Chapter

16

Growth and Development

THE VEGETATIVE PHASE OF DEVELOPMENT begins with embryogenesis, but development continues throughout the life of a plant. Plant developmental biologists are concerned with questions such as, How does a zygote give rise to an embryo, an embryo to a seedling? How do new plant structures arise from preexisting structures? Organs are generated by cell division and expansion, but they are also composed of tissues in which groups of cells have acquired specialized functions, and these tissues are arranged in specific patterns. How do these tissues form in a particular pattern, and how do cells differentiate? What are the basic principles that govern the size increase (growth) that occurs throughout plant development?

Understanding how growth, cell differentiation, and pattern formation are regulated at the cellular, biochemical, and molecular levels is the ultimate goal of developmental biologists. Such an understanding also must include the genetic basis of development. Ultimately, development is the unfolding of genetically encoded programs. Which genes are involved, what is their hierarchical order, and how do they bring about developmental change?

In this chapter we will explore what is known about these questions, beginning with embryogenesis. Embryogenesis initiates plant development, but unlike animal development, plant development is an ongoing process. Embryogenesis establishes the basic plant body plan and forms the meristems that generate additional organs in the adult.

After discussing the formation of the embryo, we will examine root and shoot meristems. Most plant development is postembryonic, and it occurs from meristems. Meristems can be considered to be cell factories in which the ongoing processes of cell division, expansion, and differentiation generate the plant body. Cells derived from meristems become the tissues and organs that determine the overall size, shape, and structure of the plant.

Vegetative meristems are highly repetitive—they produce the same or similar structures over and over again—and their activity can con-

tinue indefinitely, a phenomenon known as *indeterminate growth*. Some long-lived trees, such as bristlecone pines and the California redwoods, continue to grow for thousands of years. Others, particularly annual plants, may cease vegetative development with the initiation of flowering after only a few weeks or months of growth. Eventually the adult plant undergoes a transition from vegetative to reproductive development, culminating in the production of a zygote, and the process begins again. Reproductive development will be discussed in Chapter 24.

Cells derived from the apical meristems exhibit specific patterns of cell expansion, and these expansion patterns determine the overall shape and size of the plant. We will examine how plant growth is analyzed after discussing meristems, with an emphasis on growth patterns in space (relationship of plant structures) and time (when events occur).

Finally, despite their indeterminate growth habit, plants, like all other multicellular organisms, senesce and die. At the end of the chapter we will consider death as a developmental phenomenon, at both the cellular and organismal levels. Foe an historical overviw of the study of plant development, see **Web Essay 16.1**.

EMBRYOGENESIS

The developmental process known as **embryogenesis** initiates plant development. Although embryogenesis usually begins with the union of a sperm with an egg, forming a single-celled *zygote*, somatic cells also may undergo embryogenesis under special circumstances. Fertilization also initiates three other developmental programs: endosperm, seed, and fruit development. Here we will focus on embryogenesis because it provides the key to understanding plant development.

Embryogenesis transforms a single-celled zygote into a multicellular, microscopic, embryonic plant. A completed **embryo** has the basic body plan of the mature plant and many of the tissue types of the adult, although these are present in a rudimentary form.

Double fertilization is unique to the flowering plants (see **Web Topics 1.1 and 1.2**). In plants, as in all other eukaryotes, the union of one sperm with the egg forms a single-celled zygote. In angiosperms, however, this event is accompanied by a second fertilization event, in which another sperm unites with two polar nuclei to form the triploid endosperm nucleus, from which the ***endosperm*** (the tissue that supplies food for the growing embryo) will develop.

Embryogenesis occurs within the **embryo sac** of the ovule while the ovule and associated structures develop into the **seed**. Embryogenesis and endosperm development typically occur in parallel with seed development, and the embryo is part of the seed. Endosperm may also be part of the mature seed, but in some species the endosperm disappears before seed development is completed. Embryogenesis and seed development are highly ordered, integrated processes, both of which are initiated by double fertilization. When completed, both the seed and the embryo within it become dormant and are able to survive long periods unfavorable for growth. The ability to form seeds is one of the keys to the evolutionary success of angiosperms as well as gymnosperms.

The fact that a zygote gives rise to an organized embryo with a predictable and species-specific structure tells us that the zygote is genetically programmed to develop in a particular way, and that cell division, cell expansion, and cell differentiation are tightly controlled during embryogenesis. If these processes were to occur at random in the embryo, the result would be a clump of disorganized cells with no definable form or function.

In this section we will examine these changes in greater detail. We will focus on molecular genetic studies that have been conducted with the model plant *Arabidopsis* that have provided insights into plant development. It is most likely that most angiosperms probably use similar developmental mechanisms that appeared early in the evolution of the flowering plants and that the diversity of plant form is brought about by relatively subtle changes in the time and place where the molecular regulators of development are expressed, rather than by different mechanisms altogether (Doebley and Lukens 1998).

Arabidopsis thaliana is a member of the Brassicaceae, or mustard family (Figure 16.1). It is a small plant, well suited for laboratory culture and experimentation. It has been called the *Drosophila* of plant biology because of its widespread use in the study of plant genetics and molecular genetic mechanisms, particularly in an effort to understand plant developmental change. It was the first higher plant to have its genome completely sequenced. Furthermore, there is a concerted international effort to understand the function of every gene in the *Arabidopsis* genome by the year 2010. As a result, we are much closer to an understanding of the molecular mechanisms governing *Arabidopsis* embryogenesis than of those for any other plant species.

Embryogenesis Establishes the Essential Features of the Mature Plant

Plants differ from most animals in that embryogenesis does not directly generate the tissues and organs of the adult. For example, angiosperm embryogenesis forms a rudimentary plant body, typically consisting of an embryonic axis and two cotyledons (if it is a dicot). Nevertheless, embryogenesis establishes the two basic developmental patterns that persist and can easily be seen in the adult plant:

