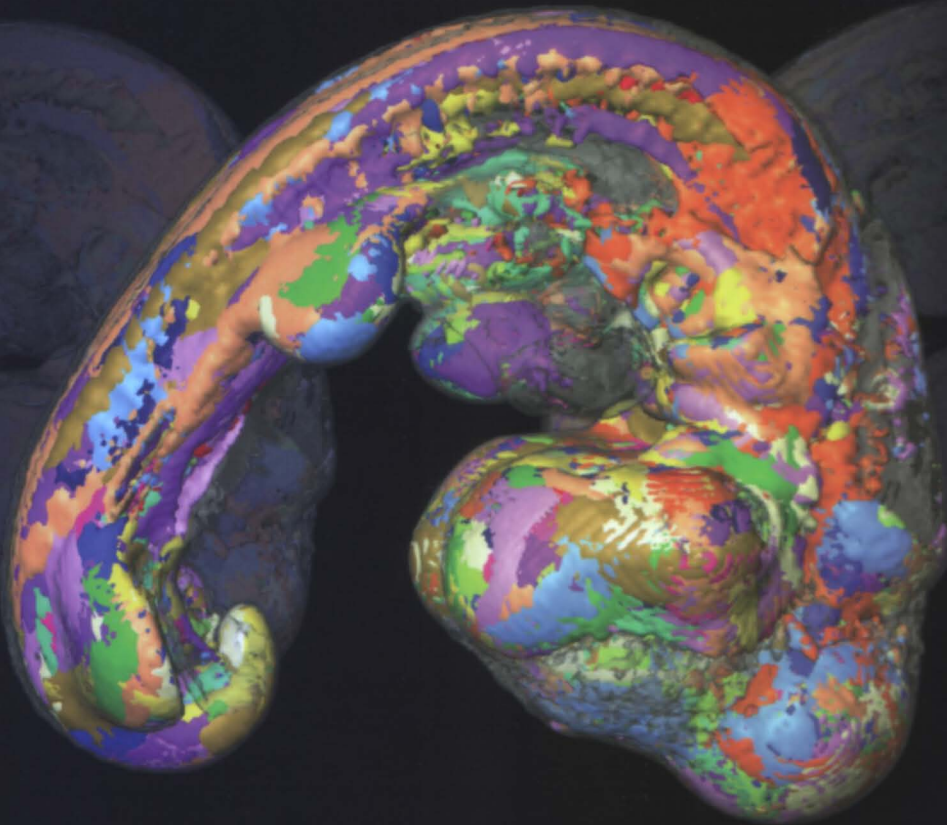OXFORD

Principles of
Development

Fourth edition

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Plant development

- **■ Embryonic development**
- **■ Meristems**
- **■ Flower development and control of flowering**

Because of plant cells' rigid cell walls and lack of cell movement within tissues, a plant's architecture is very much the result of patterns of oriented cell divisions. Despite this apparent invariance, however, cell fate in development is largely determined by similar means as in animals—by a combination of positional signals and intercellular communication. As well as communicating by extracellular signals and cell-surface interactions, plant cells are interconnected by cytoplasmic channels, which allow movement of proteins such as transcription factors directly from cell to cell.

The plant kingdom is very large, ranging from the algae, many of which are unicellular, to the multicellular land plants, which exist in a prodigious variety of forms. Plants and animals probably evolved the process of multicellular development independently, their last common ancestor being a unicellular eukaryote some 1.6 billion years ago. Plant development is, therefore, of interest not simply for its own sake and for its agricultural importance. By looking at the similarities and differences between plant and animal development, a study of plant development can shed light on the way that developmental mechanisms in two groups of multicellular organisms have evolved independently and under different sets of developmental constraints.

Do plants and animals use the same developmental mechanisms? As we shall see in this chapter, the logic behind the spatial layouts of gene expression that pattern a developing flower is similar to that of Hox gene action in patterning the body axis in animals, but the genes involved are completely different. Such similarities between plant and animal development are due to the fact that the basic means of regulating gene expression are the same in both, and thus similar general mechanisms for patterning gene expression in a multicellular tissue are bound to arise. As we shall see, many of the general control mechanisms we have encountered in animal development, such as asymmetric cell division, the response to positional signals, lateral inhibition, and changes in gene expression in response to extracellular signals, are all present in plants. Differences between plants and animals in the way development is controlled arise from some different ways in which plant cells can communicate with each other compared with animal cells, from the existence of rigid cell walls and the lack of large-scale cell movements, to the fact that environment has a much greater impact on plant development than on that of animals.

One general difference between plant and animal development is that most of the development occurs not in the embryo but in the growing plant. Unlike an animal embryo, the mature plant embryo inside a seed is not simply a smaller version of

the organism it will become. All the 'adult' structures of the plant—shoots, roots, stalks, leaves, and flowers—are produced after germination from localized groups of undifferentiated cells known as **meristems**. Two meristems are established in the embryo: one at the tip of the root and the other at the tip of the shoot. These persist in the adult plant, and almost all the other meristems, such as those in developing leaves and flower shoots, are derived from them. Cells within meristems can divide repeatedly and can potentially give rise to all plant tissues and organs. This means that developmental patterning within meristems to produce organs such as leaves and flowers continues throughout a plant's life.

Plant and animal cells share many common internal features and much basic biochemistry, but there are some crucial differences that have a bearing on plant development. One of the most important is that plant cells are surrounded by a framework of relatively rigid cell walls. There is therefore virtually no cell migration in plants, and major changes in the shape of the developing plant cannot be achieved by the movement and folding of sheets of cells. In plant development, form is largely generated by differences in rates of cell division and by division in different planes, followed by directed enlargement of the cells.
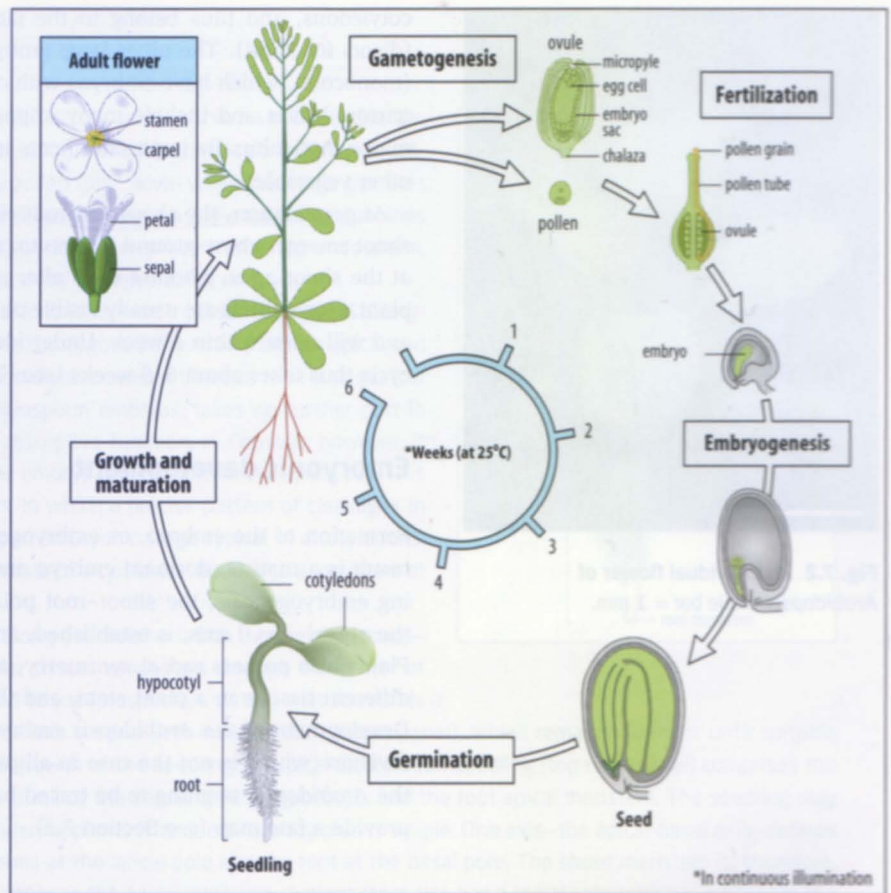
As in animal development, one of the main questions in plant development is how cell fate is determined. Many structures in plants normally develop by stereotyped patterns of cell division, but despite this observation, which implies the importance of lineage, cell fate is, in many cases, also known to be determined by factors such as position in the meristem and cell–cell signaling. The cell wall would seem to impose a barrier to the passage of large signaling molecules, such as proteins, although it is very thin in some regions, such as the meristems; but all known plant extracellular signaling molecules—such as auxins, gibberellins, cytokinins, and ethylene—are small molecules that easily penetrate cell walls. Plant cells also communicate with each other through fine cytoplasmic channels known as **plasmodesmata**, which link neighboring plant cells through the cell wall, and they may be the channels by which some developmentally important gene-regulatory proteins, and even mRNAs, move directly between cells. The size of the channel varies and those in shoot cells have the largest diameter.

Another important difference between plant and animal cells is that a complete, fertile plant can develop from a single differentiated somatic cell and not just from a fertilized egg. This suggests that, unlike the differentiated cells of adult animals, some differentiated cells of the adult plant may retain **totipotency**. Perhaps they do not become fully determined in the sense that adult animal cells do, or perhaps they are able to escape from the determined state, although how this could be achieved is as yet unknown. In any case, this difference between plants and animals illustrates the dangers of the wholesale application to plant development of concepts derived from animal development. Nevertheless, the genetic analysis of development in plants is turning up instances of genetic strategies for developmental patterning rather similar to those of animals.

The small cress-like weed *Arabidopsis thaliana* has become the model plant for genetic and developmental studies, and will provide many of the examples in this chapter. We will begin by describing the main features of its morphology, life cycle, and reproduction.

7.1 The model plant *Arabidopsis thaliana* has a short life-cycle and a small diploid genome

The equivalent of *Drosophila* in the study of plant development is the small crucifer *Arabidopsis thaliana*, commonly called thale cress, which is well-suited to genetic and developmental studies. It is a diploid (unlike many plants, which are polyploid)

Fig. 7.1 Life cycle of *Arabidopsis*. In flowering plants, egg cells are contained separately in ovules inside the carpels. Fertilization of an egg cell by a male nucleus from a pollen grain takes place inside the ovary. The egg then develops into an embryo contained within the ovule coat, forming a seed. *Arabidopsis* is a dicotyledon, and the mature embryo has two wing-like cotyledons (storage organs) at the apical (shoot) end of the main axis—the hypocotyl—which contains a shoot meristem at one end and a root meristem at the other. Following germination, the seedling develops into a plant with roots, a stem, leaves, and flowers. The photograph shows five mature *Arabidopsis* plants.

and has a relatively small, compact genome that has been sequenced and which contains about 27,000 protein-coding genes. It is an annual, flowering in the first year of growth, and develops as a small ground-hugging rosette of leaves, from which a branched flowering stem is produced with a flowerhead, or **inflorescence**, at the end of each branch. It develops rapidly, with a total life cycle in laboratory conditions of some 6–8 weeks, and like all flowering plants, mutant strains and lines can easily be stored in large quantities in the form of seeds. The life cycle of *Arabidopsis* is shown in Fig. 7.1.

Each *Arabidopsis* flower (Fig. 7.2) consists of four sepals surrounding four white petals; inside the petals are six stamens, the male sex organs, which produce pollen containing the male gametes. Petals, sepals, and the other floral organs are thought to derive evolutionarily from modified leaves. At the center of the flower are the female sex organs, which consist of an ovary of two carpels, which contain the **ovules**. Each ovule contains an egg cell. Fertilization of an egg cell occurs when a pollen grain deposited on the carpel surface grows a tube that penetrates the carpel and delivers two haploid pollen nuclei to an ovule. One nucleus fertilizes the egg cell, while the other fuses with two other nuclei in the ovule. This forms a triploid cell that will develop into a specialized nutritive tissue—the **endosperm**—that surrounds the fertilized egg cells and provides the food source for embryonic development.

Following fertilization, the embryo develops inside the ovule, taking about 2 weeks to form a mature seed, which is shed from the plant. The seed will remain dormant until suitable external conditions trigger germination. The early stages of germination and seedling growth rely on food supplies stored in the **cotyledons** (seed leaves), which are storage organs developed by the embryo. *Arabidopsis* embryos have two

Fig. 7.2 An individual flower of Arabidopsis. Scale bar = 1 mm.

cotyledons, and thus belong to the large group of plants known as dicotyledons (dicots for short). The other large group of flowering plants is the monocotyledons (monocots), which have embryos with one cotyledon; monocots typically have long, narrow leaves and include many important staple crops, such as wheat, rice, and maize. Agriculturally important dicots include potato, tomato, sugar beet, and most other vegetables.

At germination, the shoot and root elongate and emerge from the seed. Once the shoot emerges above ground it starts to photosynthesize and forms the first true leaves at the shoot apex. About 4 days after germination the seedling is a self-supporting plant. Flower buds are usually visible on the young plant 3–4 weeks after germination and will open within a week. Under ideal conditions, the complete *Arabidopsis* life cycle thus takes about 6–8 weeks (see Fig. 7.1).

Embryonic development

Formation of the embryo, or embryogenesis, occurs inside the ovule, and the end result is a mature, dormant embryo enclosed in a seed awaiting germination. During embryogenesis, the shoot–root polarity of the plant body, which is known as the **apical–basal** axis, is established, and the shoot and root meristems are formed. Plants also possess **radial symmetry**, as seen in the concentric arrangement of the different tissues in a plant stem, and this **radial axis** is also set up in the embryo. Development of the *Arabidopsis* embryo involves a rather invariant pattern of cell division (which is not the case in all plant embryos) and this enables structures in the *Arabidopsis* seedling to be traced back to groups of cells in the early embryo to provide a fate map (see Section 7.2).

7.2 Plant embryos develop through several distinct stages

Arabidopsis belongs to the angiosperms, or flowering plants, one of the two major groups of seed-bearing plants; the other is the gymnosperms, or conifers. The typical course of embryonic development in angiosperms is outlined in Box 7A. Like an animal zygote, the fertilized plant egg cell undergoes repeated cell divisions, cell growth, and differentiation to form a multicellular embryo. The first division of the zygote is at right angles to the long axis, dividing it into an apical cell and a basal cell, and establishing an initial polarity that is carried over into the apical–basal polarity of the embryo and into the apical–basal axis of the plant. In many species, the first zygotic division is unequal, with the basal cell larger than the apical cell. The basal cell divides to give rise to the **suspensor**, which may be several cells long (see Box 7A). This attaches the embryo to maternal tissue and is a source of nutrients. The apical cell divides vertically to form a two-celled **proembryo**, which will give rise to the rest of the embryo. In some species, the basal cell contributes little to the further development of the embryo, but in others, such as *Arabidopsis*, the topmost suspensor cell is recruited into the embryo, where it is known as the **hypophysis**, and contributes to the embryonic root meristem and root cap.

The next two divisions produce an eight-cell **octant-stage** embryo, which develops into a **globular-stage** embryo of around 32 cells (Fig. 7.3). The embryo elongates and the cotyledons start to develop as wing-like structures at one end, while an embryonic root forms at the other. This stage is known as the **heart stage**. Two groups of undifferentiated cells capable of continued division are located at each end of this axis; these are known as the **apical meristems**. The meristem lying between the cotyledons gives rise to the shoot, while the one at the opposite end of the axis, towards the end of the embryonic root, will drive root growth at germination. The region in between the embryonic root and the future shoot will become the seedling stem or

## Box 7A Angiosperm embryogenesis

In flowering plants (angiosperms), the egg cell is contained within an ovule inside the ovary in the flower (right inset in the bottom panel). At fertilization, a pollen grain deposited on the surface of the stigma puts out a pollen tube, down which two male gametes migrate into the ovule. One male gamete fertilizes the egg cell while the other combines with another cell inside the ovule to form a specialized nutritive tissue, the endosperm, which surrounds, and provides the food source for, the developing embryo.

The small annual weed *Capsella bursa-pastoris* (shepherd's purse) is a typical dicotyledon. The first, asymmetric, cleavage divides the zygote transversely into an apical and a basal cell (bottom panel). The basal cell then divides several times to form a single row of cells—the suspensor—which, in many angiosperm embryos, takes no further part in embryonic development, but may have an absorptive function; in *Capsella*, however, it contributes to the root meristem. Most of the embryo is derived from the apical cell. This undergoes a series of stereotyped divisions, in which a precise pattern of cleavages in different planes gives rise to the heart-shaped embryonic stage typical of dicotyledons. This develops into a mature embryo that consists of a cylindrical body with a meristem at either end, and two cotyledons.

The early embryo becomes differentiated along the radial axis into three main tissues—the outer epidermis, the prospective vascular tissue, which runs through the center of the main axis and cotyledons, and the ground tissue (prospective cortex) that surrounds it.

