. (1992) Changes in the pattern of cell arrangement at the surface of the shoot apical meristem in *Hedera helix* L. following gibberellin treatment. *Planta,* **186** 503-510.

Mathan, D.S. and Jenkins, J.A. (1962) A morphogenetic study of lanceolate, a leaf-shape mutant in the tomato. *Am J Bot,* **49** 505-514.

Mayer, K.F.X., Schoof, H., Haecker, A., Lenhard, M., Jurgens, G. and Laux, T. (1998) Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell,* **95** 805-815.

McConnell, J.R. and Barton, M.K. (1995) Effect of mutations in the *PINHEAD* gene of *Arabidopsis* on the formation of shoot apical meristems. *Dev Gen,* **16** 358-366.

McQueen-Mason, S.J. and Cosgrove, D.J. (1994) Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. *Proc Natl Acad Sci USA,* **91** 6574-6578.

McQueen-Mason, S.J. and Cosgrove, D.J. (1995) Expansin mode of action on cell walls—analysis of wall hydrolysis, stress relaxation and binding. *Plant Physiol,* **107** 87-100.

Medford, J.I. (1992) Vegetative apical meristems. *Plant Cell,* **4** 1029-1039.

Medford, J.I., Behringer, F.J., Callos, J.D. and Feldmann, K.A. (1992) Normal and abnormal development in the *Arabidopsis* vegetative shoot apex. *Plant Cell,* **4** 631-643.

Meicenheimer, R.D. (1981) Changes in *Epilobium* phyllotaxy induced by *N*-1-naphthylphthalamic acid and α-4-chlorophenoxyisobutyric acid. *Am J Bot,* **68** 1139-1154.

Meicenheimer, R.D. (1982) Change in *Epilobium* phyllotaxy during reproductive transition. *Am J Bot,* **69** 1108-1118.

Meinhardt, H. (1994) Models of pattern formation and their application to plant development, in *Positional Controls in Plant Development* (eds. P.W. Barlow and D.J. Carr), Cambridge University Press, Cambridge, UK, pp. 1-32.

Meinhardt, H. (1996) Models of biological pattern formation: common mechanism in plant and animal development. *Int J Dev Biol,* **40** 123-134.

Meyerowitz, E.M. (1997) Genetic control of cell division patterns in developing plants. *Cell*, **88** 299-308.

Mitchison, G.J. (1977) Phyllotaxis and the Fibonacci series. Science, **196** 270-275.

Moussian, B., Schoof, H., Haecker, A., Jurgens, G. and Laux, T. (1998) Role of the zwille gene in the regulation of central shoot meristem fate during Arabidopsis embryogenesis. EMBO *J*, **17** 1799-1809.

Nick, **P.,** Schafer, E. and Furuya, M. (1992) Auxin redistribution during first positive phototropism in corn coleoptiles — microtubule reorientation and the Cholodny-Went theory. Plant *Physiol*, **99** 1302-1308.

Nishimura, A., Tamaoki, M., Sato, Y. and Matsuoka, M. (1999) The expression of tobacco *knotted1*-type class 1 homeobox genes correspond to regions predicted by the cytohistological zonation model. Plant *J*, **18** 337-347.

Nougarède, A. (1967) Experimental cytology of the shoot apical cells during vegetative growth and flowering. Int *Rev* Cytol, **21** 203-351.

Okada, K. and Shimura, Y. (1994) The *pin-formed* gene, in Arabidopsis, An Atlas of Morphology and Development (ed. J.J. Bowman) Springer Verlag, New York, pp. 180-183.

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J. and Shimura, Y. (1991) Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell, **3** 677-684.

Parsons, R.L., Behringer, F.J. and Medford, J.I. (2000) The SCHIZOID gene regulates differentiation and cell division in Arabidopsis *thaliana* shoots. *Planta*, **211** 34-42.

Picket, F.B., Marino Champagne, M. and Meeks-Wagner, D.R. (1996) Temperature-sensitive mutations that arrest Arahidopsis shoot development. *Development*, **122** 3799-3807.

Pilkington, M. (1929) The regeneration of the stem apex. New Phytol, **28** 37-53.