1. The apical–basal axial developmental pattern.
2. The radial pattern of tissues found in stems and roots.

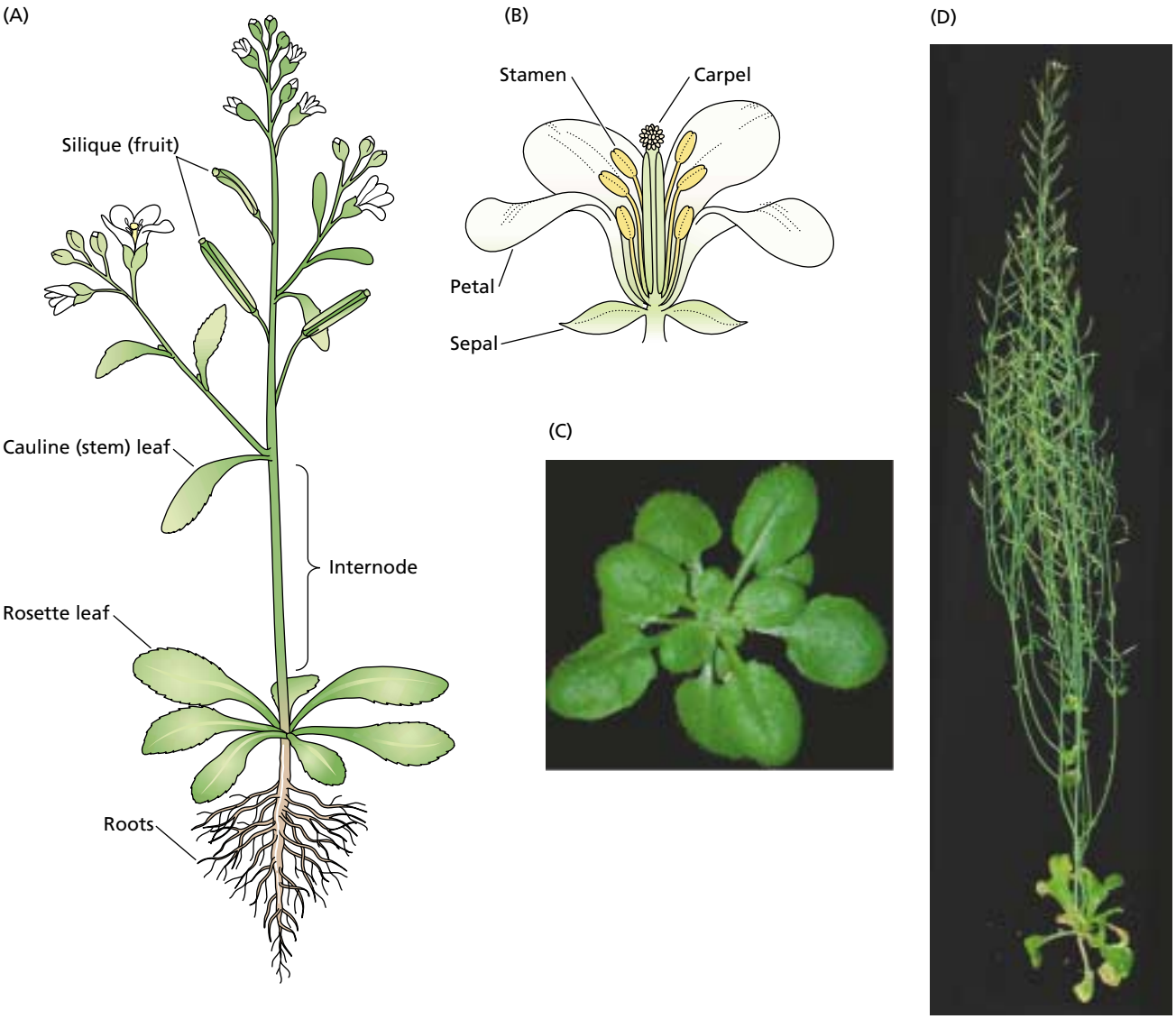


FIGURE 16.1 *Arabidopsis thaliana*. (A) Drawing of a mature *Arabidopsis* plant showing the various organs. (B) Drawing of a flower showing the floral organs. (C) An immature vegetative plant consisting of basal rosette leaves and a root system (not shown). (D) A mature plant after most of the flowers have matured and the siliques have developed. (A and B after Clark 2001; C and D courtesy of Caren Chang.)

Embryogenesis also establishes the **primary meristems**. Most of the structures that make up the adult plant are generated after embryogenesis through the activity of meristems. Although these primary meristems are established during embryogenesis, only upon germination will they become active and begin to generate the organs and tissues of the adult.

Axial patterning. Almost all plants exhibit an *axial polarity* in which the tissues and organs are arrayed in a precise order along a linear, or polarized, axis. The shoot apical meristem is at one end of the axis, the root apical meristem at the other. In the embryo and seedling, one or two cotyledons are attached just below the shoot apical meristem. Next in this linear array is the hypocotyl, followed by the root, the root apical meristem, and the root cap. This axial pattern is established during embryogenesis.

What may not be so obvious is the fact that any individual segment of either the root or the shoot also has apical and basal ends with different, distinct physiological and structural properties. For example, whereas adventitious roots develop from the basal ends of stem cuttings, buds develop from the apical ends, even if they are inverted (see Figure 19.12).

Radial patterning. Different tissues are organized in a precise pattern within plant organs. In stems and roots the tissues are arranged in a radial pattern extending from the outside of a stem or a root into its center. If we examine a root in cross section, for example, we see three concentric rings of tissues arrayed along a radial axis: An outermost

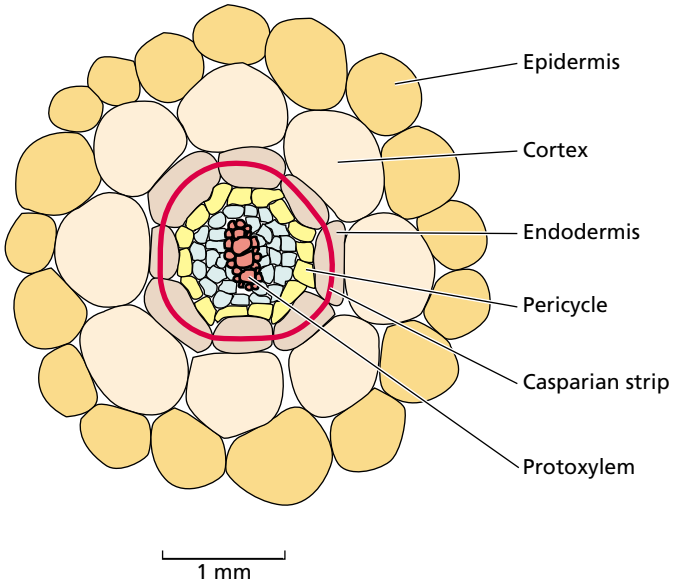


FIGURE 16.2 The radial pattern of tissues found in plant organs can be observed in a crosssection of the root. This crosssection of an *Arabidopsis* root was taken approximately 1 mm back from the root tip, a region in which the different tissues have formed.

layer of epidermal cells (the epidermis) covers a cylinder of cortical tissue (the cortex), which in turn overlies the vascular cylinder (the endodermis, pericycle, phloem, and xylem) (Figure 16.2) (see Chapter 1).

The **protoderm** is the meristem that gives rise to the epidermis, the **ground meristem** produces the future cortex and endodermis, and the **procambium** is the meristem that gives rise to the primary vascular tissue and vascular cambium.

Arabidopsis Embryos Pass through Four Distinct Stages of Development

The *Arabidopsis* pattern of embryogenesis has been studied extensively and is the one we will present here, but keep in mind that angiosperms exhibit many different patterns of embryonic development, and this is only one type.