The ovule containing the embryo matures into a seed (left inset in the bottom panel), which remains dormant until suitable external conditions trigger germination and growth of the seedling. A typical dicotyledon seedling (top right panel) comprises the shoot apical meristem, two cotyledons, the trunk of the seedling (the hypocotyl), and the root apical meristem. The seedling may be thought of as the phylotypic stage of flowering plants. The seedling body plan is simple. One axis—the apical-basal axis—defines the main polarity of the plant. The shoot forms at the apical pole and the root at the basal pole. The shoot meristem is, therefore, referred to in the seedling and adult plant simply as the apical meristem. A plant stem also has a radial axis, which is evident in the radial symmetry in the hypocotyl and is continued in the root and shoot. In the center is the vascular tissue, which is surrounded by cortex, and an outer covering of epidermis. At later stages, structures such as leaves and other organs have a dorso-ventral axis running from the upper surface to the lower surface.

| Globular stage | Early heart stage | Late heart stage | Mature embryo stage |
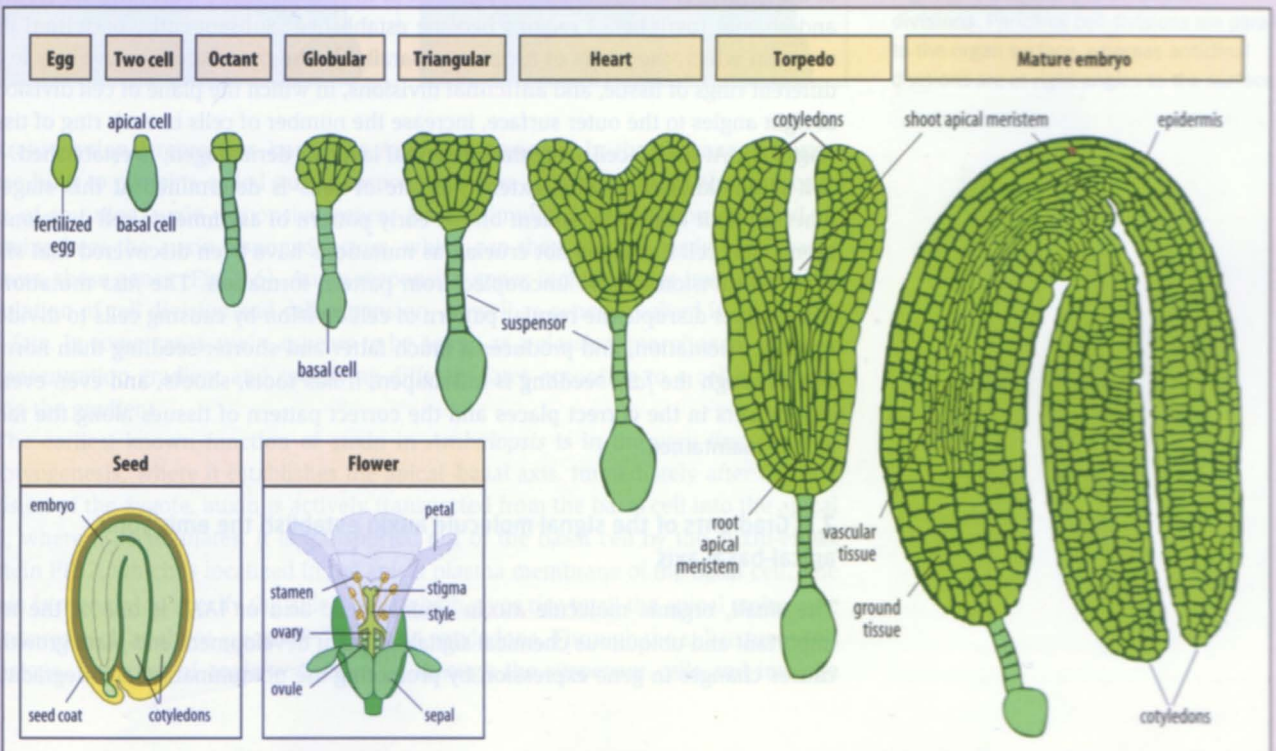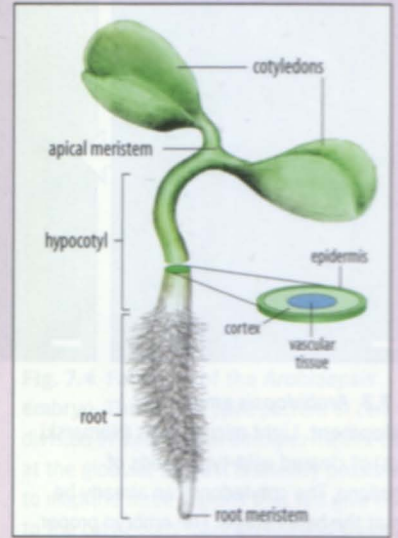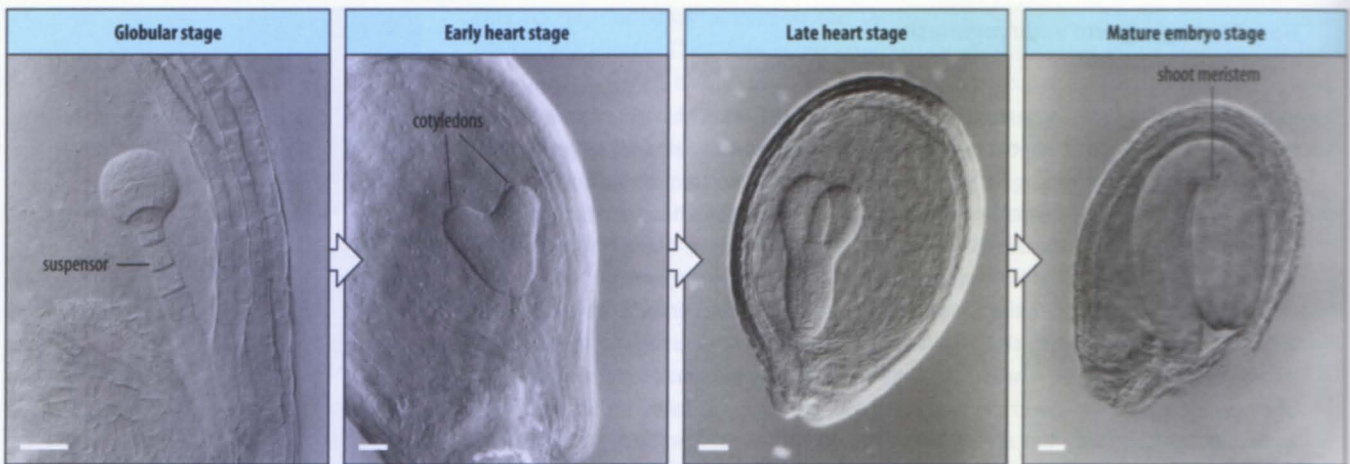
Fig. 7.3 *Arabidopsis* **embryonic development.** Light micrographs (Nomarski optics) of cleared wild-type seeds of *A. thaliana*. The cotyledons can already be seen at the heart stage. The embryo proper is attached to the seed coat through a filamentous suspensor. Scale bar = 20 μm.

Photographs courtesy of D. Meinke, from Meinke, D.W.: 1994.

hypocotyl. Almost all above ground adult plant structures are derived from the apical meristems. The main exception is radial growth in the stem, which is most evident in woody plants, and which is produced by the **cambium**, a ring of secondary meristematic tissue in the stem. After the embryo is mature, the apical meristems remain quiescent until germination.

Seedling structures can be traced back to groups of cells in the early embryo to provide a fate map (Fig. 7.4). In *Arabidopsis*, patterns of cleavage up to the 16-cell stage are highly reproducible, and even at the octant stage it is possible to make a fate map for the major regions of the seedling along the apical–basal axis. The upper tier of cells gives rise to the cotyledons and the shoot meristem, the next tier is the origin of the hypocotyl, and the bottom tier together with the region of the suspensor where it joins the embryo will give rise to the root (see Section 7.3). At the heart stage, the fate map is clear.

The radial pattern in the embryo comprises three concentric rings of tissue: the outer **epidermis**, the ground tissue (**cortex** and **endodermis**), and the vascular tissue at the center. This radial axis appears first at the octant stage, when adaxial (central) and abaxial (peripheral) regions become established. Subsequently, **periclinal** divisions, in which the plane of division is parallel to the outer surface, give rise to the different rings of tissue, and **anticlinal** divisions, in which the plane of cell division is at right angles to the outer surface, increase the number of cells in each ring of tissue (Fig. 7.5). At the 16-cell stage the epidermal layer, or dermatogen, is established.

It is not known to what extent the fate of cells is determined at this stage or whether their fate is dependent on the early pattern of asymmetric cell divisions. It seems that cell lineage is not crucial, as mutations have been discovered that show that cell division can be uncoupled from pattern formation. The *fass* mutation in *Arabidopsis* disrupts the regular pattern of cell division by causing cells to divide in random orientation, and produces a much fatter and shorter seedling than normal. But although the *fass* seedling is misshapen, it has roots, shoots, and even eventually flowers in the correct places and the correct pattern of tissues along the radial axis is maintained.

7.3 Gradients of the signal molecule auxin establish the embryonic apical–basal axis

The small, organic molecule **auxin** (indoleacetic acid or IAA) is one of the most important and ubiquitous chemical signals in plant development and plant growth. It causes changes in gene expression by promoting the ubiquitination and degradation
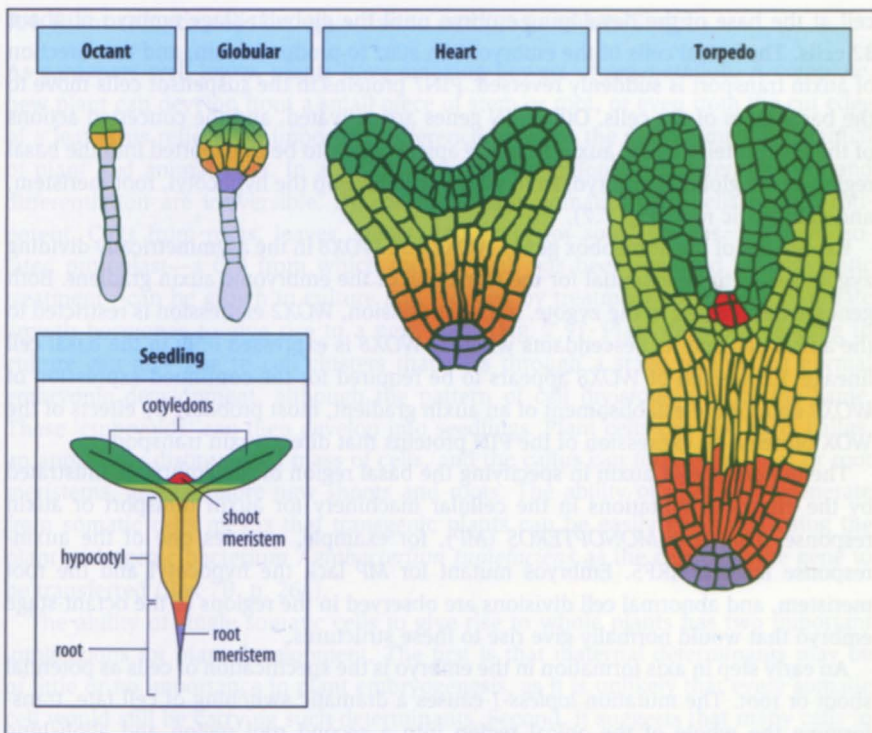
Fig. 7.4 Fate map of the *Arabidopsis* embryo. The stereotyped pattern of cell division in dicotyledon embryos means that at the globular stage it is already possible to map the three regions that will give rise to the cotyledons (dark green) and shoot meristem (red), the hypocotyl (yellow), and the root meristem (purple) in the seedling. *After Scheres, B., et al.: 1994.*
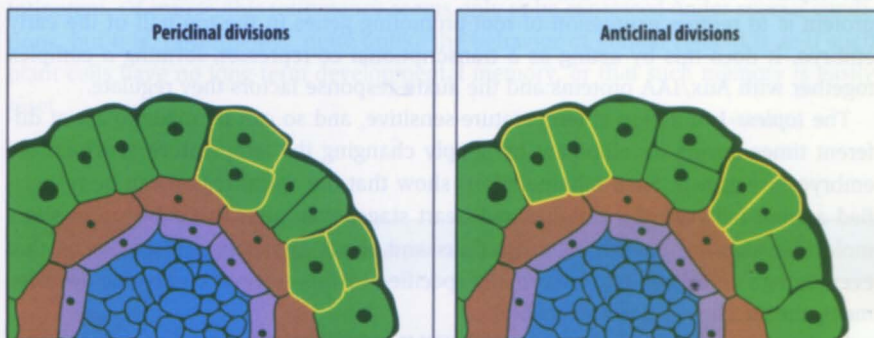

Fig. 7.5 Periclinal and anticlinal divisions. Periclinal cell divisions are parallel to the organ surface, whereas anticlinal divisions are at right angles to the surface.

of transcriptional repressors known as **Aux/IAA proteins**. In the absence of auxin, these bind to proteins called **auxin-response factors** (**ARFs**) to block the transcription of so-called auxin-responsive genes. Auxin-stimulated degradation of Aux/IAA proteins frees the auxin-response factors, which can then activate, or in some cases repress, these genes (Fig. 7.6). Auxin-responsive genes include genes involved in the regulation of cell division and cell expansion, as well as genes involved in specifying cell fate. In some cases auxin appears to be acting as a classical morphogen, forming a concentration gradient and specifying different fates according to a cell's position along the gradient.
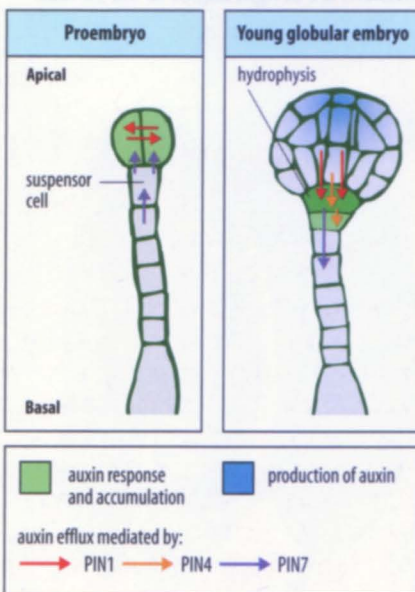
The earliest known function of auxin in *Arabidopsis* is in the very first stage of embryogenesis, where it establishes the apical–basal axis. Immediately after the first division of the zygote, auxin is actively transported from the basal cell into the apical cell, where it accumulates. It is transported out of the basal cell by the auxin-efflux protein PIN7, which is localized in the apical plasma membrane of the basal cell. The auxin is required to specify the apical cell, which gives rise to all the apical embryonic structures such as shoot apical meristem and cotyledons. Through the subsequent cell divisions, transport of auxin continues up through the suspensor cells and into the
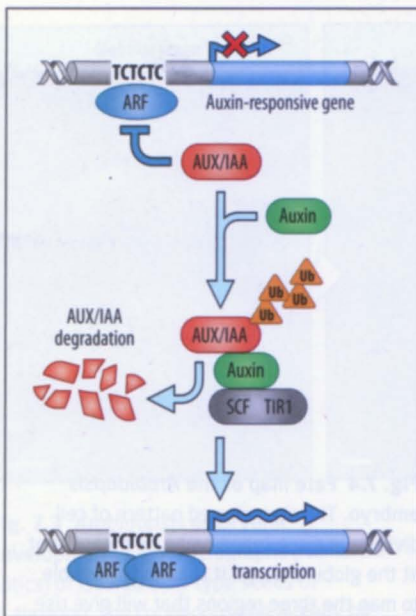
Fig. 7.6 Auxin signaling pathway. In the absence of auxin, AUX/IAA proteins bind to and repress the activity of transcription factors in the AUXIN RESPONSE FACTOR (ARF) family, which bind TGTCTC-containing DNA sequence elements in the promoters of auxin-responsive genes. Auxin targets the ubiquitin ligase complex SCF/TIR1 to the AUX/IAA protein, causing it to be ubiquitinated (Ub). This modification targets AUX/IAA for degradation and thus lifts the repression of the auxin-responsive gene, which can then be transcribed.

Adapted from Chapman, E.J. and Estelle, M.: 2009.

Fig. 7.7 The role of auxin in patterning the early embryo. Left panel: auxin produced in the original basal cell accumulates in the two-celled proembryo (green) through transport in the basal to apical direction mediated by the protein PIN7, which is located in the apical membranes of the basal and suspensor cells (purple arrows). Another PIN protein, PIN1, transports auxin between the two cells of the proembryo (red arrows). Cell division distributes auxin into the developing embryo. Right panel: at the globular stage, free auxin starts to be produced at the apical pole and the direction of auxin transport is reversed. PIN7 becomes localized in basal membranes of the suspensor cells and the proteins PIN1 and PIN4 (orange arrows) transport auxin from the apical region into the most basal cell of the embryo, known as the hypophysis, where it accumulates.

cell at the base of the developing embryo until the globular-stage embryo of about 32 cells. The apical cells of the embryo then start to produce auxin, and the direction of auxin transport is suddenly reversed. PIN7 proteins in the suspensor cells move to the basal faces of the cells. Other *PIN* genes are activated, and the concerted actions of the PIN proteins cause auxin from the apical region to be transported into the basal region of the globular embryo, from which will develop the hypocotyl, root meristem, and embryonic root (Fig. 7.7).

Expression of the homeobox genes *WOX2* and *WOX8* in the asymmetrically dividing zygote seems to be essential for the formation of the embryonic auxin gradient. Both genes are expressed in the zygote, but after division, *WOX2* expression is restricted to the apical cell and its descendants whereas *WOX8* is expressed only in the basal cell lineage. Expression of *WOX8* appears to be required for the continued expression of *WOX2* and for the establishment of an auxin gradient, most probably by effects of the WOX proteins on expression of the PIN proteins that direct auxin transport.

The importance of auxin in specifying the basal region of the embryo is illustrated by the effects of mutations in the cellular machinery for auxin transport or auxin response. The gene *MONOPTEROS* (*MP*), for example, encodes one of the auxin-response factors, ARF5. Embryos mutant for *MP* lack the hypocotyl and the root meristem, and abnormal cell divisions are observed in the regions of the octant-stage embryo that would normally give rise to these structures.

An early step in axis formation in the embryo is the specification of cells as potential shoot or root. The mutation *topless-1* causes a dramatic switching of cell fate, transforming the whole of the apical region into a second root region and abolishing development of cotyledons and shoot meristem. The normal function of the TOPLESS protein is to repress expression of root-promoting genes in the top half of the early embryo. It does this by acting as a transcriptional co-repressor, forming a complex together with Aux/IAA proteins and the auxin-response factors they regulate.

The *topless-1* mutation is temperature-sensitive, and so can be made to act at different times during development by simply changing the temperature at which the embryos are grown. Such manipulations show that the apical region can be respecified as root between the globular and heart stage, even after it has begun to show molecular signs of developing cotyledons and shoot meristem. This suggests that even though apical cell fate is normally specified by this stage of embryonic development, the decision is not irreversible.

Mutations in the gene *SHOOT MERISTEMLESS* (*STM*) completely block the formation of the shoot apical meristem, but have no effect on the root meristem or other parts of the embryo. *STM* encodes a transcription factor that is also required to maintain cells in the pluripotent state in the adult shoot meristem. The pattern of *STM* expression develops gradually, which is also typical of several other genes that characterize the shoot apical meristem. Expression is first detected in the globular stage in one or two cells and only later in the central region between the two cotyledons (Fig. 7.8).

## 7.4 Plant somatic cells can give rise to embryos and seedlings

As gardeners well know, plants have amazing powers of regeneration. A complete, new plant can develop from a small piece of stem or root, or even from the cut edge of a leaf. This reflects an important difference between the developmental potential of plant and animal cells. In animals, with few exceptions, cell determination and differentiation are irreversible. By contrast, many somatic plant cells remain toti-potent. Cells from roots, leaves, stems, and even, for some species, a single, iso-lated protoplast—a cell from which the cell wall has been removed by enzymatic treatment—can be grown in culture and induced by treatment with the appropriate growth hormones to give rise to a new plant (Fig. 7.9). Plant cells proliferating in culture can give rise to cell clusters that pass through a stage resembling normal embryonic development, although the pattern of cell divisions are not the same. These 'embryoids' can then develop into seedlings. Plant cells can also form callus, an apparently disorganized mass of cells, and the callus can form new shoot or root meristems, and therefore new shoots and roots. The ability of plants to regenerate from somatic cells means that transgenic plants can be easily generated using the plant pathogenic bacterium *Agrobacterium tumefaciens* as the carrier of the gene to be transferred (Box 7B, p. 264).

The ability of single somatic cells to give rise to whole plants has two important implications for plant development. The first is that maternal determinants may be of little or no importance in plant embryogenesis, as it is unlikely that every somatic cell would still be carrying such determinants. Second, it suggests that many cells in the adult plant body are not fully determined with respect to their fate, but remain totipotent. Of course, this totipotency seems only to be expressed under special condi-tions, but it is, nevertheless, quite unlike the behavior of animal cells. It is as if such plant cells have no long-term developmental memory, or that such memory is easily reset.

Fig. 7.8 Section through a late heart-stage *Arabidopsis* embryo showing expression of **SHOOT MERISTEMLESS (STM)**. At this stage, the STM RNA (stained red) is expressed in cells located between the cotyledons. Scale bar = 25 µm.