Popham, R.A. (1951) Principle types of shoot apex organization in vascular plants. *Ohio J* Sci, **51** 249-270.

Przemeck, G.K.H., Mattson, J., Hardtke, **C.S.,** Sung, Z.R. and Berleth, T. (1996) Studies on the role of the Arabidopsis gene, MONOPTEROS, in vascular development and plant cell axialization. *Planta*, **200** 229-237.

Ratcliff, O.J., Riechmann, J.L. and Zhang, J.Z. (2000) INTERFASCICULAR FIBERLESS is the same gene as REVOLUTA. Plant Cell, **12** 315-317.

Reinhardt, D., Wittwer, F., Mandel, T. and Kuhlemeier, C. (1998) Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. Plant Cell, **10** 1427-1437.

Reinhardt, D., Mandel., T. and Kuhlemeier, C. (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant *Cell*, **12** 507-518.

Richards, F.J. (1951) Phyllotaxis: its quantitative expression and relation to growth in the apex. Phil Trans R *Soc Lond B*, **235** 509-564.

Roe, J.L., Rivin, C.J., Sessions, R.A., Feldmann, **K.A.** and Zambryski, P.C. (1993) The Tousled gene in **A.** thaliana encodes a protein kinase homolog that is required for leaf and flower development. Cell, **75** 939-950.

Rose, J.K.C., Lee, H.H. and Bennet, A.B. (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proc *Natl* Acad *Sci USA*, **94** 5955-5960.

Running, M.P. and Meyerowitz, E.M. (1996) Mutations in the PERIANTHIA gene of Arabidopsis specifically alter floral organ number and initiation pattern. Development, **122** 1261-1269.

Sachs, T. (1991a) Cell polarity and tissue patterning in plants. Development, (Suppl.) **1** 83-93.

Sachs, T. (1991b) Pattern Formation in Plant Tissues, Cambridge University Press, New York, NY.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F., Jurgens, G. and Laux, T. (2000) The stem cell popupation of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell, **100** 635-644.

Schoute, J.C. (1913) Beiträge zur Blattstellungslehre. *Réc* Trav Bot *Néerl*, **10** 153-235.

Schuepp, **O.** (1938) Ueber periodische Formbildung bei Pflanzen. Biol Rev, **13** 59-92.

Schumacher, K., Schmitt, T., Rossberg, M., Schmitz, G. and Theres, K. (1999) The lateral suppressor (*Ls*) gene of tomato encodes a new member of the VKIID protein family. Proc *Natl* Acad Sci *USA*, **96** 290-295.

Schwabe, W.W. (1971) Chemical modification of phyllotaxis and its implications. *Symp Soc Exp Biol*, **25** 301-322.

Schwabe, W.W. (1984) Phyllotaxis, in *Positional Controls in Plant Development* (eds. P.W. Barlow and D.J. Carr), Cambridge University Press, Cambridge, UK, pp. 403-440.

Segers, G., de Almeida Engler, J., Bergounioux, C., Gadisseur, I., Jaqmard, A., Van Montagu, M. and Inzé, D. (1996) The *Arabidopsis* cyclin-dependent kinase gene, *cdc2bAt*, is preferentially expressed during S and G2 phases of the cell cycle. *Plant J*, **10** 601-612.

Selker, J.M.L., Steucek, G.L. and Green, P.B. (1992) Biophysical mechanisms for morphogenetic progression at the shoot apex. *Dev Biol*, **153** 29-43.

Sessions, A., Nemhauser, J.L., McColl, A., Roe, J.L., Feldmann, K.A. and Zambryski, P.C. (1997) *ETTIN* patterns the *Arahidopsis* floral meristem and reproductive organs. *Development*, **124** 4481-4491.

Sessions, A., Weigel, D. and Yanofsky, M.F. (1999) The *Arabidopsis thaliana MERISTEM LAYER 1* promoter specifies epidermal expression in meristems and young primordia. *Plant J*, **20** 259-263.

Sharman, B.C. (1942) Developmental anatomy of the shoot of *Zea mays* L. *Ann Bot*, **6** 245-282.