The most important stages of embryogenesis in *Arabidopsis*, and many other angiosperms, are these:

1. *The **globular stage** embryo*. After the first zygotic division, the apical cell undergoes a series of highly ordered divisions, generating an eight-cell (**octant**) globular embryo by 30 hours after fertilization (Figure 16.3C). Additional precise cell divisions

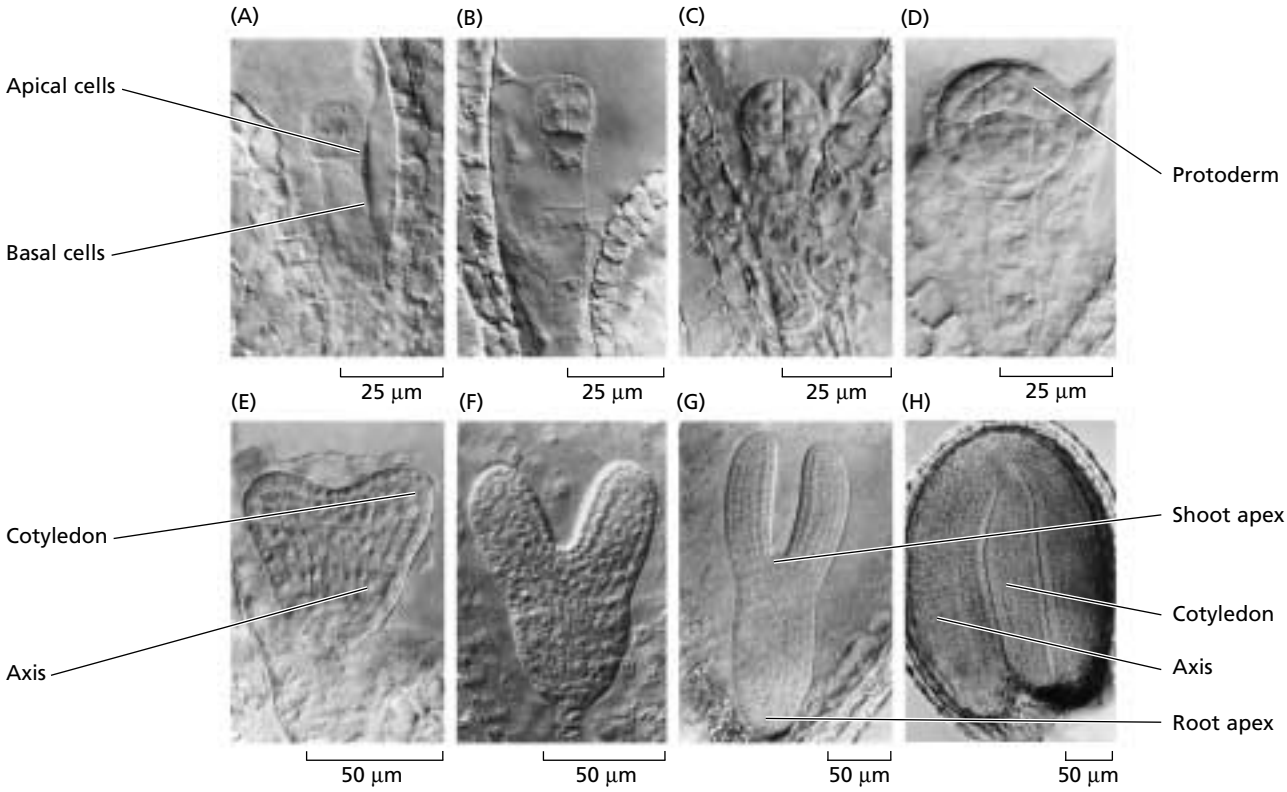


FIGURE 16.3 *Arabidopsis* embryogenesis is characterized by a precise pattern of cell division. Successive stages of embryogenesis are depicted here. (A) One-cell embryo after the first division of the zygote, which forms the apical and basal cells; (B) two-cell embryo; (C) eight-cell embryo; (D) early globular stage, which has developed a distinct protoderm (surface layer); (E) early heart stage; (F) late heart stage; (G) torpedo stage; (H) mature embryo. (From West and Harada 1993 photographs taken by K. Matsudaira Yee; courtesy of John Harada, © American Society of Plant Biologists, reprinted with permission.)

increase the number of cells in the sphere (Figure 16.3D).

2. *The **heart stage** embryo*. This stage forms through rapid cell divisions in two regions on either side of the future shoot apex. These two regions produce outgrowths that later will give rise to the cotyledons and give the embryo bilateral symmetry (Figure 16.3E and F).
3. *The **torpedo stage** embryo*. This stage forms as a result of cell elongation throughout the embryo axis and further development of the cotyledons (Figure 16.3G).
4. *The **maturation stage** embryo*. Toward the end of embryogenesis, the embryo and seed lose water and become metabolically quiescent as they enter dormancy (Figure 16.3H).

Cotyledons are food storage organs for many species, and during the cotyledon growth phase, proteins, starch, and lipids are synthesized and deposited in the cotyledons to be utilized by the seedling during the heterotrophic (nonphotosynthetic) growth that occurs after germination. Although food reserves are stored in the *Arabidopsis* cotyledons, the growth of the cotyledons is not as extensive in this species as it is in many other dicots. In monocots, the food reserves are stored mainly in the endosperm. In *Arabidopsis* and many other dicots, the endosperm develops rapidly early in embryogenesis but then is reabsorbed, and the mature seed lacks endosperm tissue.

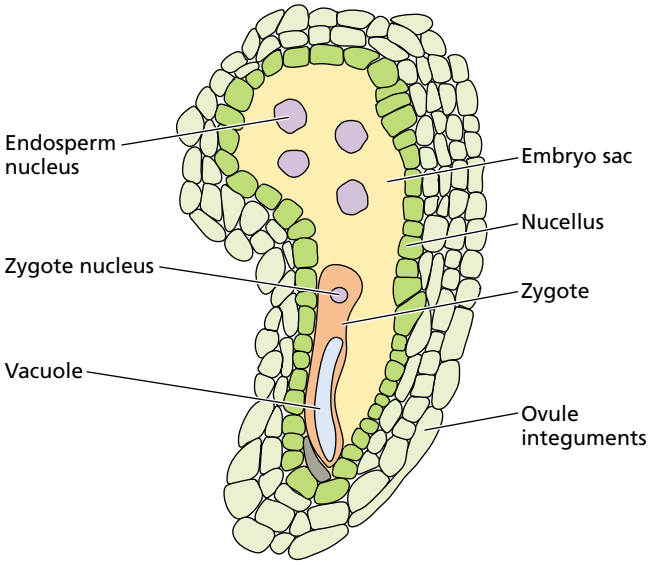


FIGURE 16.4 *Arabidopsis* ovule containing the embryo sac at about 4 hours after double fertilization. The zygote exhibits a marked polarization. The terminal half of the zygote has dense cytoplasm and a single large nucleus, while a large central vacuole occupies the basal half of the cell. At this stage, the embryo sac surrounding the zygote also contains 4 endosperm nuclei.

The Axial Pattern of the Embryo Is Established during the First Cell Division of the Zygote

Axial polarity is established very early in embryogenesis (see **Web Topic 16.1**). In fact, the zygote itself becomes polarized and elongates approximately threefold before its first division. The apical end of the zygote is densely cytoplasmic, but the basal half of the cell contains a large central vacuole (Figure 16.4).

The first division of the zygote is asymmetric and occurs at right angles to its long axis. This division creates two cells—an apical and a basal cell—that have very different fates (see Figure 16.3A). The smaller, apical daughter cell receives more cytoplasm than the larger, basal cell, which inherits the large zygotic vacuole. Almost all of the structures of the embryo, and ultimately the mature plant, are derived from the smaller apical cell. Two vertical divisions and one horizontal division of the apical cell generate the eight-celled (octant) globular embryo (see Figure 16.3C).