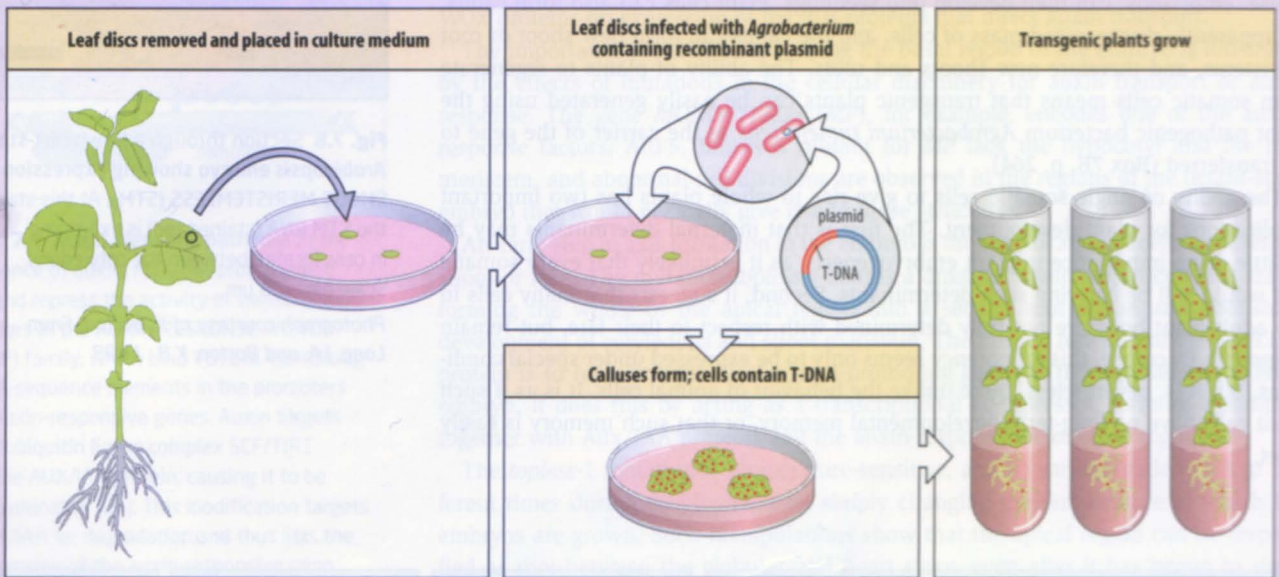*Photograph courtesy of K. Barton. From Long, J.A. and Barton, K.B.: 1998.*
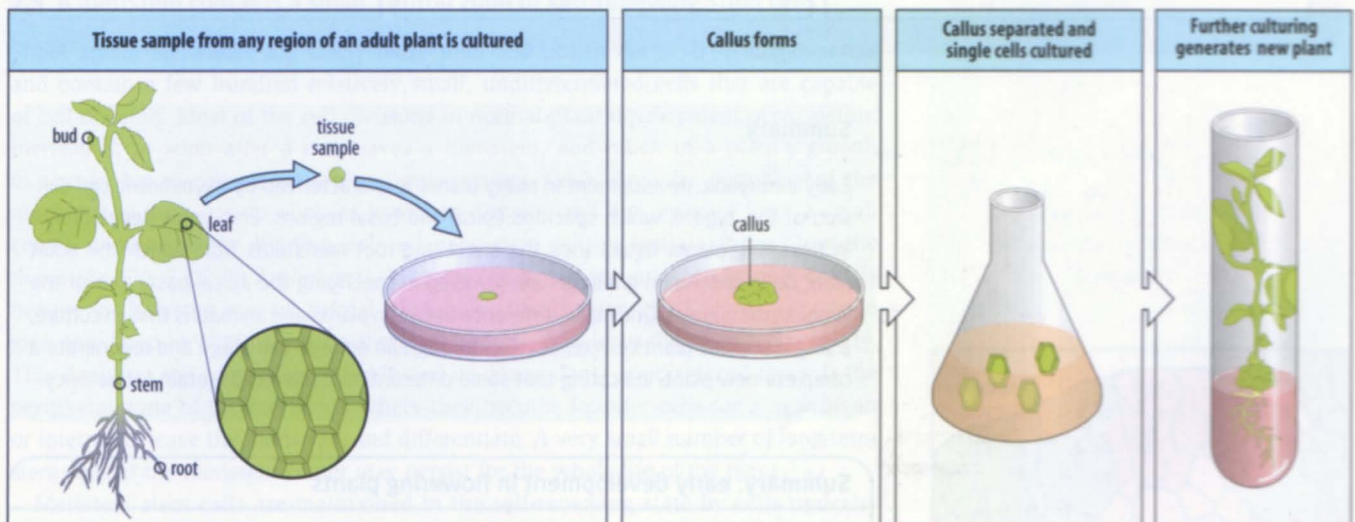
Fig. 7.9 Cultured somatic cells from a mature plant can form an embryo and regenerate a new plant. The illustration shows the generation of a plant from single cells. If a small piece of tissue from a plant stem or leaf is placed on a solid agar medium containing the appropriate nutrients and growth hormones, the cells will start to divide to form a disorganized mass of cells known as a callus.

The callus cells are then separated and grown in liquid culture, again containing the appropriate growth hormones. In suspension culture, some of the callus cells divide to form small cell clusters. These cell clusters can resemble the globular stage of a dicotyledon embryo, and with further culture on solid medium, develop through the heart-shaped and later stages to regenerate a complete new plant.

Box 7B Transgenic plants

One of the most common ways of generating transgenic plants containing new and modified genes is through infection of plant tissue in culture with the bacterium *Agrobacterium tumefaciens*, the causal agent of crown gall tumors. *Agrobacterium* is a natural genetic engineer. It contains a **plasmid**—the Ti plasmid—that contains the genes required for the transformation and proliferation of infected cells to form a callus. During infection, a portion of this plasmid—the T-DNA (shown in red below)—is transferred into the genome of the plant cell, where it becomes stably integrated. Genes experimentally inserted into the T-DNA will therefore also be transferred into the plant cell chromosomes. Ti plasmids, modified so that they do not cause tumors but still retain the ability to transfer T-DNA, are widely used as vectors for gene transfer in dicotyledonous plants. The genetically modified plant cells of the callus can then be grown into a complete new transgenic plant that carries the introduced gene in all its cells and can transmit it to the next generation.

Leaf discs removed and placed in culture medium	Leaf discs infected with *Agrobacterium* containing recombinant plasmid	Transgenic plants grow

plasmid

T-DNA

Calluses form; cells contain T-DNA

Summary

Early embryonic development in many plants is characterized by asymmetric cell division of the zygote, which specifies apical and basal regions. Embryonic development in flowering plants establishes the shoot and root meristems from which the adult plant develops. Auxin gradients are involved in specifying the apical-basal axis of the *Arabidopsis* embryo. One major difference between plants and animals is that, in culture, a single somatic plant cell can develop through an embryo-like stage and regenerate a complete new plant, indicating that some differentiated plant cells retain totipotency.

Summary: early development in flowering plants

first asymmetric cell division and auxin signaling in embryo establishes apical–basal axis

⇩

embryonic cell fate is determined by position

⇩

shoot and root meristems of seedling give rise to all adult plant structures

Meristems

In plants, most of the adult structures are derived from just two regions of the embryo, the embryonic shoot and root meristems, which are maintained after germination. The embryonic shoot meristem, for example, becomes the shoot apical meristem of the growing plant, giving rise to all the stems, leaves, and flowers. As the shoot grows, lateral outgrowths from the meristem give rise to leaves and to side shoots. In flowering shoots, the vegetative meristem becomes converted into one capable of producing **floral meristems** that make flowers, not leaves. In *Arabidopsis*, for example, the shoot apical meristem changes from a vegetative meristem that makes leaves around it in a spiral pattern to an **inflorescence meristem** that then produces floral meristems, and thus flowers, around it in a spiral pattern. The first stages of future organs are known as **primordia** (singular **primordium**). Each primordium consists of a small number of **founder cells** that produce the new structure by cell division and cell enlargement, accompanied by differentiation.

There is usually a time delay between the initiation of two successive leaves in a shoot apical meristem, and this results in a plant shoot being composed of repeated modules. Each module consists of an **internode** (the cells produced by the meristem between successive leaf initiations), a **node** and its associated leaf, and an axillary bud (Fig. 7.10). The axillary bud itself contains a meristem, known as the **lateral shoot meristem**, which forms at the base of the leaf, and can produce a side shoot when the inhibitory influence of the main shoot tip is removed. Root growth is not so obviously modular, but similar considerations apply, as new lateral meristems initiated behind the root apical meristem give rise to lateral roots.
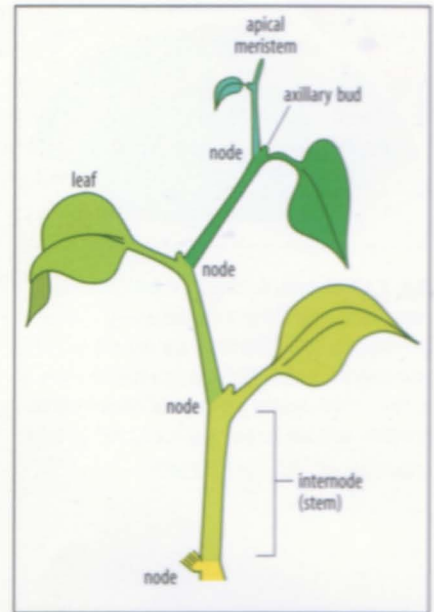
Shoot apical meristems and root apical meristems operate on the same principles, but there are some significant differences between them. We will use the shoot apical meristem to illustrate the basic principles of meristem structure and properties, and then discuss roots.

7.5 A meristem contains a small, central zone of self-renewing stem cells

Shoot apical meristems are rarely more than 250 μm in diameter in angiosperms and contain a few hundred relatively small, undifferentiated cells that are capable of cell division. Most of the cell divisions in normal plant development occur within meristems, or soon after a cell leaves a meristem, and much of a plant's growth in size is due to cell elongation and enlargement. Cells leave the periphery of the meristem to form organs such as leaves or flowers, and are replaced from a small central zone of slowly dividing, self-renewing stem cells or **initials** at the tip of the meristem (Fig. 7.11). In *Arabidopsis* this zone comprises around 12 to 20 cells. Initials behave in the same way as animal stem cells (see Chapter 10). They can divide to give one daughter that remains a stem cell and one that loses the stem-cell property. This daughter cell continues to divide and its descendants are displaced towards the peripheral zone of the meristem, where they become founder cells for a new organ or internode, leave the meristem, and differentiate. A very small number of long-term stem cells at the meristem center may persist for the whole life of the plant.
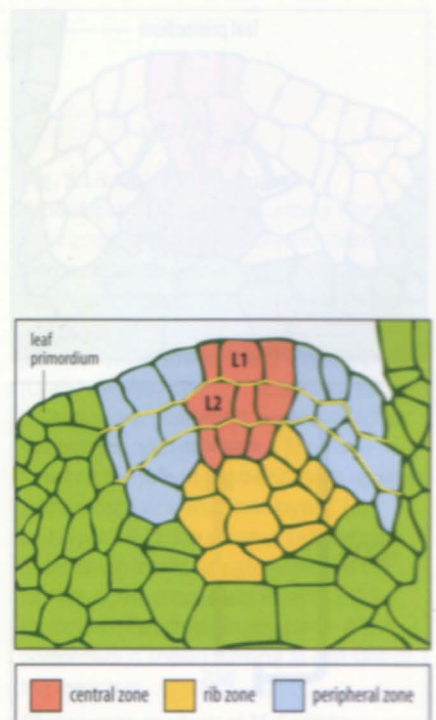
Meristem stem cells are maintained in the self-renewing state by cells underlying the central zone that form the **organizing center**. As we shall see, it is the

Fig. 7.10 Plant shoots grow in a modular fashion. The shoot apical meristem produces a repeated basic structural module. The vegetative shoot module typically consists of internode, node, leaf, and axillary bud (from which a side branch may develop). Successive modules are shown here in different shades of green. As the plant grows, the internodes behind the meristem lengthen and the leaves expand.
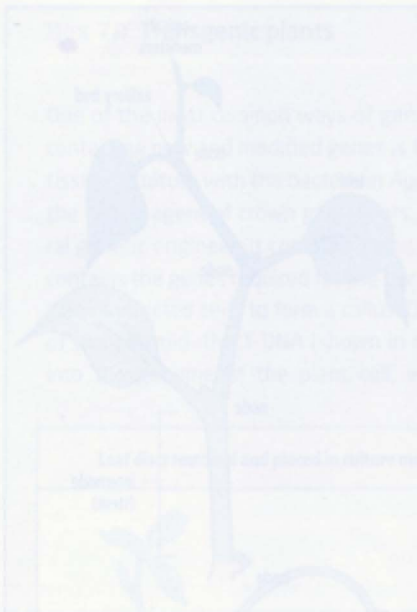
After Alberts, B., et al.: 2002.

Fig. 7.11 Organization of the *Arabidopsis* shoot meristem. A longitudinal section is shown. The meristem has three main layers, L1, L2, and an inner layer, as indicated by the yellow lines, and is divided functionally into a central zone (red), a rib zone (yellow), and a peripheral zone (blue). The stem cells or initials lie in the central zone, while the peripheral zone contains proliferating cells that will give rise to leaves and side shoots. The rib zone gives rise to the central tissues of the plant stem.

microenvironment maintained by the organizing center that gives stem cells their identity.

The undetermined state of meristem stem cells is confirmed by the fact that meristems are capable of regulation. If, for example, a seedling shoot meristem is divided into two or four parts by vertical incision, each part becomes reorganized into a complete meristem, which gives rise to a normal shoot. Provided some subpopulation of organizing center cells plus overlying stem cells is present, a normal meristem will regenerate. If a shoot apical meristem is completely removed, no new apical meristem form, but the incipient meristem at the base of the leaf is now able to develop and form a new side shoot. In the presence of the original meristem, this prospective meristem remains inactive, as active meristems inhibit the development of other meristems nearby—as a result of auxin transport from the shoot apex, among other factors. This regulative behavior is in line with cell–cell interactions being a major determinant of cell fate in the meristem.

7.6 The size of the stem-cell area in the meristem is kept constant by a feedback loop to the organizing center

Numerous genes that control the behavior of the cells in the meristem are known. The gene STM, which is involved in specifying the shoot meristem in the development of the Arabidopsis embryo (see Section 7.3) is, for example, expressed throughout adult shoot meristems but is suppressed as soon as cells become part of an organ primordium. Its role seems to be to maintain meristematic cells in an undifferentiated state, as loss of STM function results in all the meristem cells being incorporated into organ primordia. In contrast, mutations in the Arabidopsis CLAVATA (CLV) genes increase the size of the meristem as a result of an increase in the number of stem cells. Normally, despite the continual exit of cells from the stem-cell pool, the number of stem cells in a meristem is kept roughly the same throughout a plant's life, by division of the remaining stem cells. The role of the CLV genes in regulating stem-cell numbers is understood in some detail, and involves feedback between the stem cells and the organizing center that underlies them.

In Arabidopsis, cells of the organizing center express a homeobox transcription factor, WUSCHEL (WUS). This is required to produce a signal (as yet unknown) that gives the overlying cells their stem-cell identity. Mutations in WUS result in termination of the shoot meristem and cessation of growth as a result of the loss of stem cells, while its overexpression increases stem-cell numbers. The stem cells express CLAVATA3 (CLV3), which encodes a secreted protein that acts indirectly to repress WUS. This feedback loop could control WUS activity in the organizing center and suppress WUS activation in neighboring cells, thus limiting the extent of WUS expression (Fig. 7.12, top panel). In turn, this would regulate the extent of the stem-cell zone
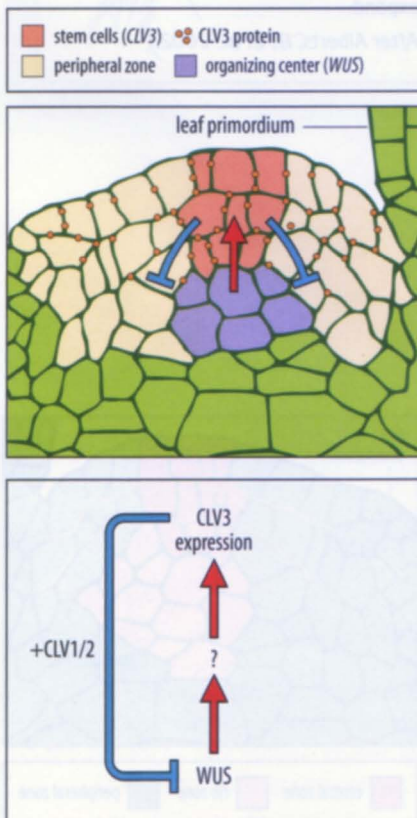
Fig. 7.12 Regulation of the stem-cell population in a shoot meristem. Intercellular signals control the position and size of the stem-cell population in the Arabidopsis shoot meristem. Top panel: the organizing center (purple) expresses the transcription factor WUS, which induces the production of an as yet unknown intercellular signal (red arrow) that maintains the overlying stem cells (orange). The stem cells express and secrete the signal protein CLAVATA3 (CLV3; orange dots), which moves laterally and downwards and indirectly represses transcription of the WUS gene in the surrounding cells, acting through its cell-surface receptor proteins CLV1 and CLV2. CLV3 thus limits the extent of the area specified as stem cells. The descendants of the stem cells are continuously displaced into the peripheral zone of the meristem (pale yellow), where they are recruited into leaf primordia. Bottom panel: the negative-feedback loop whereby WUS expression is restricted by CLV3. Expression of WUS in the organizing center is responsible for the production of an unknown signal that induces the expression of CLV3. CLV3 in turn signals through CLV1/2 to suppress the expression of WUS.
Adapted from Brand, U., et al.: 2000.

above the organizing center. If stem-cell numbers temporarily fall, for example, less CLV3 is produced and *WUS* activity increases, with a consequent increase in stem-cell numbers. More CLV3 is then produced and limits the extent of *WUS* activity. Other CLAVATA proteins are involved in the feedback loop (Fig. 7.12, bottom panel).

Meristems can be induced elsewhere in the plant by the misexpression of genes involved in specifying stem-cell identity, yet another indication that stem-cell identity is conferred by cell–cell interactions and not by an embryonically specified cell lineage.