Sharman, B.C. (1947) The biology and developmental morphology of the shoot apex in Gramineae. *New Phytol*, **46** 20-34.

Shibaoka, H. (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. *Annu Rev Plant Physiol Plant Mol Biol*, **45** 527-544.

Slack, J.M.W. (1991) *From Egg to Embryo*. 2nd edn., Cambridge University Press, Cambridge, UK.

Smith, L.G., Hake, S. and Sylvester, A.W. (1996) The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape. *Development*, **122** 481-489.

Smith, R.H. and Murashige, T. (1970) *In vitro* development of the isolated shoot apical meristem of angiosperms. *Am J Bot*, **57** 562-568.

Snow, M. and Snow, R. (1931) Experiments on phyllotaxis. I. The effect of isolating a primordium. *Phil Trans R Soc Lond B*, **221** 1-43.

Snow, M. and Snow, R. (1933) Experiments on phyllotaxis. II. The effect of displacing a primordium. *Phil Trans R Soc Lond B*, **222** 354-400.

Snow, M. and Snow, R. (1937) Auxin and leaf formation. *New Phytol*, **36** 1-18.

Snow, M. and Snow, R. (1962) A theory of the regulation of phyllotaxis based on *Lupinus albus*. *Phil Trans R Soc Lond B*, **244** 483-514.

Souer, E., van Houwelingen, **A.,** Kloos, D., Mol, J. and Koes, R. (1996) The *No Apical Meristem* gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell*, **85** 159-170.

Steeves, T.A. and Sussex, I.M. (1989) *Patterns in Plant Development*. Cambridge University Press, New York, NY.

Stewart, R.N. and Dermen, **H.** (1970) Determination of number and mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. *Am J Bot*, **57** 816-826.

Stewart, R.N. and Dermen, H. (1975) Flexibility in ontogeny as shown by the contribution of the shoot apical layers to leaves of periclinal chimeras. *Am J Bot*, **62** 935-947.

Talbert, P.B., Adler, H.T., Parks, D.W. and Comai, L. (1995) The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana*. *Development*, **121** 2723-2735.

Torres-Ruiz, **R.A.** and Jürgens, G. (1994) Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. *Development*, **120** 2967-2978.

Traas, J., Bellini, C., Nacry, P., Kronenberger, J., Bouchez, D. and Caboche, M. (1995) Normal differentiation patterns in plants lacking microtubular preprophase bands. *Nature*, **375** 676-677.

van Iterson, G., (1907) *Mathematische und mikroskopisch-anatomische Studien über Blattstellung, nebst Betrachtungen über den Schalenbau der Miliolinen*. Gustav Fischer Verlag, Jena.

Veen, A.H. and Lindenmayer, A. (1977) Diffusion mechanism for phyllotaxis: theoretical, physicochemical and computer study. *Plant Physiol*, **60** 127-139.

Vernoux, T., Kronenberger, J., Greandjean, O., Laufs, P. and Traas, J. (2000) *PZN-FORMED1* regulates cell fate at the periphery of the shoot apical meristem. *Development,* **127** 5157-5165.

Veit, B., Briggs, S.P., Schmidt, R.J., Yanofsky, M.F. and Hake, S. (1998) Regulation of leaf initiation by *Terminal Ear1* gene of maize. *Nature,* **393** 166-168.

Wardlaw, C.W. (1949) Further experimental observation on the shoot apex of *Dryopteris aristata* Druce. *Phil Trans R Soc Lond B,* **233** 415-451.

Weberling, F. (1989) Morphology of flowers and inflorescences. Cambridge University Press, Cambridge, UK.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) LEAFY controls floral meristem identity in *Arabidopsis. Cell,* **69** 843-859.

Williams, R.F. (1975) The shoot apex and leaf growth. Cambridge University Press, Cambridge, UK.

Ziegelhoffer, E.C., Medrano, L.J. and Meyerowitz, E.M. (2000) Cloning of the *WIGGUM* gene identifies a role for farnesylation in meristem development. *Proc Natl Acad Sci USA,* **97** 7633-7638.