The basal cell also divides, but all of its divisions are horizontal, at right angles to the long axis. The result is a filament of six to nine cells known as the **suspensor** that attaches the embryo to the vascular system of the plant. Only one of the basal cell derivatives contributes to the embryo. The basal cell derivative nearest the embryo is known as the **hypophysis** (plural *hypophyses*), and it forms the **columella,** or central part of the root cap, and an essential part of the root apical meristem known as the *quiescent center*, which will be discussed later in the chapter (Figure 16.5).

Even though the embryo is spherical throughout the globular stage of embryogenesis (see Figure 16.3A–D), the cells within the apical and basal halves of the sphere have different identities and functions. As the embryo continues to grow and reaches the heart stage, its axial polarity becomes more distinct (see Figure 16.5), and three axial regions can readily be recognized:

1. The *apical region* gives rise to the cotyledons and shoot apical meristem.
2. The *middle region* gives rise to the hypocotyl, root, and most of the root meristem.
3. The *hypophysis* gives rise to the rest of the root meristem (see Figure 16.5).

The cells of the upper and lower tiers of the early globular stage embryo differ, and the embryo is divided into apical and basal halves, reflecting the axial pattern imposed on the embryo in the zygote.

The Radial Pattern of Tissue Differentiation Is First Visible at the Globular Stage

The radial pattern of tissue differentiation is first observed in the octant embryo (Figure 16.6). As cell division continues in the globular embryo, transverse divisions divide the

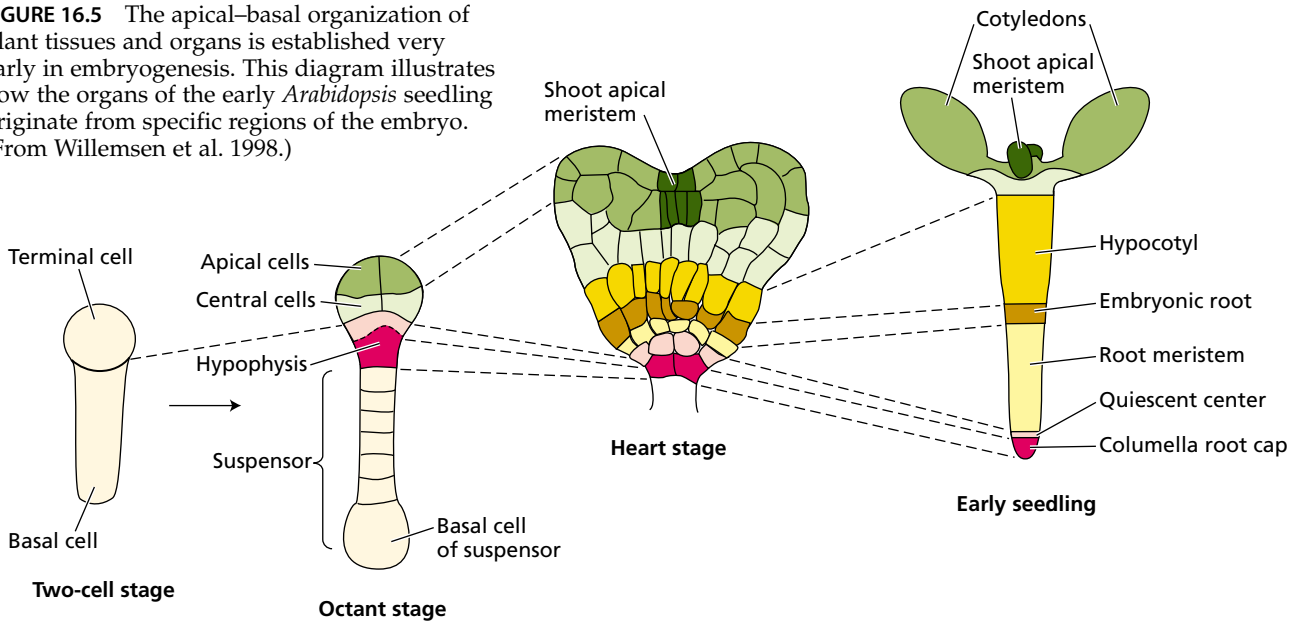


FIGURE 16.5 The apical–basal organization of plant tissues and organs is established very early in embryogenesis. This diagram illustrates how the organs of the early *Arabidopsis* seedling originate from specific regions of the embryo. (From Willemsen et al. 1998.)