7.7 The fate of cells from different meristem layers can be changed by changing their position

Other evidence that the fate of a meristematic cell is determined by its position in the meristem, and thus the intercellular signals it is exposed to, comes from observing the fates of cells in the different meristem layers. As well as being organized into central and peripheral zones, the apical meristem of a dicotyledon, such as *Arabidopsis*, is composed of three distinct layers of cells (Fig. 7.13). The outermost layer, L1, is just one cell thick. Layer L2, just beneath L1, is also one cell thick. In both L1 and L2, cell divisions are anticlinal—that is, the new wall is in a plane perpendicular to the
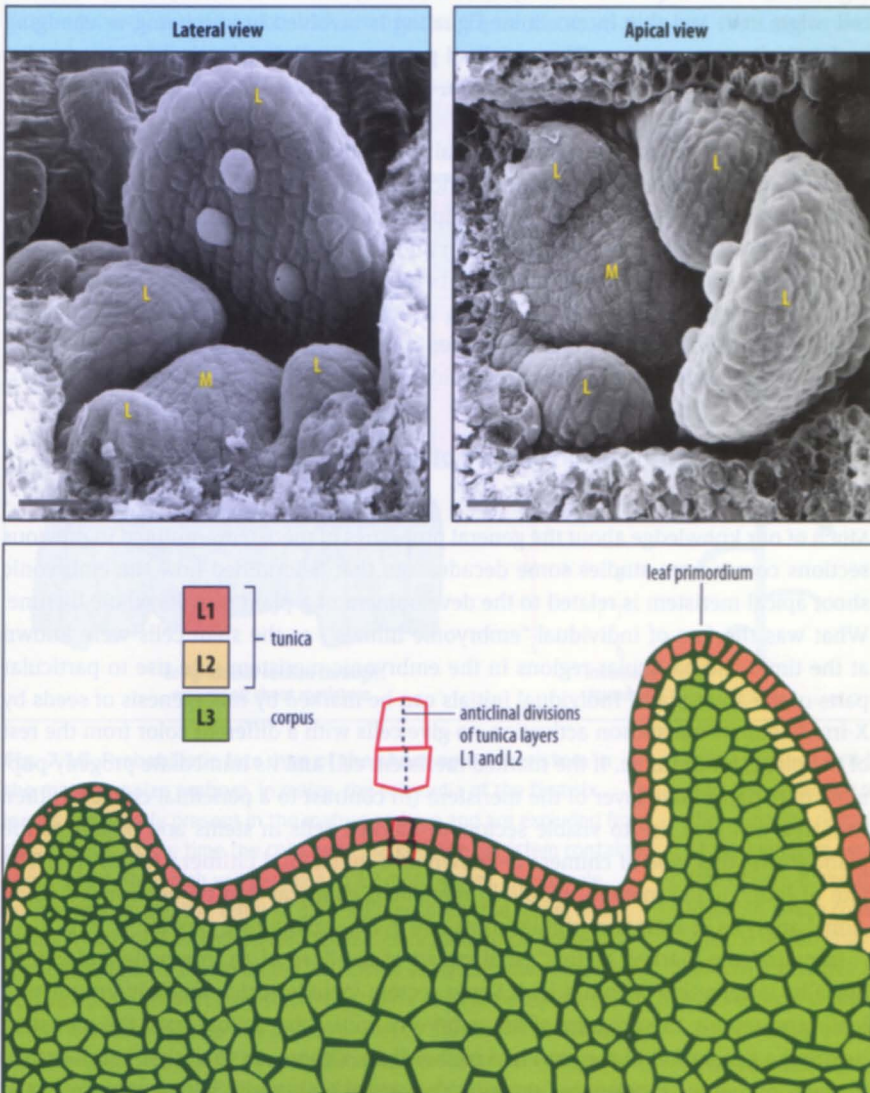

Fig. 7.13 Apical meristem of *Arabidopsis*. Top panels: scanning electron micrographs showing the organization of the meristem at the young vegetative apex of *Arabidopsis*. The plant is a *clavata1* mutant, which has a broadened apex that allows for a clearer visualization of the leaf primordia (L) and the meristem (M). Scale bar = 10 μm. Bottom panel: diagram of a vertical section through the apex of a shoot. The three-layered structure of the meristem is apparent in the most apical region. In layer 1 (L1) and layer 2 (L2), the plane of cell division is anticlinal; that is, at right angles to the surface of the shoot. Cells in layer 3 (L3, the corpus) can divide in any plane. A leaf primordium is shown forming at one side of the meristem.

Photographs courtesy of M. Griffiths.
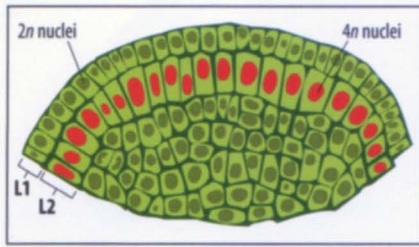
Fig. 7.14 A periclinal meristem chimera composed of cells of two different genotypes. In L1 the cells are diploid whereas the cells of L2 are tetraploid—that is, they have double the normal chromosome number—and are larger and easily recognized. *After Steeves, T.A., et al.: 1989.*

layer—thus maintaining the two-layer organization. The innermost layer is L3, in which the cells can divide in any plane. L1 and L2 are often known as the tunica, and L3 as the corpus.

To find which tissues each layer can give rise to, the fates of cells in the different layers can be followed by marking one layer with a distinguishable mutation, such as a change in pigmentation or a polyploid nucleus. When a complete layer is genetically different from the others in this way, the organism is known as a **periclinal chimera** (Fig. 7.14) and the fate of cells from this layer can be traced. As we saw in relation to animal embryos, chimeras are organisms composed of cells of two different genotypes (see Section 3.9). Plant chimeras can be made by inducing mutation in the apical meristem of a seed or shoot tip by X-irradiation, or by treatment with chemicals such as colchicine that induce polyploidy.

Layer L1 gives rise to the epidermis that covers all structures produced by the shoot, while L2 and L3 both contribute to cortex and vascular structures. Leaves and flowers are produced mostly from L2; L3 contributes mainly to the stem. Although the three layers maintain their identity in the central region of the meristem over long periods of growth, cells in either L1 or L2 occasionally divide periclinally; new cell walls are formed parallel to the surface of the meristem, and thus one of the new cells invades an adjacent layer. This migrant cell now develops according to its new position, showing that cell fate is not necessarily determined by the meristem layer in which the cell originated, and that intercellular signaling is involved in specifying or changing its fate in its new position. The anticlinal pattern of cell division in L2 becomes disrupted when leaf primordia start to form, when the cells divide periclinally, as well as anticlinally.

The transcription factor Knotted-1 in maize is homologous to *Arabidopsis* STM and, like STM, it is expressed throughout the shoot meristem to keep cells in an undifferentiated state. It is one example of a transcription factor that moves directly from cell to cell. The *KNOTTED-1* gene is expressed in all layers except L1, but the protein is also found in L1, suggesting that it can move between cells, perhaps via plasmodesmata. In *Knotted-1* gain-of-function mutants, in which the gene is misexpressed in leaves, Knotted-1 protein fused to green fluorescent protein has also been observed to move from the inner layers of the leaf to the epidermis, but not in the opposite direction.

7.8 A fate map for the embryonic shoot meristem can be deduced using clonal analysis

Much of our knowledge about the general properties of meristems outlined in previous sections comes from studies some decades ago that determined how the embryonic shoot apical meristem is related to the development of a plant over its whole lifetime. What was the fate of individual 'embryonic initials', as the stem cells were known at the time? Did particular regions in the embryonic meristem give rise to particular parts of the adult plant? Individual initials can be marked by mutagenesis of seeds by X-irradiation or transposon activation, to give cells with a different color from the rest of the plant, for example. If the marked meristem cell and its immediate progeny populate only part of one layer of the meristem (in contrast to a periclinal chimera), then this area will give rise to visible sectors of marked cells in stems and organs as the plant grows; this type of chimera is known as a **mericlinal chimera** (Fig. 7.15). The fate of individually marked initials in mericlinal chimeras can be determined using clonal analysis in a manner similar to its use in *Drosophila* (see Box 2E, p. 73).

In maize, the marked sectors usually start at the base of an internode and extend apically, terminating within a leaf. Some sectors include just a single internode and leaf, representing the progeny of an embryonic initial that is lost from the meristem before the generation of the next leaf primordium. Others, on the other hand, extend through numerous internodes, showing that some embryonic initials remain in the


Fig. 7.15 Tobacco plant mericlinal chimera. This plant has grown from an embryonic shoot meristem in which an albino mutation has occurred in a cell of the L2 layer. The affected area occupies about a third of the total circumference of the shoot, suggesting that there are three apical initial cells in the embryonic shoot meristem. *Photograph courtesy of S. Poethig.*

meristem for a long time, contributing to a succession of nodes and internodes. In sunflowers, marked clones have been observed to extend through several internodes up into the flower, showing that a single initial can contribute to both leaves and flowers.

From the analysis of hundreds of mericlinal chimeras, fate maps of the embryonic shoot meristems of several species were constructed, which shed light on the properties of the shoot meristem and how it behaves during normal development. These fate maps are probabilistic because it was not possible to know the location of the marked cell in the embryonic meristem, which is inaccessible inside the seed.

The probabilistic fate map for the maize embryonic shoot apical meristem indicates that the three most apical cells in L1 give rise to the male inflorescence (the tassel and spike; Fig. 7.16). The remainder of the maize meristem can be divided into five tiers of cells that produce internodes and leaves, and which form overlapping concentric domains on the fate map. The outermost domain contributes to the earliest internode–leaf modules, while the inner domains give rise to internodes and leaves successively higher up the stem. A fate map has been similarly constructed for the embryonic shoot apical meristem of *Arabidopsis* (Fig. 7.17). Most of the *Arabidopsis* embryonic meristem gives rise to the first six leaves, whereas the remainder of the shoot, including all the flowerheads, is derived from a very small number of embryonic cells at the center of the meristem. Unlike maize, the number of leaves in *Arabidopsis* is not fixed—growth is said to be **indeterminate**. There is no relation between particular
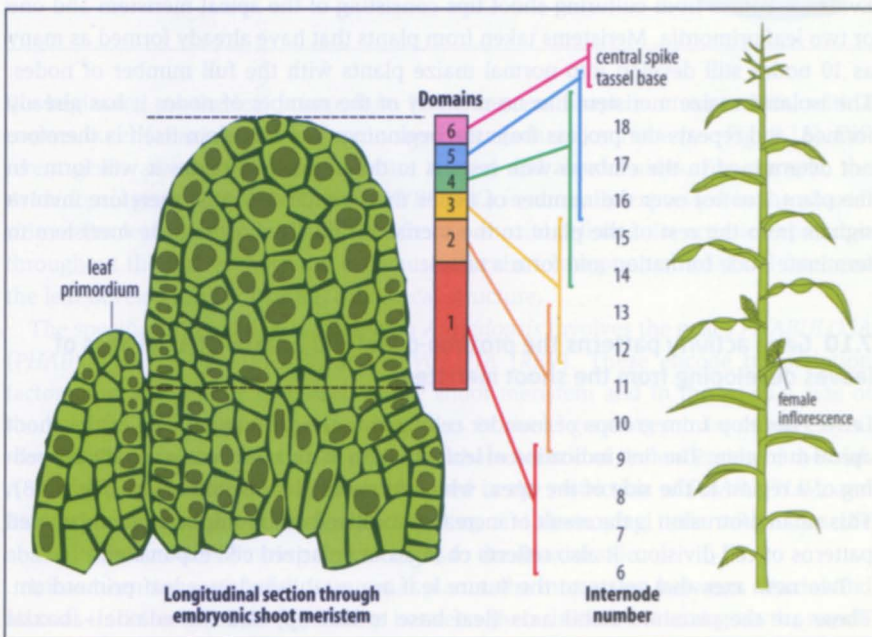
Fig. 7.16 Probabilistic fate map of the shoot apical meristem in the mature maize embryo. In maize, the primordia of the first six leaves are already present in the mature embryo and are excluded from the analysis. At the time the cells were marked, the meristem contained about 335 cells, which will give rise to 12 more leaves, the female inflorescences, and the terminal male inflorescence (the tassel and spike). A longitudinal section through the embryonic apical dome—the shoot meristem—is shown on the left. Clonal analysis shows that it can be divided into six vertically stacked domains, each comprising a set of initials that can give rise to a particular part of the plant. The number of initials in layers L1 and L2 of each domain at the embryonic stage can be estimated from the final extent of the corresponding marked sectors in the mature plant (see text). The fate of each domain, as estimated from the collective results of clonal analysis of many different plants, is shown on the right. Domain 6, comprising the three most apical L1 cells of the meristem, will give rise to the terminal male inflorescence. The fate of cells in the other domains is less circumscribed. Domain 5, for example, which consists of around eight L1 cells surrounding domain 6 and the underlying four or so L2 cells, can contribute to nodes 16-18; domain 4 to nodes 14-18; and domain 3 to nodes 12-15. The female inflorescences, which develop in the leaf axils, are derived from the corresponding domains.

After McDaniel, C.N., et al.: 1988.
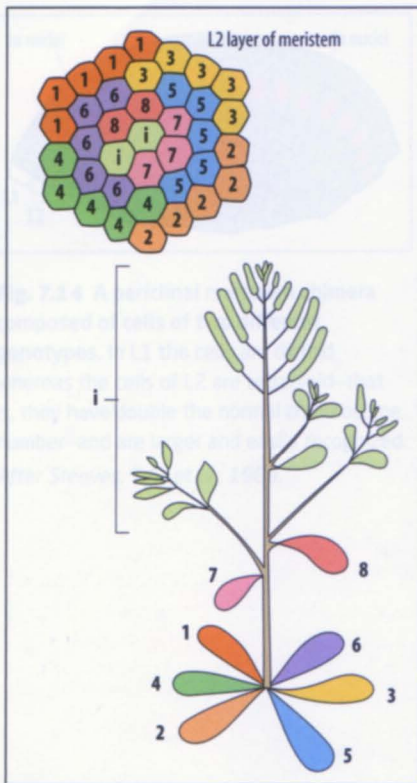
Fig. 7.17 Probabilistic fate map of the embryonic shoot meristem of *Arabidopsis*. The L2 layer of the meristem is depicted as if flattened out and viewed from above. The numbers indicate the leaf, as shown on the plant below, to which each group of meristem cells contributes, and indicate the sequence in which the leaves are formed. The inflorescence shoot (i) is derived from a small number of cells in the center of the layer.

After Irish, V.E.: 1991.

cell lineages and particular structures, which indicates that position in the meristem is crucial in determining cell fate. One exception is that germ cells always arise from L2. The L2 layer is a clone, and so there is, in the case of germ cells, a relation between fate and a particular cell lineage.

The conclusions from the clonal analysis experiments are that the initials that contribute to a particular structure are simply those that happen to be in the appropriate region of the meristem at the time; they have not been prespecified in the embryo as, say, flower or leaf.

7.9 Meristem development is dependent on signals from other parts of the plant

To what extent does the behavior of a meristem depend on other parts of the plant? It seems to have some autonomy, because if a meristem is isolated from adjacent tissues by excision it will continue to develop, although often at a much slower rate. Excised shoot apical meristems of a variety of plants can be grown in culture, where they will develop into shoots complete with leaves if the growth hormones auxin and cytokinin are added. The behavior of the meristem *in situ*, however, is influenced in more subtle ways by interactions with the rest of the plant.

As we saw earlier, the apical meristem of maize gives rise to a succession of nodes, and terminates in the male flower. The number of nodes before flowering is usually between 16 and 22. This number is not controlled by the meristem alone, however. Evidence comes from culturing shoot tips consisting of the apical meristem and one or two leaf primordia. Meristems taken from plants that have already formed as many as 10 nodes still develop into normal maize plants with the full number of nodes. The isolated maize meristem has no memory of the number of nodes it has already formed, and repeats the process from the beginning. The meristem itself is therefore not determined in the embryo with respect to the number of nodes it will form. In the plant, control over the number of nodes that are formed must therefore involve signals from the rest of the plant to the meristem, finally directing the meristem to terminate node formation and form a tassel.

7.10 Gene activity patterns the proximo-distal and adaxial–abaxial axes of leaves developing from the shoot meristem

Leaves develop from groups of founder cells within the peripheral zone of the shoot apical meristem. The first indication of leaf initiation in the meristem is usually a swelling of a region to the side of the apex, which forms the **leaf primordium** (Fig. 7.18). This small protrusion is the result of increased localized cell multiplication and altered patterns of cell division. It also reflects changes in polarized cell expansion.

Two new axes that relate to the future leaf are established in a leaf primordium. These are the **proximo-distal axis** (leaf base to leaf tip) and the **adaxial–abaxial axis** (upper surface to lower surface, sometimes called dorsal to ventral). The latter is termed adaxial–abaxial as it is related to the radial axis of the shoot. The upper surface of the leaf derives from cells near the center of this axis (adaxial), while the lower surface derives from more peripheral cells (abaxial). The two leaf surfaces carry out different functions and have different structures, with the top surface being specialized for light capture and photosynthesis. In *Arabidopsis*, flattening of the leaf along the adaxial–abaxial axis occurs after leaf primordia begin developing, but in monocots like maize the leaf is flattened as it emerges. The establishment of adaxial–abaxial polarity is likely to make use of positional information along the radial axis of the meristem.

Arabidopsis leaf primordia emerge from the shoot meristem with distinct programs of development in the adaxial and abaxial halves. This asymmetry can be seen from
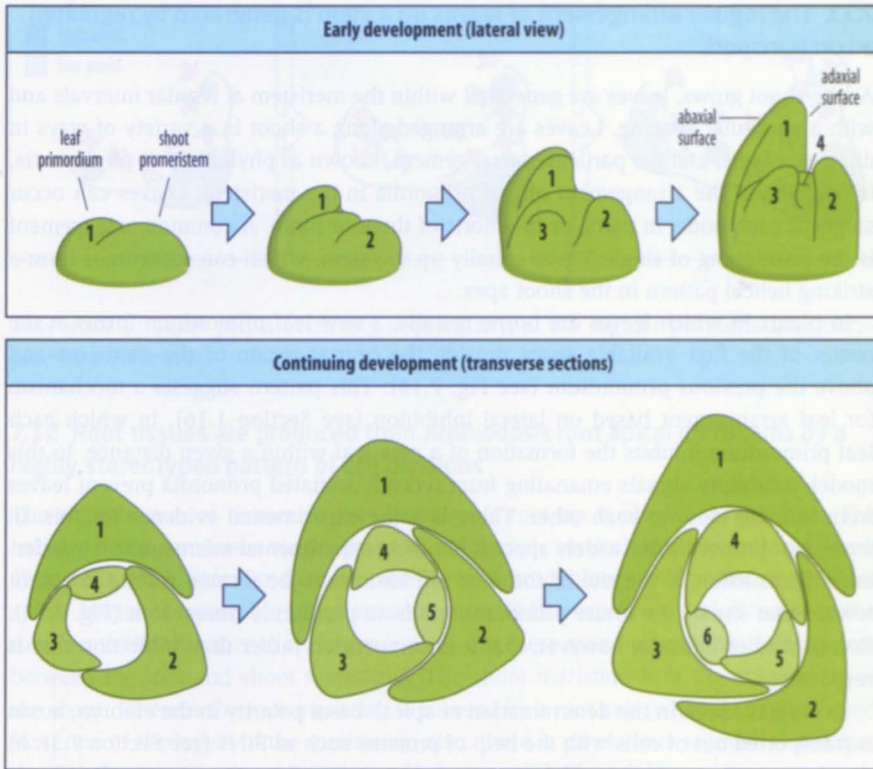
Early development (lateral view)

leaf primordium
shoot promeristem

adaxial surface
abaxial surface

Continuing development (transverse sections)

the beginning, as the leaf primordium has a crescent shape in cross-section, with a convex outer (abaxial) side and a concave inner (adaxial) surface (see Fig. 7.18). Different genes are expressed in the future adaxial and abaxial sides. For example, the *Arabidopsis* gene *FILAMENTOUS FLOWER* (*FIL*) is normally expressed in the abaxial side of the leaf primordium, and specifies an abaxial cell fate. Its ectopic expression throughout the leaf primordium can cause all cells to adopt an abaxial cell fate, and the leaf develops as an arrested cylindrical structure.

The specification of adaxial cell fate in *Arabidopsis* involves the genes *PHABULOSA* (*PHAB*), *PHAVOLUTA* (*PHAV*), and *REVOLUTA* (*REV*). These encode transcription factors and are initially expressed in the shoot meristem and in the adaxial side of the primordium. Loss-of-function mutations in these genes result in radially symmetrical leaves with only abaxial cell types characteristic of the underside of the leaf. A microRNA (see Box 6B, p. 228) is involved in the restriction of *PHAB*, *PHAV*, and *REV* expression to the adaxial side, targeting and destroying their mRNAs on the abaxial side and thus limiting their activity to the adaxial side.

It has been suggested that, in normal plants, the interaction between adaxial and abaxial initial cells at the boundary between them initiates lateral growth, resulting in the formation of the leaf blade and the flattening of the leaf. The importance of boundaries in controlling pattern and form has already been seen in the parasegments of *Drosophila* (see Section 2.24) and further examples will be found in Chapter 11.

Development along the leaf proximo-distal axis also appears to be under genetic control. Like other grasses, a maize leaf primordium is composed of prospective leaf-sheath tissue proximal to the stem and prospective leaf-blade tissue distally. Mutations in certain genes result in distal cells taking on more proximal identities; for example, making sheath in place of blade. Similar proximo-distal shifts in pattern occur in *Arabidopsis* as a result of mutation. Positional identity along the proximo-distal axis may reflect the developmental age of the cells, distal cells adopting a different fate from proximal cells because they mature later.
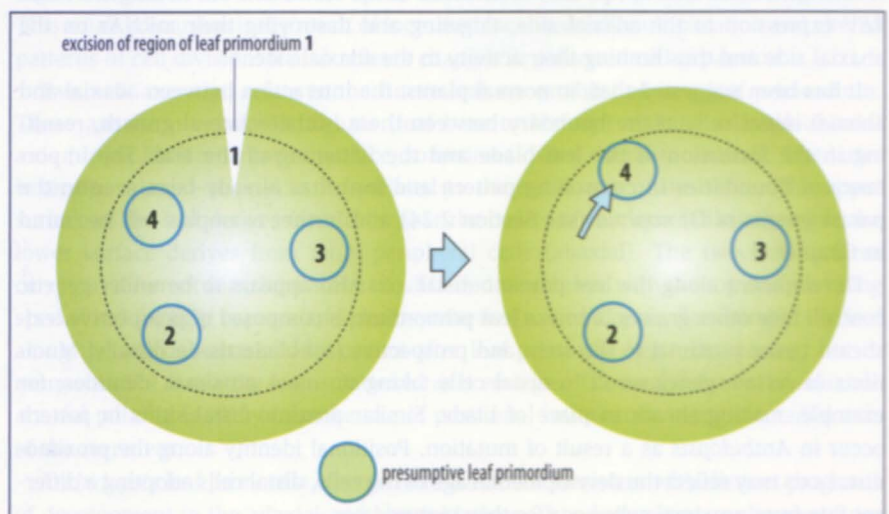
7.11 The regular arrangement of leaves on a stem is generated by regulated auxin transport
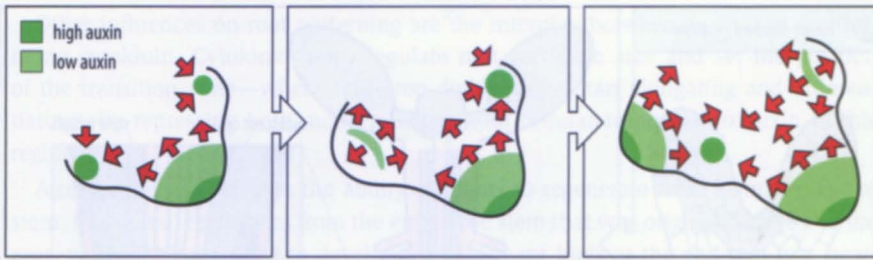
As the shoot grows, leaves are generated within the meristem at regular intervals and with a particular spacing. Leaves are arranged along a shoot in a variety of ways in different plants, and the particular arrangement, known as phyllotaxy or **phyllotaxis**, is reflected in the arrangement of leaf primordia in the meristem. Leaves can occur singly at each node, in pairs, or in whorls of three or more. A common arrangement is the positioning of single leaves spirally up the stem, which can sometimes form a striking helical pattern in the shoot apex.

In plants in which leaves are borne spirally, a new leaf primordium forms at the center of the first available space outside the central region of the meristem and above the previous primordium (see Fig. 7.18). This pattern suggests a mechanism for leaf arrangement based on lateral inhibition (see Section 1.16), in which each leaf primordium inhibits the formation of a new leaf within a given distance. In this model, inhibitory signals emanating from recently initiated primordia prevent leaves from forming close to each other. There is some experimental evidence for this. In ferns, leaf primordia are widely spaced, allowing experimental microsurgical interference. Destruction of the site of the next primordium to be formed results in a shift toward that site by the future primordium, whose position is closest to it (Fig. 7.19). Recent studies indicate, however, that it is competition rather than inhibition that is responsible.

As we have seen in the determination of apical–basal polarity in the embryo, auxin is transported out of cells with the help of proteins such as PIN1 (see Section 7.3). In the shoot, auxin produced in the shoot tip below the meristem is transported upwards into the meristem, through the epidermis and the outermost meristem layer. The direction of auxin flow in the shoot apex is controlled by PIN1 and follows a simple rule: the side of the cell on which PIN1 is found is the side nearest the neighbor cell with the highest auxin concentration. Thus auxin transport is always towards a region of higher concentration. A high concentration of auxin is a primordium activator, and initially, auxin is pumped towards a new primordium that is developing at a site of high auxin concentration. This, however, depletes a zone of cells around the primordium of auxin, such that cells nearer the center of the meristem now have more auxin than the cells adaxial to the new primordium. This activates a feedback mechanism that causes PIN1 to move to the other side of these cells, and auxin now flows out of the new primordium towards the meristem, creating a new spot of high auxin concentration in the meristem farthest away from any new primordium (Fig. 7.20).

Fig. 7.19 Leaf primordia may be positioned by lateral inhibition or by competition. Leaf primordia on a fern shoot tip form in a regular order in positions 1 to 4. Primordia appear to form as far as possible from existing primordia, so 2 forms almost opposite 1. Normally, 4 will develop between 1 and 2, but if 1 is excised, 4 forms much further away from 2. This result can be interpreted either by the removal of lateral inhibition by primordium 1 or by the removal of the competitive effect of 1 for some primordium-inducing factor such as auxin.

excision of region of leaf primordium **1**

presumptive leaf primordium

■	high auxin
■	low auxin

Fig. 7.20 Auxin-dependent mechanism of phyllotaxis in *Arabidopsis*. Auxin is transported through PIN proteins towards areas of high auxin concentration (dark green), at which an organ primordium will form. Cells around the developing primordium become depleted of auxin (light green), which causes the polarity of the PIN proteins to reverse so that auxin then flows away from the primordium. The red arrows indicate the direction of PIN polarity. It is proposed that this pattern of auxin circulation could set up the regular pattern of leaf and flower formation from meristems.

This leads to auxin peaks occurring sequentially, at the regular positions later occupied by new leaves.

7.12 Root tissues are produced from *Arabidopsis* root apical meristems by a highly stereotyped pattern of cell divisions

The organization of tissues in the *Arabidopsis* root tip is shown in Fig. 7.21. The radial pattern comprises single layers of epidermal, cortical, endodermal, and pericycle cells, with vascular tissue in the center (protophloem and protoxylem). Root apical meristems resemble shoot apical meristems in many ways and give rise to the root in a similar manner to shoot generation. But there are some important differences between the root and shoot meristems. The shoot meristem is at the extreme tip of the shoot, whereas the root meristem is covered by a root cap (which is itself derived from one of the layers of the meristem); also, there is no obvious segmental arrangement at the root tip resembling the node–internode–leaf module.
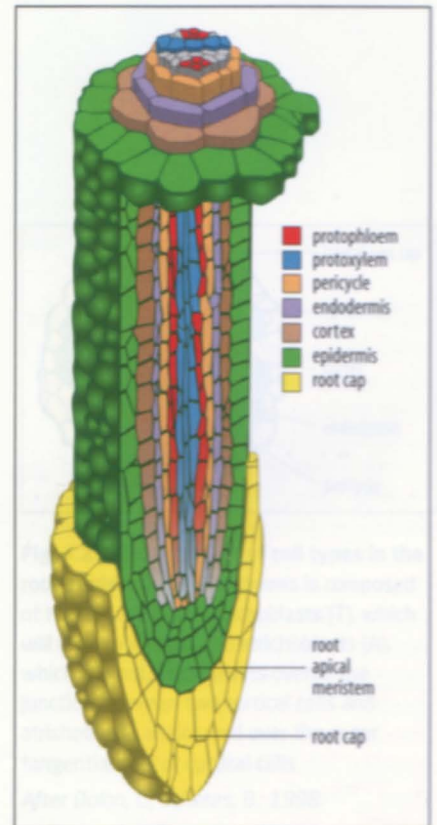
The root is set up early (see Section 7.2) and a well-organized embryonic root can be identified in the late heart-stage embryo (Fig. 7.22). An antagonistic interaction between auxin and cytokinin controls the establishment of the root stem-cell niche. Clonal analysis has shown that the seedling root meristem can be traced back to a set of embryonic initials that arise from a single tier of cells in the heart-stage embryo.

As in the shoot meristem, a root meristem is composed of an organizing center, called the **quiescent center** in roots, in which the cells divide only very rarely, and which is surrounded by stem-cell-like initials that give rise to the root tissue (see Fig. 7.22). The quiescent center is essential for meristem function. When parts of the meristem are removed by microsurgery, it can regenerate, but regeneration is always preceded by the formation of a new quiescent center. Laser destruction of individual quiescent-center cells shows that, as in the shoot meristem, a key function of the quiescent center is to maintain the immediately adjacent initials in the stem-cell state and prevent them from differentiating.

Each initial undergoes a stereotyped pattern of cell divisions to give rise to a number of columns, or **files**, of cells in the growing root (see Fig. 7.21); each file of cells in the root thus has its origin in a single initial. Some initials give rise to both endodermis and cortex, whereas others give rise to both epidermis and the root cap. Before it leaves the meristem, therefore, the undifferentiated progeny of an endodermis/cortex initial, for example, will divide asymmetrically to give one daughter that produces cortex and one that produces endodermis. The gene *SCARECROW* is necessary to confer this asymmetry on the dividing cell, and mutations in this gene give roots with no distinct endodermis or cortex but with a tissue layer with characteristics of both.

The normal pattern of cell divisions is not obligatory, however. As discussed earlier, *fass* mutants, which have disrupted cell divisions, still have relatively normal

Fig. 7.21 The structure of the root tip in *Arabidopsis*. Roots have a radial organization. In the center of the growing root tip is the future vascular tissue (protoxylem and protophloem). This is surrounded by further tissue layers.

■	protophloem
■	protoxylem
■	pericycle
■	endodermis
■	cortex
■	epidermis
■	root cap

root
apical
meristem

root cap

Fig. 7.22 Fate map of root regions in the heart-stage *Arabidopsis* embryo. The root grows by the division of a set of initial cells. The root meristem comes from a small number of cells in the heart-shaped embryo. Each tissue in the root is derived from the division of a particular initial cell. At the center of the root meristem is a quiescent center, which does not divide.

After Scheres, B., et al.: 1994.
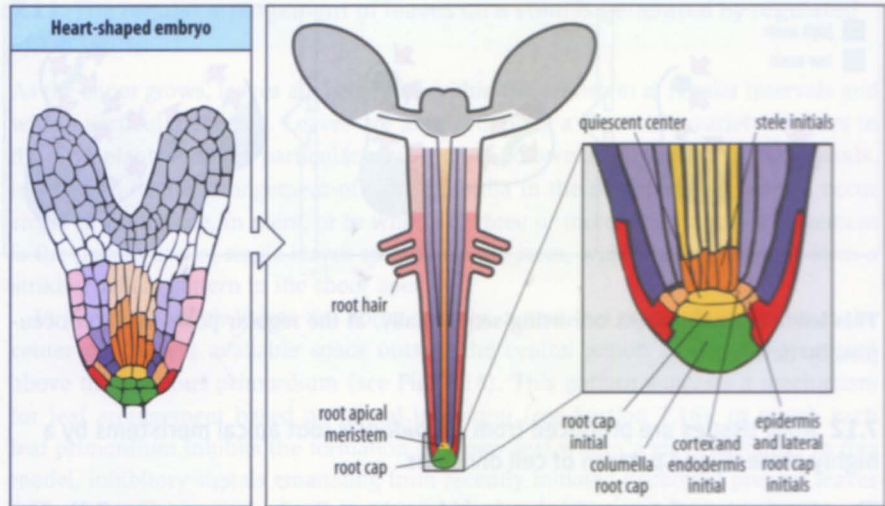
patterning in the root. In addition, laser ablation of individual meristem cells does not lead to an abnormal root. The remaining initials undergo new patterns of cell division that replace the progeny of the cells that have been destroyed. Such observations show that, as in the shoot meristem, the fate of cells in the developing root meristem depends on their recognition of positional signals and not on their lineage.

As we saw in Section 7.3, auxin gradients play a major role in patterning the embryo and specifying the root region, and mutations that affect auxin localization lead to root defects. At the globular stage of embryonic development, the auxin-transport protein PIN1 is localized in cells in the future root region and the highest level of auxin is found adjacent to where the quiescent center will develop.

The role of auxin in root development continues into the adult plant. Auxin in the root is transported out of cells via the PIN proteins, and enters adjacent cells. Cells with raised auxin levels transport auxin better, probably due to an increase in the number of PIN proteins in the membrane as auxin prevents their endocytosis and recycling, and so there is a positive feedback loop that raises the local concentration.

Auxin plays a key role in patterning the growing root. Modeling of auxin movement, using the known distribution of PIN proteins in the membranes of different cell types, has shown that PIN-directed flow can explain the formation and maintenance of a stable auxin maximum at the quiescent center. Auxin is transported down the central vascular tissue of the root tip, forming a concentration maximum at the quiescent center, and then outwards and upwards through the outer layers of the root tip, forming gradients along both the basal-apical axis and laterally in the root. This modeling successfully simulates the effects of such auxin gradients on pattern formation, cellular differentiation and root growth in real time.

An idea of how the auxin gradients might be translated into effects on cell fate and cell behavior is provided by the graded distribution of the four PLETHORA-family transcription factors in the root. These transcription factors are required for proper root development and are expressed in a graded manner along the apical–basal axis, with expression maxima for all at the quiescent center—the region of maximum auxin concentration. Here they are essential for stem-cell maintenance and function. Lower concentrations of PLETHORA proteins correspond to the meristem region, where cells are proliferating, while even lower concentrations appear to be necessary for exit from the meristem and cell differentiation in the elongation zone. Although not yet proven experimentally, *PLETHORA* gene expression could provide a graded read-out of the auxin gradient that helps direct root patterning.

Other influences on root patterning are the interplay between auxin and the hormone cytokinin. Cytokinin helps regulate root meristem size and set the position of the transition zone—where cells stop dividing and start elongating and differentiating—by repressing both auxin transport and cellular responses to auxin in this region.

Auxin is also involved in the ability of plants to regenerate from a small piece of stem. In general, roots form from the end of the stem that was originally closest to the root, whereas shoots tend to develop from dormant buds at the end that was nearest to the shoot. This polarized regeneration is related to vascular differentiation and to the polarized transport of auxin. Transport of auxin from its source in the shoot tip toward the root leads to an accumulation of auxin at the 'root' end of the stem cutting, where it induces the formation of roots. One hypothesis suggests that polarity is both induced and expressed by the oriented flow of auxin.

One of the best examples of developmentally important transcription factor movement from one cell to another is found in roots. As noted earlier, expression of the gene *SCARECROW* is required for root cells to adopt an endodermal fate. This expression requires the transcriptional activator SHORT-ROOT (SHR). SHR is, however, not synthesized in the prospective endodermal cells, but in the adjacent cells on the inner side. SHR protein is transported from these cells outwards into the prospective endodermis, and this movement appears to be regulated and not simply due to diffusion.

7.13 Root hairs are specified by a combination of positional information and lateral inhibition

Root hairs are formed from epidermal cells at regular intervals around the root, and this regularity is thought to be achieved by a combination of responses to positional information and lateral inhibition by the movement of transcription factors between cells. Files of cells that will make root hairs alternate with files of non-hair-producing cells on the surface of the developing root. The importance of position is shown by the fact that if an epidermal cell overlies a junction between two cortical cells it forms a root hair, whereas if it contacts just one cortical cell it does not (Fig. 7.23). And if a cell changes its position in relation to the cortex, its fate will also change, from a potential hair-forming cell to a non-hair-forming cell and vice versa. Most cell divisions in the future epidermis are horizontal, increasing the number of cells per file, but occasionally a vertical anticlinal division occurs, pushing one of the daughter cells into an adjacent file. The daughter cell then assumes a fate corresponding to its new position in relation to the adjacent cortical cells.
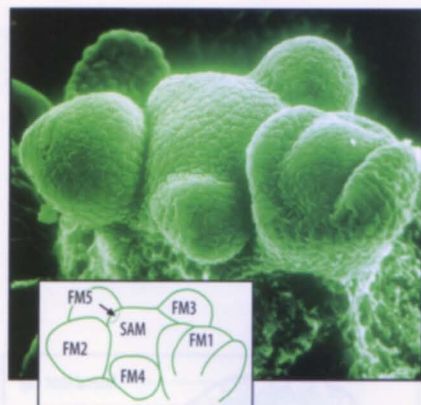
The positional cues, as yet unknown, are thought to be detected by the epidermal cells through the SCRAMBLED protein, which is a receptor-like protein kinase. SCRAMBLED activity influences the activity of a network of transcription factors that control cell fate. Two groups of transcription factors have been identified by mutation experiments, one group promoting a root-hair fate and one suppressing it. A key transcription factor that seems to be regulated by SCRAMBLED activity is WEREWOLF, whose expression is suppressed in presumptive hair-forming cells, presumably in response to the positional signal. As well as promoting an atrichoblast fate, however, WEREWOLF is also required for the expression of transcription factors (CAPRICE, TRYPTYCHON, and ENHANCER OF TRYPTYCHON) that are needed to specify hair cells. After positional signaling, these proteins will only be produced in the presumptive atrichoblasts, but they are thought to move laterally into the adjacent epidermal cells and promote these cells' differentiation as trichoblasts by inhibiting genes that would otherwise give an atrichoblast fate.
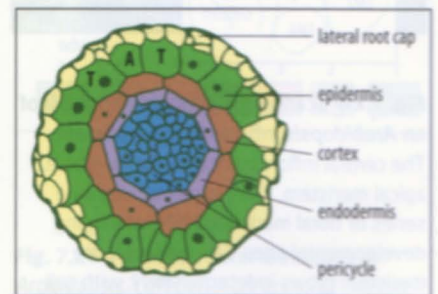
Fig. 7.23 Organization of cell types in the root epidermis. The epidermis is composed of two types of cells: trichoblasts (T), which will form root hairs, and atrichoblasts (A), which will not. Trichoblasts overlie the junction between two cortical cells and atrichoblasts are located over the outer tangential wall of cortical cells.

After Dolan, L., Scheres, B.: 1998.

Summary

Meristems are the growing points of a plant. The apical meristems, found at the tips of shoots and roots, give rise to all the plant organs—roots, stem, leaves, and flowers. They consist of small groups of a few hundred undifferentiated cells that are capable of repeated division. The center of the meristem is occupied by self-renewing stem cells, which replace the cells that are lost from the meristem when organs are formed. The fate of a cell in the shoot meristem depends upon its position in the meristem and interactions with its neighbors, as when a cell is displaced from one layer to another it adopts the fate of its new layer. Meristems can also regulate when parts are removed, in line with cell-cell interactions determining cell fate. Fate maps of embryonic shoot meristems show that they can be divided into domains, each of which normally contributes to the tissues of a particular region of the plant, but the fate of the embryonic initials is not fixed. The shoot meristem gives rise to leaves in species-specific patterns—phyllotaxy—which seem best accounted for by regulated transport of auxin. Lateral inhibition is involved in the regular spacing of hairs on root and leaf surfaces. In the root meristem, the cells are organized rather differently from those in the shoot meristem, and there is a much more stereotyped pattern of cell division. A set of initial cells maintains root structure by dividing along different planes.