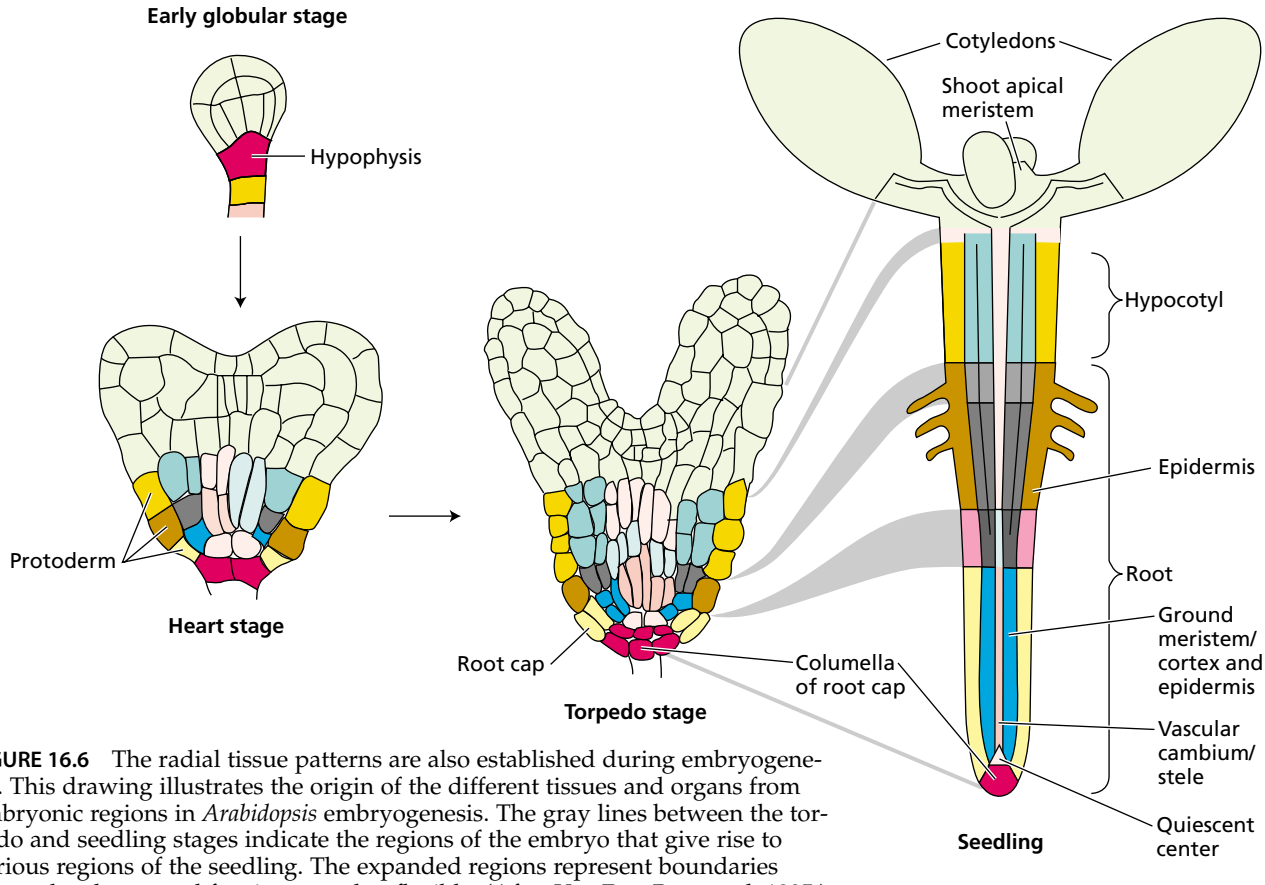


FIGURE 16.6 The radial tissue patterns are also established during embryogenesis. This drawing illustrates the origin of the different tissues and organs from embryonic regions in *Arabidopsis* embryogenesis. The gray lines between the torpedo and seedling stages indicate the regions of the embryo that give rise to various regions of the seedling. The expanded regions represent boundaries where developmental fate is somewhat flexible. (After Van Den Berg et al. 1995.)

lower tier of cells radially into three regions. These regions will become the radially arranged tissues of the root and stem axes. The outermost cells form a one-cell-thick surface layer, known as the **protoderm**. The protoderm covers both halves of the embryo and will generate the epidermis.

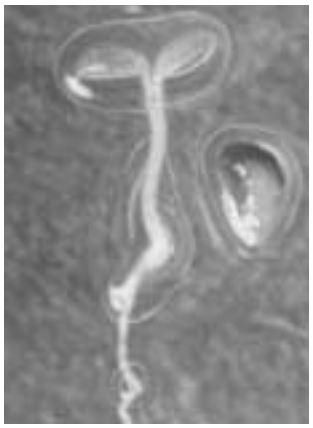
Cells that will become the ground meristem underlie the protoderm. The ground meristem gives rise to the **cortex** and, in the root and hypocotyl, it will also produce the **endodermis**. The procambium is the inner core of elongated cells that will generate the **vascular tissues** and, in the root, the **pericycle** (see Figure 16.2).

Embryogenesis Requires Specific Gene Expression

Analysis of *Arabidopsis* mutants that either fail to establish axial polarity or develop abnormally during embryogenesis has led to the identification of genes whose expression participates in tissue patterning during embryogenesis.

The* GNOM *gene: Axial patterning. Seedlings homozygous for mutations in the *GNOM* gene lack both roots and cotyledons (Figure 16.7A) (Mayer et al. 1993). Defects in *gnom* embryos first appear during the initial division of the zygote, and they persist throughout embryogenesis. In the most extreme mutants, *gnom* embryos are spherical and lack axial polarity entirely. We can conclude that *GNOM* gene expression is required for the establishment of axial polarity.[1]

(A) Wild type *gnom mutant*



GNOM genes control apical–basal polarity

(B) Wild type *monopteros* mutant

MONOPTEROS genes control formation of the primary root

FIGURE 16.7 Genes whose functions are essential for *Arabidopsis* embryogenesis have been identified by the selection of mutants in which a stage of embryogenesis is blocked, such as *gnom* and *monopteros*. The development of mutant seedlings is contrasted here with that of the wild type at the same stage of development. (A) The *GNOM* gene helps establish apical–basal polarity. A plant homozygous for *gnom* is shown on the right. (B) The *MONOPTEROS* gene is necessary for basal patterning and formation of the primary root. Plants homozygous for the *monopteros* mutation have a hypocotyl, a normal shoot apical meristem, and cotyledons, but they lack the primary root. (A from Willemsen et al. 1998; B from Berleth and Jürgens 1993.)

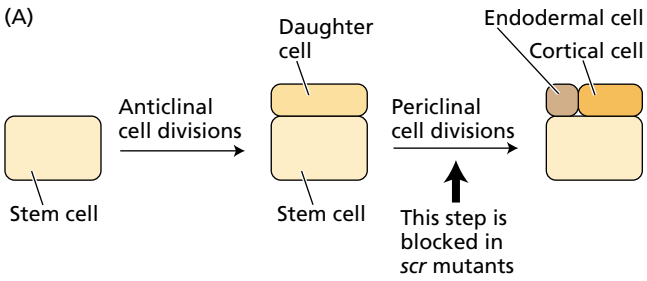
The* MONOPTEROS *gene: Primary root and vascular tissue. Mutations in the *MONOPTEROS* (*MP*) gene result in seedlings that lack both a hypocotyl and a root, although they do produce an apical region. The apical structures in the *mp* mutant embryos are not structurally normal, however, and the tissues of the cotyledons are disorganized (Figure 16.7B) (Berleth and Jürgens 1993). Embryos of *mp* mutants first show abnormalities at the octant stage, and they do not form a procambium in the lower part of the globular embryo, the part that should give rise to the hypocotyl and root. Later some vascular tissue does form in the cotyledons, but the strands are improperly connected.

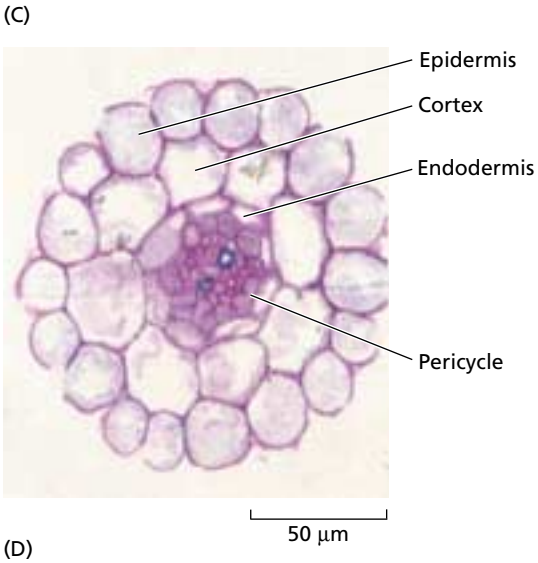
Although the *mp* mutant embryos lack a primary root when they germinate, they will form adventitious roots as the seedlings grow into adult plants. The vascular tissues in all organs of these mutant plants are poorly developed, with frequent discontinuities. Thus the *MP* gene is required for the formation of the embryonic primary root, but not for root formation in the adult plant. The *MP* gene is important for the formation of vascular tissue in postembryonic development (Przemeck et al. 1996).

The* SHORT ROOT *and* SCARECROW *genes: Ground tissue development. Genes have been identified that function in the establishment of the radial tissue pattern in the root and hypocotyl during embryogenesis. These genes also are required for maintenance of the radial pattern during postembryonic development (Scheres et al. 1995; Di Laurenzio et al. 1996). To identify these genes, investigators isolated *Arabidopsis* mutants that caused roots to grow slowly (Figure 16.8B). Analysis of these mutants identified several that have defects in the radial tissue pattern. Two of the affected genes, *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*), are necessary for tissue differentiation and cell differentiation not only in the embryo, but also in both primary and secondary roots and in the hypocotyl.

Mutants of *SHR* and *SCR* both produce roots with a single-celled layer of ground tissue (Figure 16.8D). Cells making up the single-celled layer of ground tissue have a mixed identity and show characteristics of both endodermal and cortical cells in plants with the *scr* mutation. These *scr* mutants also lack the cell layer called the **starch sheath**, a structure that is involved in the growth response to gravity (see Chapter 19). Roots of plants with the *shr* mutation also

[1] In discussions of plant and yeast genetics, wild-type (normal) genes are capitalized and italicized (in this case *GNOM*), and mutations are set in lowercase letters (here *gnom*).





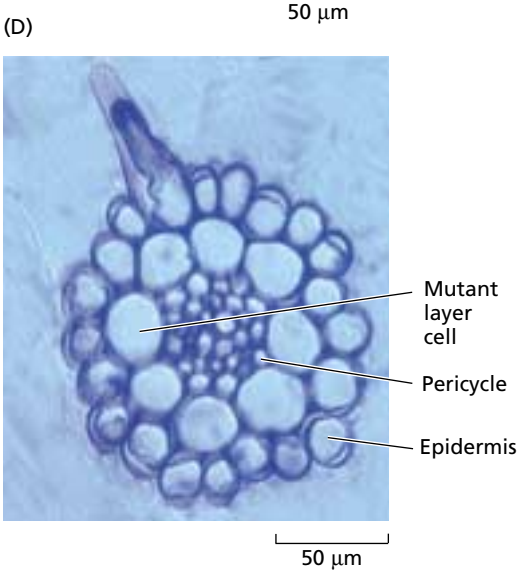


FIGURE 16.8 Mutations in the *Arabidopsis* gene *SCARECROW* (*SCR*) alter the pattern of tissues in the root. (A) The cell divisions forming the endodermis and cortex. The endodermal cells and cortical cells are derived from the same initial cells as a result of two asymmetric cell divisions. The cortical–endodermal stem cell (uncommitted cell) expands and then divides anticlinally, reproducing itself and a daughter cell. The daughter cell then divides periclinally to produce a small cell that develops endodermal characteristics and a larger cell that becomes a cortical cell. The second asymmetric division does not occur in *scr* mutants, and the daughter cell formed as a result of the anticlinal division of the initial has characteristics of both cortical and endodermal cells. (B) The growth of a 12-day-old wild-type seedling (left) is compared with that of two 12-day-old seedlings homozygous for a mutation in the *SCARECROW* (*SCR*) gene (middle and right). (C) Cross section of the primary root of a wild-type seedling. (D) Cross section of the primary root of a seedling homozygous for the *scr* mutant. (From Di Laurenzio et al. 1996; photos © Cell Press, courtesy of P. Benfey.)

have a single layer of ground tissue, but it has only cortical cell characteristics and lacks endodermal characteristics.

The* HOBBIT *gene: The root meristem. The primary root and shoot meristems are established during embryogenesis. Because in most cases they do not become active at this time, the term *promeristem* may be more appropriate to describe these structures. A **promeristem** may be defined as an embryonic structure that will become a meristem upon germination.

A molecular marker for the root promeristem has not yet been identified, but it appears to be determined early in embryogenesis. Root cap stem cells (the cells that divide to produce the root cap) are formed from the hypophysis at the heart stage of embryogenesis, indicating that the root promeristem is established at least by this stage of embryogenesis (Figure 16.9). The expression of the *HOBBIT* gene may be an early marker of root meristem identity (Willemsen et al. 1998).

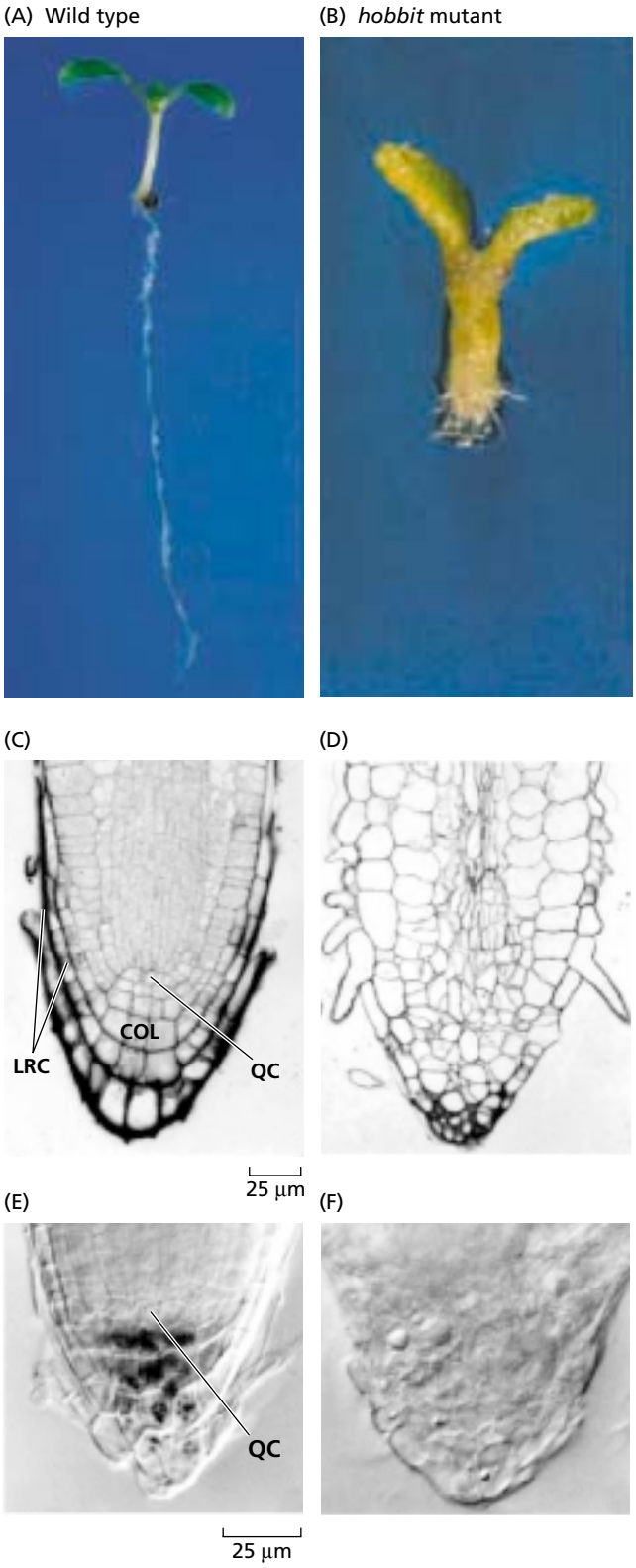


FIGURE 16.9 The *HOBBIT* (*HBT*) gene is important for the development of a functional root apical meristem. (A) Wild-type *Arabidopsis* seedling; (B) *hobbit* mutant seedling; (C) root tip of wild type showing quiescent center (QC), columella (COL) and lateral root cap (LRC); (D) root tip of *hobbit* mutant; (E) quiescent center and columella of wild-type; (F) absence of quiescent center and columella in *hobbit*. The seedlings in A and B are both shown 7 days after germination (4× magnification). Staining with iodine reveals starch grains in the columella cells of the root cap in the wild type (E). No starch grains are present in the *hbt* mutant root tip (F). (From Willemsen et al. 1998.)