Summary: meristems give rise to all adult tissues

Shoot meristem	Root meristem
cell fate is determined by position	stereotyped division of initials along different planes
⇩	⇩
shoot meristem gives rise to stem internodes, leaves, and flowers	root meristem gives rise to all root structures
⇩	
arrangement of leaves on stem may be due to an auxin-based mechanism at shoot apex	

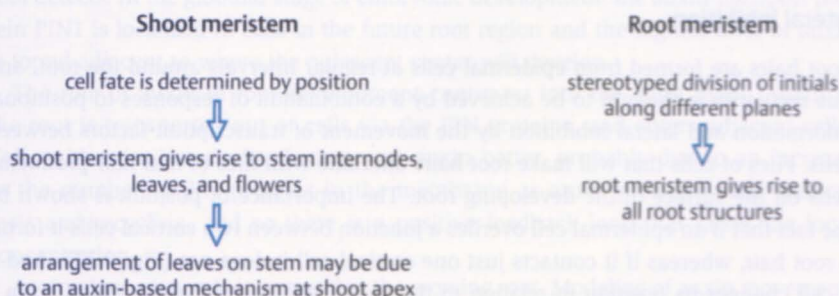**Fig. 7.24 Scanning electron micrograph of an *Arabidopsis* inflorescence meristem.** The central inflorescence meristem (shoot apical meristem, SAM) is surrounded by a series of floral meristems (FM) of varying developmental ages. The inflorescence meristem grows indeterminately, with cell divisions providing new cells for the stem below, and new floral meristems on its flanks. The floral meristems (or floral primordia) arise one at a time in a spiral pattern. The most mature of the developing flowers is on the right (FM1), showing the initiation of sepal primordia surrounding a still-undifferentiated floral meristem. Eventually such a floral meristem will also form petal, stamen, and carpel primordia.
Photograph from Meyerowitz, E.M., et al.: 1991.

Flower development and control of flowering

Flowers contain the reproductive cells of higher plants and develop from the shoot meristem. In most plants, the transition from a vegetative shoot meristem to a floral meristem that produces a flower is largely, or absolutely, under environmental control, with daylength and temperature being important determining factors. In a plant such as *Arabidopsis*, in which each flowering shoot produces multiple flowers, the vegetative shoot meristem first becomes converted into an inflorescence meristem, which then forms floral meristems, each of which develops completely into a single flower (Fig. 7.24). Floral meristems are thus determinate, unlike the indeterminate shoot apical meristem. Flowers, with their arrangement of floral organs (sepals, petals, stamens, and carpels), are rather complex structures, and it is a major challenge to understand how they arise from the floral meristem.

The conversion of a vegetative shoot meristem into one that makes flowers involves the induction of so-called **meristem identity genes**. A key regulator of floral induction in *Arabidopsis* is the meristem identity gene *LEAFY* (*LFY*); a related gene in *Antirrhinum* is *FLORICAULA* (*FLO*). How environmental signals, such as daylength,

Fig. 7.25 Structure of an Arabidopsis flower. *Arabidopsis* flowers are radially symmetrical and have an outer ring of four identical green sepals, enclosing four identical white petals, within which is a ring of six stamens, with two carpels in the center. Bottom: floral diagram of the *Arabidopsis* flower representing a cross-section taken in the plane indicated in the top diagram. This is a conventional representation of the arrangement of the parts of the flower, showing the number of flower parts in each whorl and their arrangement relative to each other. *After Coen, E.S., et al.: 1991.*

influence floral induction is discussed later. We will first consider the mechanisms that pattern the flower, in particular those that specify the identity of the floral organs.

7.14 Homeotic genes control organ identity in the flower

The individual parts of a flower each develop from a **floral organ primordium** produced by the floral meristem. Unlike leaf primordia, which are all identical, the floral organ primordia must each be given a correct identity and be patterned according to it. An *Arabidopsis* flower has four concentric whorls of structures (Fig. 7.25), which reflect the arrangement of the floral organ primordia in the meristem. The sepals (whorl 1) arise from the outermost ring of meristem tissue, and the petals (whorl 2) from a ring of tissue lying immediately inside it. An inner ring of tissue gives rise to the male reproductive organs—the stamens (whorl 3). The female reproductive organs—the carpels (whorl 4)—develop from the center of the meristem. In a floral meristem of *Arabidopsis*, there are 16 separate primordia, giving rise to a flower with four sepals, four petals, six stamens and a pistil made up of two carpels (see Fig. 7.25).

The primordia arise at specific positions within the meristem, where they develop into their characteristic structures. After the emergence of the primordia in *Antirrhinum*, cell lineages become restricted to particular whorls, rather like the lineage restriction to compartments in *Drosophila* (see Section 2.23). Lineage restriction occurs at the time when the pentagonal symmetry of the flower becomes visible and genes that give the different floral organs their identity are expressed. The lineage compartments within the floral meristem appear to be delineated by narrow bands of non-dividing cells.

Like the homeotic selector genes that specify segment identity in *Drosophila*, mutations in floral identity genes cause homeotic mutations in which one type of flower part is replaced by another. In the *Arabidopsis* mutant *apetala2*, for example, the sepals are replaced by carpels and the petals by stamens; in the *pistillata* mutant, petals are replaced by sepals and stamens by carpels. These mutations identified the floral organ identity genes, and have enabled their mode of action to be determined.

Homeotic floral mutations in *Arabidopsis* fall into three classes, each of which affects the organs of two adjacent whorls (Fig. 7.26). The first class of mutations, of
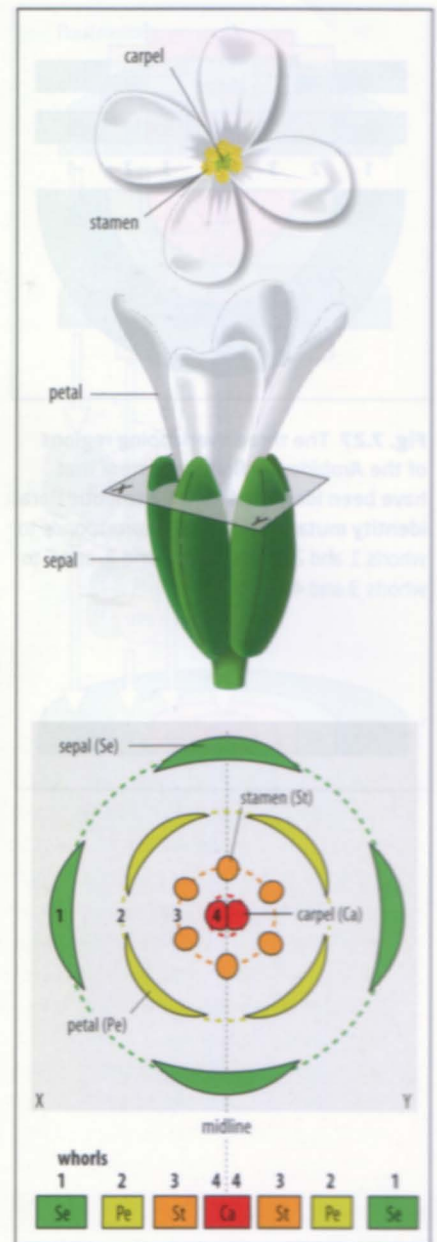
Fig. 7.26 Homeotic floral mutations in Arabidopsis. Left panel: an *apetala2* mutant has whorls of carpels and stamens in place of sepals and petals. Center panel: an *apetala3* mutant has two whorls of sepals and two of carpels. Right panel: *agamous* mutants have a whorl of petals and sepals in place of stamens and carpels. Transformations of whorls are shown inset, and can be compared to the wild-type arrangement, as shown in Fig. 7.25.

Photographs from Meyerowitz, E.M., et al.: 1991 (left panel), and Bowman, J.L., et al.: 1989 (center panel).
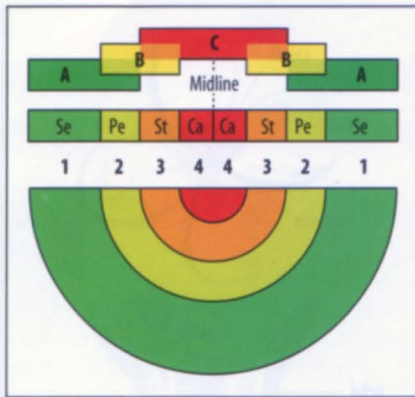
Fig. 7.27 The three overlapping regions of the *Arabidopsis* floral meristem that have been identified by the homeotic floral identity mutations. Region A corresponds to whorls 1 and 2, B to whorls 2 and 3, and C to whorls 3 and 4.

which *apetala2* is an example, affect whorls 1 and 2, giving carpels instead of sepals in whorl 1, and stamens instead of petals in whorl 2. The phenotype of the flower, going from the outside to the center, is therefore carpel, stamen, stamen, carpel. The second class of homeotic floral mutations affects whorls 2 and 3. In this class, *apetala3* and *pistillata* give sepals instead of petals in whorl 2 and carpels instead of stamens in whorl 3, with a phenotype sepal, sepal, carpel, carpel. The third class of mutations affects whorls 3 and 4, and gives petals instead of stamens in whorl 3 and sepals or variable structures in whorl 4. The mutant *agamous*, which belongs to this class, has an extra set of sepals and petals in the center instead of the reproductive organs.

These mutant phenotypes can be accounted for by an elegant model in which overlapping patterns of gene activity specify floral organ identity (Fig. 7.27) in a manner highly reminiscent of the way in which *Drosophila* homeotic genes specify segment identity along the insect's body. In detail, however, there are many differences, and quite different genes are involved. In this instance, plants and animals have, perhaps not surprisingly, independently evolved a similar approach to patterning a multicellular structure, but have recruited different proteins to carry it out.

In essence, the floral meristem is divided by the expression patterns of the homeotic genes into three concentric overlapping regions, A, B, and C, which partition the meristem into four non-overlapping regions corresponding to the four whorls. Each of the A, B, and C regions corresponds to the zone of action of one class of homeotic genes and the particular combinations of A, B, and C functions give each whorl a unique identity and so specify organ identity. Of the genes mentioned in Fig. 7.26, *APETALA1* (*AP1*) and *APETALA2* (*AP2*) are A-function genes, *APETALA3* (*AP3*) and *PISTILLATA* (*P1*) are B-function genes, and *AGAMOUS* (*AG*) is a C-function gene. The expression of *AP3* and *AG* in the developing flower is shown in Fig. 7.28. All the homeotic genes, also known as **floral organ identity genes**, encode transcription factors, and the B- and C-function proteins such as AP3 and AG contain a conserved DNA-binding sequence known as the MADS box. MADS-box genes are present in animals and yeast, but a role in development is known mainly in plants—although a MADS-box transcription factor, MEF2, is involved in muscle differentiation in animals. The original simple model for specifying floral organ identity is presented in more detail in Box 7C. Since the model was first proposed, more has become known about the activities and functions of the genes identified by the homeotic mutations, more genes controlling flower development have been discovered, and more 'functions' added.

One question that arose when the original floral organ identity model was investigated experimentally was why the ABC genes only showed their homeotic properties in the floral meristem and did not convert leaves into floral organs when artificially overexpressed in vegetative meristems, as might be expected for homeotic genes of this type. The answer came with the discovery of the *SEPALLATA* (*SEP*) genes, which also encode MADS-box proteins. These genes are required for the B and C functions and are only active in floral meristems. The SEP proteins are thought to combine with B

Fig. 7.28 Expression of *APETALA3* and *AGAMOUS* during flower development. *In situ* hybridization shows that *AGAMOUS* is expressed in the central whorls (left panel), whereas *APETALA3* is expressed in the outer whorls that give rise to petals and stamens (right panel).
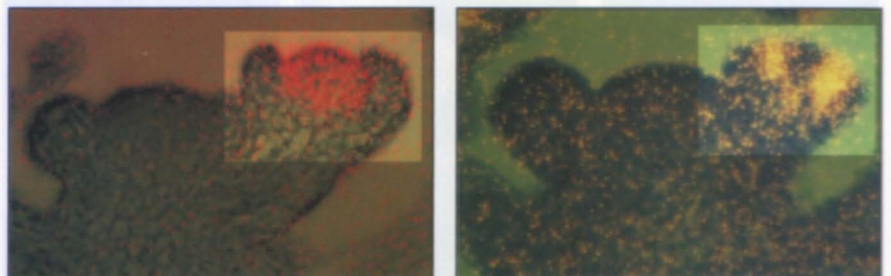
Fig. 7.29 Current status of the ABC model of floral organ identity. The regulatory genes *LEAFY*, *WUSCHEL (WUS)*, and *UNUSUAL FLORAL ORGANS (UFO)* are expressed in specific domains in the floral meristem, which, together with repression of *APETALA1* by *AGAMOUS*, results in the pattern of ABC functions. ABC proteins and the co-factor SEP proteins assemble into complexes that specify the different organ identities.

Adapted from Lohmann, J.U., Weigel, D.: 2002

and *C* gene products to form active gene-regulatory complexes. A current view of the mechanism of specifying floral organ identity is shown in Fig. 7.29.

There is a better understanding of the functions and patterning of the floral homeotic genes than when the ABC model was first proposed. The MADS-box homeotic A-class gene *AP1* has been found to have a dual role: it acts early with other genes to specify general floral meristem identity and only later contributes to A function. It is induced by the meristem identity gene *LFY*, which is expressed throughout the meristem, and *AP1* is actively inhibited in the central regions of floral meristems by *AGAMOUS*. The expression of the A-function gene *APETALA2 (AP2)* is translationally repressed by a microRNA, keeping the AP2 protein at a low level. *APETALA3* and *PISTILLATA1* are thought to be activated as a result of a meristem identity gene called *UNUSUAL FLORAL ORGANS (UFO)*, which is expressed in the meristem in a pattern similar to that of B-function genes (see Fig. 7.29). *UFO* encodes a component of ubiquitin ligase, and is thought to exert its effects on flower development by targeting specific proteins for degradation. As we saw in animals, in relation to the control of β-catenin degradation (see Chapters 4 and 6), regulated degradation of proteins can be a powerful developmental mechanism. In the center of the floral meristem, the expression of *AGAMOUS* is partly controlled by *WUS*, which as we have seen earlier, is expressed in the organizing center of the vegetative shoot meristem and continues to be expressed in floral meristems.

Another group of genes that help pattern the floral organ primordia are genes that control cell division. The gene *SUPERMAN* is one example, controlling cell proliferation in stamen and carpel primordia, and in ovules. Plants with a mutation in this gene have stamens instead of carpels in the fourth whorl. *SUPERMAN* is expressed in the third whorl, and maintains the boundary between the third and fourth whorls.

Despite the enormous variation in the flowers of different species, the mechanisms underlying flower development seem to be very similar. For example, there are striking similarities between the genes controlling flower development of *Arabidopsis* and *Antirrhinum*, despite the quite different final morphology of the snapdragon flower. In developing *Arabidopsis* flowers, the patterns of activity of the corresponding genes fit well with the cell-lineage restriction to whorls seen in *Antirrhinum*.

7.15 The *Antirrhinum* flower is patterned dorso-ventrally as well as radially

Like *Arabidopsis* flowers, those of *Antirrhinum* consist of four whorls, but unlike *Arabidopsis*, they have five sepals, five petals, four stamens, and two united carpels (Fig. 7.30, left). Floral homeotic mutations similar to those in *Arabidopsis* occur in *Antirrhinum*, and floral organ identity is specified in the same way. Several of the *Antirrhinum* homeotic genes have extensive homology with those of *Arabidopsis*, the MADS box in particular being well conserved.

An extra element of patterning is required in the *Antirrhinum* flower, which has a bilateral symmetry imposed on the basic radial pattern common to all flowers. In whorl 2, the upper two petal lobes have a shape quite distinct from the lower three, giving the flower its characteristic snapdragon appearance. In whorl 3, the uppermost stamen is absent, as its development is aborted early on. *The Antirrhinum flower*
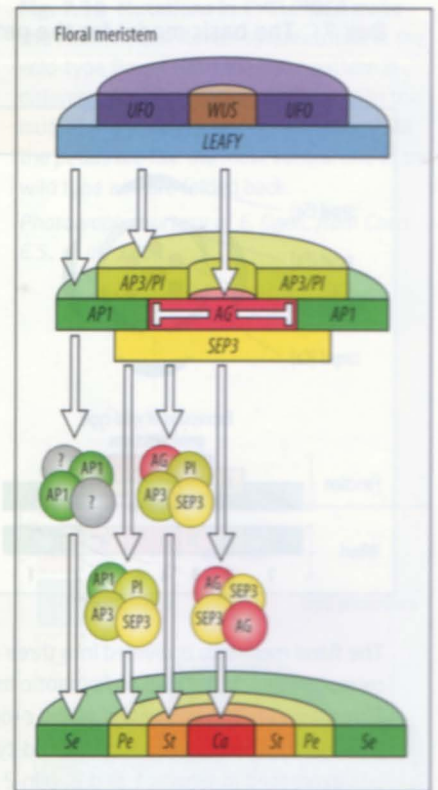
Box 7C The basic model for the patterning of the *Arabidopsis* flower

**Wild type** | **apetala2 mutant** | **agamous mutant**
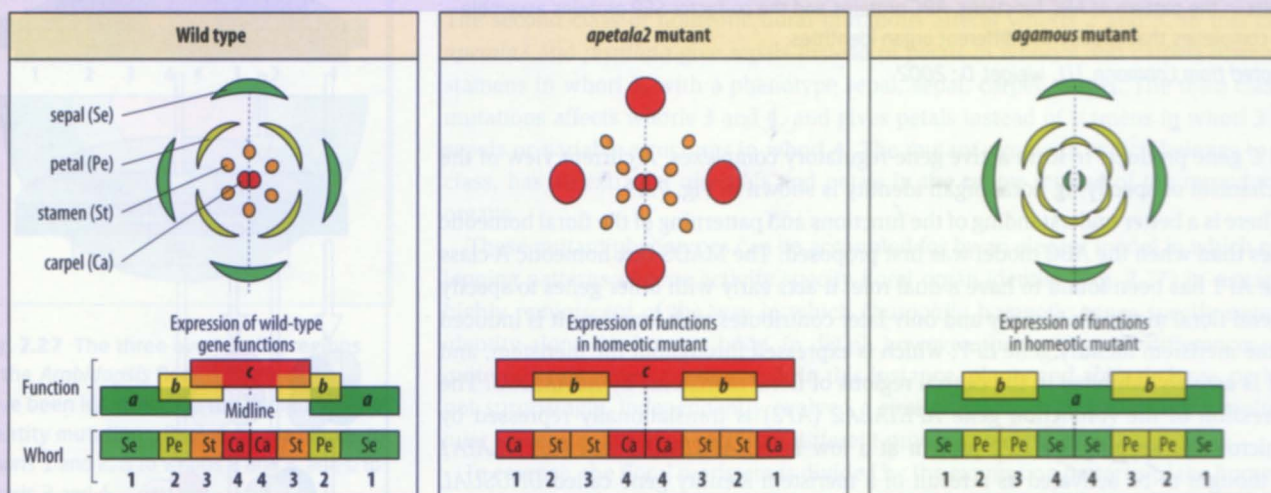
The floral meristem is divided into three overlapping regions, A, B, and C, each region corresponding to a class of homeotic mutations, as shown in Fig. 7.26 (see text). Three regulatory functions—*a*, *b*, and *c*—operate in regions A, B, and C, respectively, as shown in the panels above. In the wild-type flower (top left panel), it is assumed that *a* is expressed in whorls 1 and 2, *b* in 2 and 3, and *c* in whorls 3 and 4. In addition, *a* function inhibits *c* function in whorls 1 and 2 and *c* function inhibits *a* function in whorls 3 and 4—that is, *a* and *c* functions are mutually exclusive. *a* alone specifies sepals, *a* and *b* together specify petals, *b* and *c* stamens, and *c* alone carpels.

The homeotic mutations eliminate the functions of *a*, *b*, or *c*, and alter the regions within the meristem where the various functions are expressed. Mutations in *a*, such as *apetala2* (see center top panel), result in an absence of function *a*, and *c* spreads throughout the meristem, resulting in the half-flower pattern of carpel, stamen, stamen, carpel. Mutations in *b*, such as *apetala3* (see Fig. 7.26), result in only *a* functioning in whorls 1 and 2, and *c* in whorls 3 and 4, giving sepal, sepal, carpel, carpel. Mutations in *c* genes (such as *agamous*), result in *a* activity in all whorls, giving the phenotype sepal, petal, petal, sepal (see top right panel).