Mutants of the *HOBBIT* (*HBT*) gene are defective in the formation of a functional embryonic root, as are plants with *mp* mutants. However, these two mutations act in very different ways. The *hbt* mutants begin to show abnormalities at the two- or four-cell stage, before the formation of the globular embryo. The primary defect in *hbt* mutants is in the hypophyseal precursor, which divides vertically instead of horizontally. As a result, the hypophysis does not form, and the root meristem that subsequently forms lacks a quiescent center and the columella (see Figure 16.9F). Embryos of *hbt* mutants appear to have a root meristem, but it does not function when the seedlings germinate. Furthermore, plants grown from *hbt* mutant embryos are unable to form lateral roots.

The SHOOTMERISTEMLESS gene: The shoot promeristem. The shoot promeristem can be recognized morphologically by the torpedo stage of embryogenesis in *Arabidopsis*. Oriented cell divisions of some of the cells between the cotyledons result in a layered appearance of this region that is characteristic of the shoot apical meristem (as described later in the chapter). However, the progenitors of these cells probably acquired the molecular identity of the shoot apical meristem cells much earlier, during the globular stage.

The *SHOOTMERISTEMLESS* (*STM*) gene is expressed specifically in the cells that will become the shoot apical meristem, and its expression in these cells is required for the formation of the shoot promeristem. *Arabidopsis* plants homozygous for a mutated, loss-of-function *STM* gene do not form a shoot apical meristem, and instead all the cells in this region differentiate (Lincoln et al. 1994). The product of the wild-type *STM* gene appears to suppress cell differentiation, ensuring that the meristem cells remain undifferentiated.

STM mRNA can first be detected in one or two cells at the apical end of the midglobular embryo. By the heart stage, *STM* expression is confined to a few cells between the cotyledons (Long et al. 1996). Because *STM* acts as a marker for these cells, the shoot apical meristem must be specified long before it can be recognized morphologically. The *STM* gene is necessary not only for the formation of the embryonic shoot apical meristem, but also for the maintenance of shoot apical meristem identity in the adult plant. The role of the nucleus in controlling development was first demonstrated in the giant algal unicell, *acetabularia* (see **Web Essay 16.2**).

Embryo Maturation Requires Specific Gene Expression

The *Arabidopsis* embryo enters dormancy after it has generated about 20,000 cells. Dormancy is brought about by the loss of water and a general shutting down of gene transcription and protein synthesis, not only in the embryo, but also throughout the seed. To adapt the cell to the special conditions of dormancy, specific gene expression is required. For example, the *ABSCISIC ACID INSENSITIVE3* (*ABI3*) and *FUSCA3* genes are necessary for the initiation of dormancy and are sensitive to the hormone abscisic acid, which is the signaling molecule that initiates seed and embryo dormancy. *ABI3* also controls the expression of genes encoding the storage proteins that are deposited in the cotyledons during the maturation phase of embryogenesis (see Chapter 23).

The *LEAFY COTYLEDON1* (*LEC1*) gene also is active in late embryogenesis. Because *lec1* mutants cannot survive desiccation and do not enter dormancy, the embryos die unless they are rescued through isolation before desiccation occurs. The rescued embryos will germinate in culture and produce fertile plants, which are like wild-type plants except that they lack the 7S storage protein and they have leaflike cotyledons with trichomes on their upper surface.

The normal appearance and development of the mature *lec1* mutants indicates that the *LEC1* gene is required only during embryogenesis. Although the most obvious defects of the *lec1* mutants are seen only in the maturation phase embryo, mRNA from *LEC1* gene expression can be detected throughout embryogenesis. It has been proposed that *LEC1* is a general repressor of vegetative development and its expression is necessary throughout embryogenesis (Lotan et al. 1998).

THE ROLE OF CYTOKINESIS IN PATTERN FORMATION

One of the most striking features of tissue organization in many plants, illustrated by *Arabidopsis*, is the remarkably precise pattern of oriented, often called *stereotypic*, cell divisions. This pattern of divisions generates files of cells extending from the meristem toward the base of the plant. Although the division pattern is not as precise in all other species, the basic pattern of tissue formation is similar. How important is the plane of cell division for the establishment of the tissue patterns found in plant organs?

The Stereotypic Cell Division Pattern Is Not Required for the Axial and Radial Patterns of Tissue Differentiation

Two *Arabidopsis* mutants, *fass* and *ton*, have dramatic effects on the patterns of cell division in all stages of development