All the floral homeotic mutants discovered so far in *Arabidopsis* can be quite satisfactorily accounted for by this model (although there are small variations in gene numbers and expression patterns in other species that allow mutant phenotypes not seen in *Arabidopsis*), and particular genes can be assigned to each controlling function. Function *a* corresponds to the activity of genes such as *APETALA2*, *b* to *APETALA3* and *PISTILLATA*, and *c* to *AGAMOUS*. The model also accounts for the phenotype of double mutants, such as *apetala2* with *apetala3*, and *apetala3* with *pistillata*, as shown in the panels on the right.

This system emphasizes the similarity in function between the homeotic genes in animals and those controlling organ identity in flowers, although the genes themselves are completely different. The functional similarity with the Hox complex of *Drosophila* is further illustrated by the role of the *CURLY LEAF* gene *of Arabidopsis*, which is necessary for the stable maintenance of homeotic gene activity. *CURLY LEAF* is related to the *Polycomb* family of genes in *Drosophila* and is similarly required for stable repression of homeotic genes.
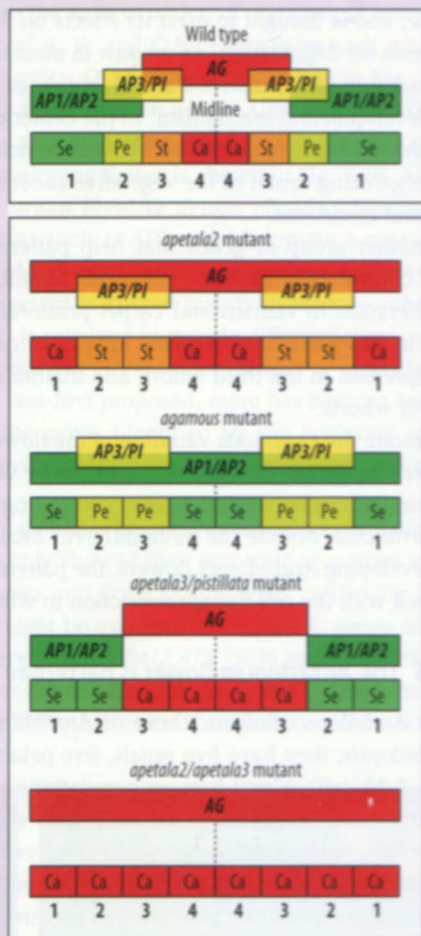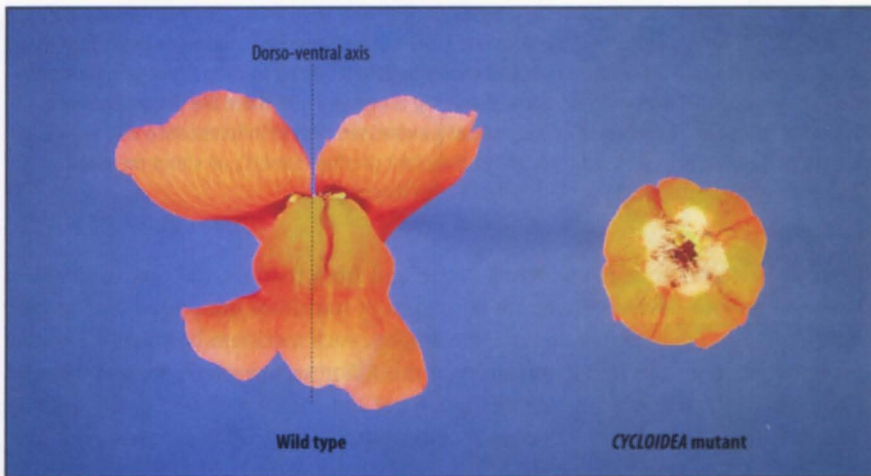
therefore has a distinct dorso-ventral axis. Another group of homeotic genes, different from those that govern floral organ identity, appear to act in this dorso-ventral patterning. For example, mutations in the gene *CYCLOIDEA*, which is expressed in the dorsal region, abolish dorso-ventral polarity and produce flowers that are more radially symmetrical (Fig. 7.30, right).

7.16 The internal meristem layer can specify floral meristem patterning

Although all three layers of a floral meristem (Fig. 7.31) are involved in organogenesis, the contribution of cells from each layer to a particular structure may be variable. Cells from one layer can become part of another layer without disrupting normal morphology, suggesting that a cell's position in the meristem is the main determinant of its future behavior. Some insight into positional signaling and patterning in the floral meristem can be obtained by making periclinal chimeras (see Section 7.7) from cells that have different genotypes and that give rise to different types of flower. From such chimeras, one can find out whether the cells develop autonomously according to their own genotype, or whether their behavior is controlled by signals from other cells.

As well as being produced by mutation, chimeras can also be generated by grafting between two plants of different genotypes. A new shoot meristem can form at the junction of the graft, and sometimes contains cells from both genotypes. Such chimeras can be made between wild-type tomato plants and tomato plants carrying the mutation *fasciated*, in which the flower has an increased number of floral organs per whorl. This phenotype is also found in chimeras in which only layer L3 contains *fasciated* cells (Fig. 7.32). The increased number of floral organs is associated with an overall increase in the size of the floral meristem, and in the chimeric plants this cannot be achieved unless the *fasciated* cells of layer L3 induce the wild-type L1 cells to divide more frequently than normal. The mechanism of intercellular signaling between L3 and L1 is not yet known. In *Antirrhinum*, the abnormal expression of *FLORICAULA* in only one meristem layer can result in flower development. These results illustrate the importance of signaling between layers in flower development.

7.17 The transition of a shoot meristem to a floral meristem is under environmental and genetic control

Flowering plants first grow vegetatively, during which time the apical meristem generates leaves. Then, triggered by environmental signals such as increasing daylength,
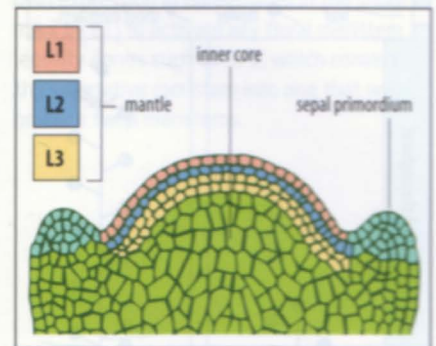
Fig. 7.31 Floral meristem. The meristem is composed of layers L1, L2, and L3. The inner core cells are derived from L3. The sepal primordia are just beginning to develop.
After Drews, G.N., et al.: 1989.
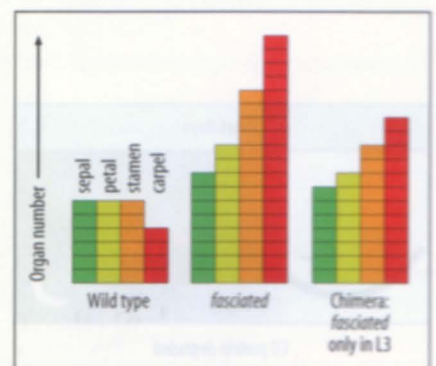
Fig. 7.32 Floral organ number in chimeras of wild-type and *fasciated* tomato plants. In the *fasciated* mutant there are more organs in the flower than in wild-type plants. In chimeras in which only layer L3 of the floral meristem contains *fasciated* mutant cells, the number of organs per flower is still increased, showing that L3 can control cell behavior in the outer layers of the meristem.

	Long day	Short day
Wild type		
LEAFY expressed throughout		

— leaf   ● flower   ↗ shoot meristem
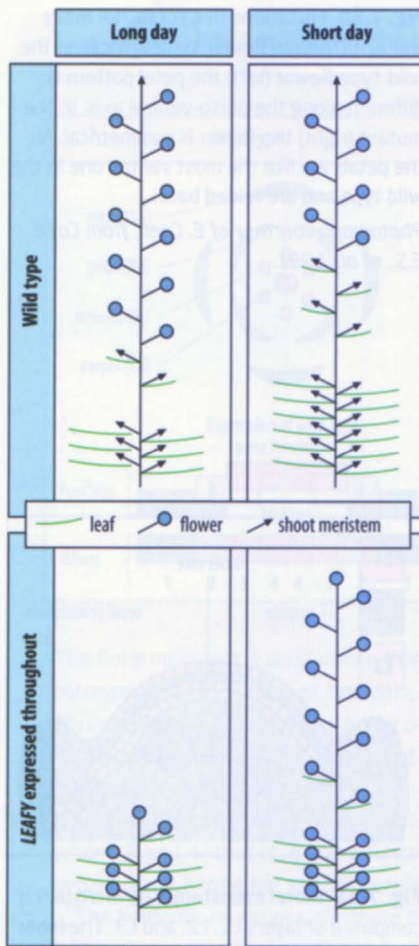
Fig. 7.33 Flowering can be controlled by daylength and LEAFY expression. As shown in the top panels, when wild-type *Arabidopsis* is grown under long-day conditions (left), few lateral shoots are formed before the apical shoot meristem begins to form floral meristems. When grown under short-day conditions, flowering is delayed and there are in consequence more lateral shoots. The gene *LEAFY* is normally expressed only in inflorescence and floral meristems, but if it is expressed throughout the plant (bottom panels), all shoot meristems produced are converted to floral meristems in both daylengths.

the plant switches to a reproductive phase and from then on the apical meristem gives rise only to flowers. There are two types of transition from vegetative growth to flowering. In the determinate type, the inflorescence meristem becomes a terminal flower, whereas in the indeterminate type the inflorescence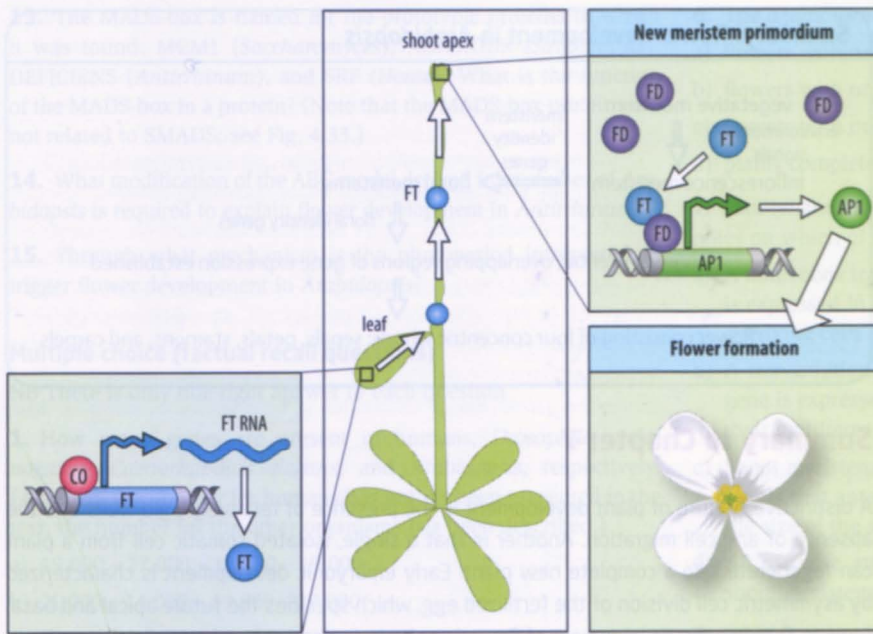 meristem gives rise to a number of floral meristems. *Arabidopsis* is of the indeterminate type (Fig. 7.33). A primary response to floral inductive signals in *Arabidopsis* is the expression of floral meristem identity genes such as *LEAFY* and the dual-function *AP1* (see Section 7.13), which are necessary and sufficient for this transition. *LEAFY* potentially activates *AP1* throughout the meristem while also activating *AGAMOUS* in the center of the flower. *AGAMOUS* then represses the expression of *AP1* in the center, helping to restrict its floral organ identity function to region A (see Fig. 7.27). Mutations in floral meristem identity genes partly transform flowers into shoots. In a *leafy* mutant, which lacks LEAFY function, the flowers are transformed into spirally arranged sepal-like organs along the stem, whereas expression of *LEAFY* throughout a plant is sufficient to confer a floral fate on lateral shoot meristems and they develop as flowers (Fig. 7.33, bottom panels).

In *Arabidopsis*, flowering is promoted by increasing daylength, which predicts the end of winter and the onset of spring and summer (see Fig. 7.33). This behavior is called **photoperiodism**. In some strains, flowering is also accelerated after the plant has been exposed to a long period of cold temperature, a cue that winter has passed. This phenomenon is known as **vernalization**. Grafting experiments have shown that daylength is sensed not by the shoot meristem itself, but by the leaves. When the period of continuous light reaches a certain length, a diffusible flower-inducing signal is produced that is transmitted through the phloem to the shoot meristem. The pathway that triggers flowering involves the plant's **circadian clock**, the internal 24-hour timer that causes many metabolic and physiological processes, including the expression of some genes, to vary throughout the day. One of the genes regulated by the circadian clock is *CONSTANS* (*CO*), which is a key gene in controlling the onset of flowering and provides the link between the plant's daylength-sensing mechanism and production of the flowering signal. The expression of *CO* oscillates on a 24-hour cycle under the control of the circadian clock, and its timing is such that the peak *CO* expression occurs towards the end of the afternoon. This means that in longer days, peak expression occurs in the light, whereas in short days, it will already be dark at this time. In the dark, the CO protein is degraded and so the circadian control ensures that CO only accumulates to high enough levels to trigger the flowering pathway when light conditions are favorable (Fig. 7.34).

CO is a transcription factor that activates a gene known as *FLOWERING LOCUS T* (*FT*), producing the FT protein, which appears to act as the flowering signal. The FT protein is thought to travel from the leaf through the phloem to the shoot apical meristem, where it acts in a complex with the transcription factor FLOWERING LOCUS D (FD),

Short days

CO protein degraded

Long days

CO protein accumulates
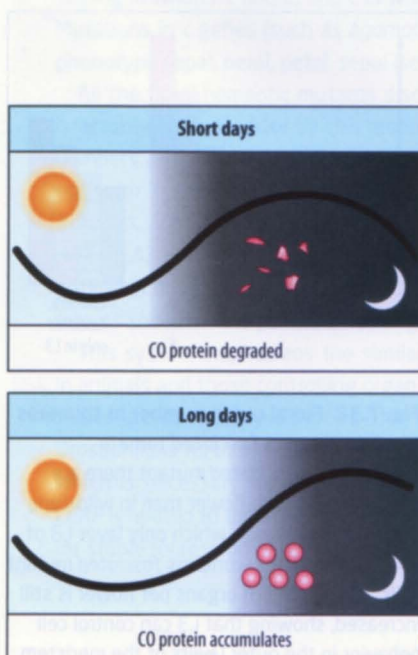
Fig. 7.34 The initiation of flowering is under the dual control of daylength and the circadian clock. The transcription factor CONSTANS (CO) is required for production of the flowering signal and is expressed in leaves under the control of the circadian clock. In short days, expression of the *CO* gene peaks in the dark and the protein is rapidly degraded. In long days, peak expression occurs in the light, and the CO protein accumulates.
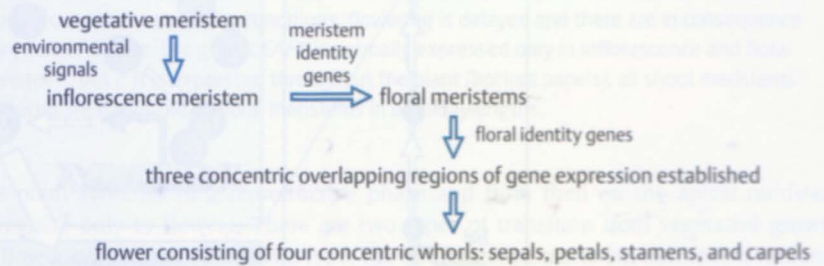
shoot apex

New meristem primordium

FT

leaf

Flower formation

FT RNA

CO

FT

FT

which is expressed in the meristem, to turn on the expression of genes such as *AP1*
that promote flowering (Fig. 7.35). If *FT* is activated in a single leaf, this is sufficient
to induce flowering. Induction of flowering also requires the downregulation of a set
of floral repressor genes such as *FLOWERING LOCUS C* (*FLC*). These suppress the
transition from a vegetative to the flowering state until the positive signals to flower
are received. FLC encodes a protein that binds to FT and suppresses its activity.
After cold exposure, for example, FLC activity is low, and the repression of FT can
be released.

Summary

Before flowering, which is triggered by environmental conditions such as daylength,
the vegetative shoot apical meristem becomes converted into an inflorescence
meristem, which either then becomes a flower or produces a series of floral mer-
istems, each of which develops into a single flower. Genes involved in the initiation
of flowering and patterning of the flower have been identified in both *Arabidopsis*
and *Antirrhinum.* Flowering is induced by daylength acting together with the plant's
natural circadian rhythms of gene expression to turn on a gene in the leaves that
produces a flowering signal that is transported to the shoot meristem. This signal
turns on the expression of meristem identity genes that are required for the trans-
formation of the vegetative shoot meristem to an inflorescence meristem and the
formation of floral meristems from the inflorescence meristem. Homeotic floral organ
identity genes, which specify the organ types found in the flowers, have been iden-
tified from mutations that transform one flower part into another. On the basis of
these mutations, a model has been proposed in which the floral meristem is divided
into three concentric overlapping regions, in each of which certain floral identity
genes act in a combinatorial manner to specify the organ type appropriate to each
whorl. Studies with chimeric plants have shown that different meristem layers com-
municate with each other during flower development and that transcription factors
can move between cells.

Summary: flower development in *Arabidopsis*

vegetative meristem

environmental signals

⬇

inflorescence meristem ➡ (meristem identity genes) ➡ floral meristems

⬇ floral identity genes

three concentric overlapping regions of gene expression established

⬇

flower consisting of four concentric whorls: sepals, petals, stamens, and carpels

Summary to Chapter 7

A distinctive feature of plant development is the presence of relatively rigid walls and the absence of any cell migration. Another is that a single, isolated somatic cell from a plant can regenerate into a complete new plant. Early embryonic development is characterized by asymmetric cell division of the fertilized egg, which specifies the future apical and basal regions. During early development of flowering plants, both asymmetric cell division and cell–cell interactions are involved in patterning the body plan. During this process, the shoot and root meristems are specified and these meristems give rise to all the organs of the plant—stems, leaves, flowers, and roots. The shoot meristem gives rise to leaves in well-defined positions, a process involving regulated transport of a morphogen, auxin. The shoot meristem eventually becomes converted to an inflorescence meristem, which either becomes a floral meristem (in determinate inflorescences) or gives rise to a series of floral meristems, retaining its shoot meristem identity indefinitely (in indeterminate inflorescences). In floral meristems, each of which develops into a flower, homeotic floral organ identity genes act in combination to specify the floral organ types. Increasing daylength induces the synthesis of a flowering signal in the leaves that is transported to the shoot meristem where it induces flower formation.

■ End of chapter questions

Long answer (concept questions)

1. What features led to the adoption of *Arabidopsis thaliana* as the predominant model for plant development?

2. Distinguish between the following parts of a plant: shoot, root, node, leaf, meristem, sepal, petal, stamen, carpel.

3. What is the role of auxin before the 32-cell stage in *Arabidopsis* embryogenesis? What is the mechanism by which a differential in auxin concentrations is generated? What is the mechanism by which auxins influence gene expression?

4. Describe the process by which transgenic plants are produced. Include the role of the Ti plasmid, and of *Agrobacterium tumefaciens*.

5. What is a meristem? Describe the structure of the shoot meristem of *Arabidopsis*.

6. Contrast the roles of the homeobox genes *WUSCHEL* (*WUS*) and *SHOOT MERISTEMLESS* (*STM*) in formation and maintenance of shoot meristems.

7. What has the study of mericlinal chimeras revealed about cell specification during plant embryogenesis? Are cells specified in the embryo to form leaves and flowers, in a way analogous, for example, to the specification of cells as dorsal mesoderm in animal embryos?

8. What are the terms applied to the top and bottom surfaces of a leaf, in reference to the radial axis of the shoot? How are the transcription factors PHAB, PHAV, and REV restricted to the top surface?

9. How does auxin control the positioning of leaf primordia? Include the role of the PIN transporter and the concept of lateral inhibition in your answer.

10. What is SHORT-ROOT? How does it come to be present in endodermal cells? Might plasmodesmata be involved? Is there an analogous process involving the SHOOT MERISTEMLESS ortholog KNOTTED-1 of maize?

11. What is the nature of the homeotic mutations that can occur in *Arabidopsis*? What genes are involved?

12. What is the ABC model for flower development? How does it illustrate combinatorial control of cellular identity?

13. The MADS-box is named for the prototypic proteins in which it was found: MCM1 (*Saccharomyces*), AGAMOUS (*Arabidopsis*), DEFICIENS (*Antirrhinum*), and SRF (*Homo*). What is the function of the MADS-box in a protein? (Note that the MADS-box proteins are not related to SMADS; see Fig. 4.33.)

14. What modification of the ABC model derived from studies of *Arabidopsis* is required to explain flower development in *Antirrhinum*?

15. Through what mechanism is the photoperiod interpreted to trigger flower development in *Arabidopsis*?

Multiple choice (factual recall questions)

NB There is only one right answer to each question

1. How many genes are present in humans, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis*, respectively. (Although the number for humans has not yet been presented in the text, the number for the other organisms has been described.)

a) 19,000 – 27,000 – 14,000 – 19,000

b) 21,000 – 14,000 – 19,000 – 27,000

c) 27,000 – 21,000 – 19,000 – 14,000

d) 14,000 – 19,000 – 21,000 – 27,000

2. Embryogenesis in plants occurs

a) in the ovule, after the seed is fertilized and shed by the plant

b) in the seed after it germinates

c) in the seed, after the seed is fertilized

d) inside the ovule, before the seed is shed by the plant

3. Which statement is true about the totipotency of cells?

a) All animal and plant cells are totipotent.

b) In plants, many cells are totipotent, whereas in animals, only the fertilized egg is totipotent.

c) Mammalian embryonic stem cells are totipotent.

d) Only the stem cells of animals, and the meristem cells of plants are totipotent.

4. One of the earliest events in *Arabidopsis* development is formation of the _____ axis, in response to a gradient of _____.

a) adaxial–abaxial, cytokinins

b) apical–basal, auxin

c) apical–basal, Pin proteins

d) dorsal–ventral, miRNA

5. The fate map of the *Arabidopsis* embryo at the heart stage indicates that

a) although none of the adult structures have formed, the regions that will give rise to the meristems, which will in turn give rise to adult structures, can be identified

b) development in plants is so indeterminate that a true fate map cannot be drawn

c) the primordia of the leaves, stems, and roots have already formed

d) the three germ layers that will give rise to roots, stems, and leaves have formed

6. The *agamous* mutation causes the formation of

a) flowers with only petals and sepals

b) flowers with only sepals and carpels

c) flowers with only stamens and carpels

d) plants completely lacking flowers

7. Maintenance of the shoot meristems in adult *Arabidopsis* plants relies on which of the following mechanisms?

a) A homeobox transcription factor encoded by the WUSCHEL gene is expressed in the organizing center and initiates a signal to the overlying cells to behave as stem cells.

b) A transcription factor encoded by the SHOOT MERISTEMLESS gene is expressed in shoot meristem cells and maintains them in their undifferentiated state.

c) Shoot meristems cells secrete proteins encoded by the CLAVATA family that antagonize WUSCHEL expression, thereby restricting the size of the shoot meristems.

d) All of these are involved in specification and maintenance of shoot meristems.

8. What is meant by the word 'whorl' in discussing floral meristems?

a) Flowers consist of four different types of organs, which occur in concentric rings called 'whorls.'

b) The floral meristem as to rotate during flower formation, giving the process the name 'whorl.'

c) The flowers of *Arabidopsis* appear as the stem elongates in a pattern called a 'whorl.'

d) The six stamens in a dicot flower like that of *Arabidopsis* form a ring that is called the flower's 'whorl.'

9. In what way are the homeotic genes of flowering plants similar to those of *Drosophila* and other animals?

a) All homeotic genes encode transcription factors of the homeobox class.

b) Homeotic genes in both plants and animals encode transcription factors of the MADS-box type.

c) Mutations in the homeotic genes of flowers cause transformation of one organ into another.

d) The homeotic genes of flowers are derived during evolution from the same primordial genes used in animals.

10. How is the ABC model for floral identity in *Arabidopsis* reminiscent of the models for homeotic gene function derived from studies in *Drosophila*?

a) In both organisms, each homeobox gene specifies the identity of a different region of the adult.

b) In both organisms, one homeotic gene is expressed at the two ends, a second expressed in domains more central to that of the first, and a third expressed most centrally, thus contributing unambiguous identities to all regions of the organism.

c) In both organisms, it is often the combination of genes present that is critical in unambiguously specifying structures in the adult.

d) The key responsibility of the homeotic genes in both organisms is the patterning of antero-posterior identity.

Multiple choice answer key

1: b, 2: d, 3: b, 4: b, 5: a, 6: a, 7: d, 8: a, 9: c, 10: c.

■ General further reading

Meyerowitz, E.M.: *Arabidopsis—a useful weed*. Cell 1989, **56**: 263–264.

Meyerowitz, E.M.: Plants compared to animals: the broader comparative view of development. Science 2002, **295**: 1482–1485.

■ Section further reading

7.1 The model plant *Arabidopsis thaliana* has a short life-cycle and a small diploid genome & 7.2 Plant embryos develop through several distinct stages

Lloyd, C.: **Plant morphogenesis: life on a different plane**. *Curr. Biol.* 1995, **5**: 1085–1087.

Mayer, U., Jürgens, G.: **Pattern formation in plant embryogenesis: a reassessment**. *Semin. Cell Dev. Biol.* 1998, **9**: 187–193.

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

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

7.3 Gradients of the signal molecule auxin establish the embryonic apical-basal axis

Breuninger, H., Rikirsch, E., Hermann, M., Ueda, M., Laux, T.: **Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo**. *Dev. Cell* 2008, **14**: 867–876.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., Jürgens, G.: **Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis***. *Nature* 2003, **426**: 147–153.

Jenik, P.D., Barton, M.K.: **Surge and destroy: the role of auxin in plant embryogenesis**. *Development* 2005, **132**: 3577–3585.

Jürgens, G.: **Axis formation in plant embryogenesis: cues and clues**. *Cell* 1995, **81**: 467–470.

Long, J.A., Moan, E.I., Medford, J.I., Barton, M.K.: **A member of the knotted class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis***. *Nature* 1995, **379**: 66–69.

Szemenyei, H., Hannon, M., Long, J.A.: **TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis**. *Science* 2008, **319**: 1384–1386.

7.4 Plant somatic cells can give rise to embryos and seedlings

Zimmerman, J.L.: **Somatic embryogenesis: a model for early development in higher plants**. *Plant Cell* 1993, **5**: 1411–1423.

7.5 A meristem contains a small central zone of self-renewing stem cells

Byrne, M.E., Kidner, C.A., Martienssen, R.A.: **Plant stem cells: divergent pathways and common themes in shoots and roots**. *Curr. Opin. Genet. Dev.* 2003, **13**: 551–557.

Großhardt, R., Laux, T.: **Stem cell regulation in the shoot meristem**. *J. Cell Sci.* 2003, **116**: 1659–1666.

Ma, H.: **Gene regulation: Better late than never?** *Curr. Biol.* 2000, **10**: R365–R368.

7.6 The size of the stem-cell area in the meristem is kept constant by a feedback loop to the organizing center

Brand, U., Fletcher, J.C., Hobe, M., Meyerowitz, E.M., Simon, R.: **Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity**. *Science* 2000, **289**: 635–644.

Carles, C.C., Fletcher, J.C.: **Shoot apical meristem maintenance: the art of dynamical balance**. *Trends Plant Sci.* 2003, **8**: 394–401.

Clark, S.E.: **Cell signalling at the shoot meristem**. *Nat Rev. Mol. Cell Biol.* 2001, **2**: 277–284.

Lenhard, M., Laux, T.: **Stem cell homeostasis in the *Arabidopsis* shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1**. *Development* 2003, **130**: 3163–3173.

Reddy, G.V., Meyerowitz, E.M.: **Stem-cell homeostasis and growth dynamics can be uncoupled in the *Arabidopsis* shoot apex**. *Science* 2005, **310**: 663–667.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jürgens, G., Laux, T.: **The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes**. *Cell* 2000, **100**: 635–644.

Vernoux, T., Benfey, P.N.: **Signals that regulate stem cell activity during plant development**. *Curr. Opin. Genet. Dev.* 2005, **15**: 388–394.

7.7 The fate of cells from different meristem layers can be changed by changing their position

Castellano, M.M., Sablowski, R.: **Intercellular signalling in the transition from stem cells to organogenesis in meristems**. *Curr. Opin. Plant Biol.* 2005, **8**: 26–31.

Gallagher, K.L., Benfey, P.N.: **Not just another hole in the wall: understanding intercellular protein trafficking**. *Genes Dev.* 2005, 19: 189–195.

Laux, T., Mayer, K.F.X.: **Cell fate regulation in the shoot meristem**. *Semin. Cell Dev. Biol.* 1998, **9**: 195–200.

Sinha, N.R., Williams, R.E., Hake, S.: **Overexpression of the maize homeobox gene *knotted-1*, causes a switch from determinate to indeterminate cell fates**. *Genes Dev.* 1993, **7**: 787–795.

Turner, I.J., Pumfrey, J.E.: **Cell fate in the shoot apical meristem of *Arabidopsis thaliana***. *Development* 1992, **115**: 755–764.

Waites, R., Simon, R.: **Signaling cell fate in plant meristems: three clubs on one tousle**. *Cell* 2000, **103**: 835–838.

7.8 A fate map for the embryonic shoot meristem can be deduced using clonal analysis

Irish, V.F.: **Cell lineage in plant development**. *Curr. Opin. Genet. Dev.* 1991, **1**: 169–173.

7.9 Meristem development is dependent on signals from other parts of the plant

Doerner, P.: **Shoot meristems: intercellular signals keep the balance**. *Curr. Biol.* 1999, **9**: R377–R380.

Irish, E.E., Nelson, T.M.: **Development of maize plants from cultured shoot apices**. *Planta* 1988, **175**: 9–12.

Sachs, T.: *Pattern Formation in Plant Tissues*. Cambridge: Cambridge University Press, 1994.

7.10 Gene activity patterns the proximo-distal and adaxial-abaxial axes of leaves developing from the shoot meristem

Bowman, J.L.: **Axial patterning in leaves and other lateral organs**. *Curr. Opin. Genet. Dev.* 2000, **10**: 399–404.

Kidner, C.A., Martienssen, R.A.: **Spatially restricted microRNA directs leaf polarity through *ARGONAUTE1***. *Nature* 2004, **428**: 81–84.

Waites, R., Selvadurai, H.R., Oliver, I.R., Hudson, A.: **The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum***. *Cell* 1998, **93**: 779–789.

7.11 The regular arrangement of leaves on a stem is generated by regulated auxin transport

Berleth, T., Scarpella, E., Prusinkiewicz, P.: **Towards the systems biology of auxin-transport-mediated patterning**. *Trends Plant Sci.* 2007, **12**: 151–159.

Heisler, M.G., Ohno, C., Das, P., Sieber, P., Reddy, G.V., Long, J.A., Meyerowitz, E.M.: **Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem**. *Curr. Biol.* 2005, **15**: 1899–1911.

Jönsson, H., Heisler, M.G., Shapiro, B.E., Meyerowitz, E.M., Mjolsness, E.: **An auxin-driven polarized transport model for phyllotaxis**. *Proc. Natl. Acad. Sci. USA* 2006, **103**: 1633–1638.

Mitchison, G.J.: **Phyllotaxis and the Fibonacci series**. *Science* 1977, **196**: 270–275.

Reinhardt, D.: **Regulation of phyllotaxis**. *Int. J. Dev. Biol.* 2005, **49**: 539–546.

Scheres, B.: **Non-linear signaling for pattern formation**. *Curr. Opin. Plant Biol.* 2000, **3**: 412–417.

Schiefelbein, J.: **Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot**. *Curr. Opin. Plant Biol.* 2003, **6**: 74–78.

Smith, L.G., Hake, S.: **The initiation and determination of leaves**. *Plant Cell* 1992, **4**: 1017–1027.

7.12 Root tissues are produced from *Arabidopsis* root apical meristems by a highly stereotyped pattern of cell divisions

Costa, S., Dolan, L.: **Development of the root pole and patterning in *Arabidopsis* roots**. *Curr. Opin. Genet. Dev.* 2000, **10**: 405–409.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., Sabatini, S.: **A genetic framework for the control of cell division and differentiation in the root meristem**. *Science* 2008, **322**: 1380–1384.

Dolan, L.: **Positional information and mobile transcriptional regulators determine cell pattern in *Arabidopsis* root epidermis**. *J. Exp. Bot.* 2006, **57**: 51–54.

Grieneisen, V.A., Xu, J., Marée, A.F.M., Hogeweg P., Scheres, B.: **Auxin transport is sufficient to generate a maximum and gradient guiding root growth**. *Nature* 2007, **449**: 1008–1013.

Sabatini, S., Beis, D., Wolkenfeldt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P.,

Scheres, B.: **An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root**. *Cell* 1999, **99**: 463–472.

Scheres, B., McKhann, H.I., van den Berg, C.: **Roots redefined: anatomical and genetic analysis of root development**. *Plant Physiol.* 1996, **111**: 959–964.

van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P., Scheres, B.: **Short-range control of cell differentiation in the *Arabidopsis* root meristem**. *Nature* 1997, **390**: 287–289.

Veit, B.: **Plumbing the pattern of roots**. *Nature* 2007, **449**: 991–992.

7.13 Root hairs are specified by a combination of positional information and lateral inhibition

Kwak, S.-H., Schiefelbein, J.: **The role of the SCRAMBLED receptor-like kinase in patterning the *Arabidopsis* root epidermis**. *Dev. Biol.* 2007, **302**: 118–131.

Schiefelbein, J., Kwak, S.-H., Wieckowski, Y., Barron, C., Bruex, A.: **The gene regulatory network for root epidermal cell-type pattern formation in *Arabidopsis***. *J. Exp. Bot.* 2009, **60**: 1515–1521.

7.14 Homeotic genes control organ identity in the flower

Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U., Meyerowitz, E.M.: **SUPERMAN, a regulator of floral homeotic genes in *Arabidopsis***. *Development* 1992, **114**: 599–615.

Breuil-Broyer, S., Morel, P., de Almeida-Engler, J., Coustham, V., Negrutiu, I., Trehin, C.: **High-resolution boundary analysis during *Arabidopsis thaliana* flower development**. *Plant J.* 2004, **38**: 182–192.

Coen, E.S., Meyerowitz, E.M.: **The war of the whorls: genetic interactions controlling flower development**. *Nature* 1991, **353**: 31–37.

Irish, V.F.: **Patterning the flower**. *Dev. Biol.* 1999, **209**: 211–220.

Krizek, B.A., Meyerowitz, E.M.: **The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function**. *Development* 1996, **122**: 11–22.

Krizek, B.A., Fletcher, J.C.: **Molecular mechanisms of flower development: an armchair guide**. *Nat Rev. Genet.* 2005, **6**: 688–698.

Lohmann, J.U., Weigel, D.: **Building beauty: the genetic control of floral patterning**. *Dev. Cell* 2002, **2**: 135–142.

Ma, H., dePamphilis, C.: **The ABCs of floral evolution**. *Cell* 2000, **101**: 5–8.

Meyerowitz, E.M., Bowman, J.L., Brockman, L.L., Drews, G.M., Jack, T., Sieburth, L.E., Weigel, D.: **A genetic and molecular model for flower development in *Arabidopsis thaliana***. *Development Suppl.* 1991, **1**: 157–167.

Meyerowitz, E.M.: **The genetics of flower development**. *Sci. Am.* 1994, **271**: 40–47.

Sakai, H., Medrano, L.J., Meyerowitz, E.M.: **Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries**. *Nature* 1994, **378**: 199–203.

Vincent, C.A., Carpenter, R., Coen, E.S.: **Cell lineage patterns and homeotic gene activity during *Antirrhinum* flower development**. *Curr. Biol.* 1995, **5**: 1449–1458.

Wagner, D., Sablowski, R.W.M., Meyerowitz, E.M.: **Transcriptional activation of *APETALA 1***. *Science* 1999, **285**: 582–584.

7.15 The *Antirrhinum* flower is patterned dorso-ventrally as well as radially

Coen, E.S.: **Floral symmetry**. *EMBO J.* 1996, **15**: 6777–6788.

Luo, D., Carpenter, R., Vincent, C., Copsey, L., Coen, E.: **Origin of floral asymmetry in *Antirrhinum***. *Nature* 1996, **383**: 794–799.

7.16 The internal meristem layer can specify floral meristem patterning

Szymkowiak, E.J., Sussex, I.M.: **The internal meristem layer (L3) determines floral meristem size and carpel number in tomato periclinal chimeras**. *Plant Cell* 1992, **4**: 1089–1100.

7.17 The transition of a shoot meristem to a floral meristem is under environmental and genetic control

An, H., Roussot, C., Suarez-Lopez, P., Corbesier, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., Coupland, G.: **CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis***. *Development* 2004, **131**: 3615–3626.

Becroft, P.W.: **Intercellular induction of homeotic gene expression in flower development**. *Trends Genet.* 1995, **11**: 253–255.

Blázquez, M.A.: **The right time and place for making flowers**. *Science* 2005, **309**: 1024–1025.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., Coupland, G.: **FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis***. *Science* 2007, **316**: 1030–1033.

Hake, S.: **Transcription factors on the move**. *Trends Genet.* 2001, **17**: 2–3.

Jaeger, K.E., Wigge, P.A.: **FT protein acts as a long-range signal in *Arabidopsis***. *Curr. Biol.* 2007, **17**: 1050–1054.

Kobayashi, Y., Weigel, D.: **Move on up, it's time for change - mobile signals controlling photoperiod-dependent flowering**. *Genes Dev.* 2007, **21**: 2371–2384.

Lohmann, J.U., Hong, R.L., Hobe, M., Busch, M.A., Parcy, F., Simon, R., Weigel, D.: **A molecular link between stem cell regulation and floral patterning in *Arabidopsis***. *Cell* 2001, **105**: 793–803.

Putterill, J., Laurie, R., Macknight, R.: **It's time to flower: the genetic control of flowering time**. *BioEssays* 2004, **26**: 363–373.

Sheldon, C.C., Hills, M.J., Lister, C., Dean, C., Dennis, E.S., Peacock, W.J.: **Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization**. *Proc. Natl. Acad. Sci. USA* 2008, **105**: 2214–2219.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., Coupland, G.: **Photoreceptor regulation of CONSTANS protein in photoperiodic flowering**. *Science* 2004, **303**: 1003–1006.