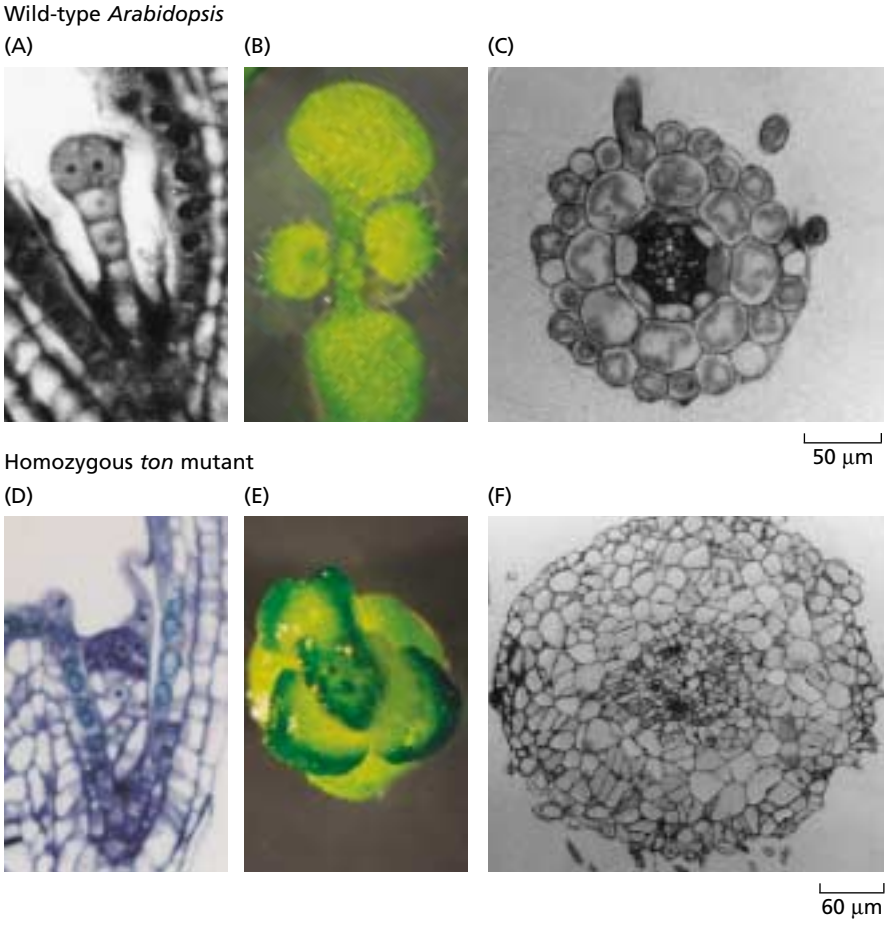


FIGURE 16.10 *Arabidopsis* plants with mutations in the *TON* gene are unable to form a preprophase band of microtubules in cells at any stage of division. Plants carrying this mutation are highly irregular in their cell division and expansion planes, and as a result they are severely deformed. However, they continue to produce recognizable tissues and organs in their correct positions. Although the organs and tissues produced by these mutant plants are highly abnormal, the radial tissue pattern is not disturbed. (A–C) Wild-type *Arabidopsis*: (A) early globular stage embryo; (B) seedling seen from the top; (C) cross section of a root. (D–F) Comparable stages of *Arabidopsis* homozygous for the *ton* mutation: (D) early embryogenesis; (E) mutant seedling seen from the top; (F) cross section of the mutant root showing the random orientation of the cells, but a near wild-type tissue order; an outer epidermal layer covers a multicellular cortex, which in turn surrounds the vascular cylinder. (From Traas et al. 1995.)

and eliminate the stereotypic divisions seen in the wild type (Torres-Ruiz and Jürgens 1994; Traas et al. 1995). These mutations probably are in the same gene, and cells in plants homozygous for the *ton* (*fass*) mutation lack a cytoplasmic structure known as the *preprophase band* of microtubules. The preprophase band appears to be essential for the orientation of the phragmoplast during cytokinesis, and thus is required for oriented cell divisions (see Chapter 1 and **Web Topic 16.2**).

The effects of the *ton* (*fass*) mutation are seen from the earliest stages of embryogenesis and persist throughout development. The plants are tiny, never reaching more than 2 to 3 cm in height. They have misshapen leaves, roots, and stems, and they are sterile (Figure 16.10D–F). Nevertheless, the mutant plants not only establish an axial pattern, but they have all the cell types and organs of the wild-type plant, and these occur in their correct positions. The precise numbers of cells found in each tissue layer are radically different in the mutants, but each tissue is present and in the proper order.

The fact that these mutations do not prevent the establishment of the radial tissue pattern is strong evidence that the stereotypic cell division pattern found in the *Arabidopsis* embryo and in the root is not essential for the radial pattern of tissue differentiation.

An *Arabidopsis* Mutant with Defective Cytokinesis Cannot Establish the Radial Tissue Pattern

The *Arabidopsis* mutant *knolle* is defective in cytokinesis, the step at the end of mitosis in which a new wall is formed partitioning the daughter nuclei into separate cells. The *KNOLLE* gene encodes a syntaxin-like protein that is important for vesicle fusion. **Syntaxins** are proteins that integrate into membranes, permitting the membranes to fuse. Vesicle fusion is essential for cytokinesis (Figure 16.11).

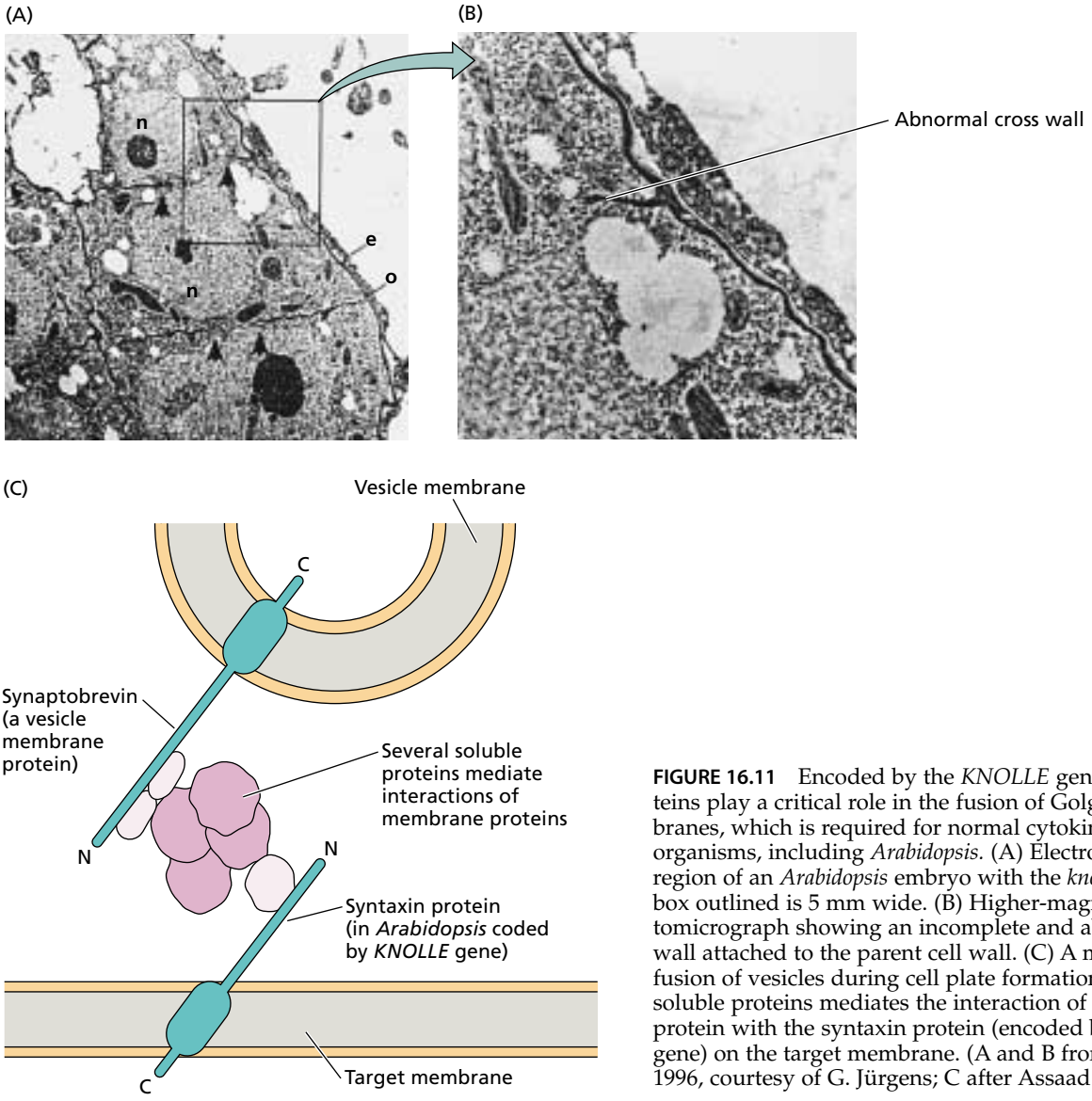


FIGURE 16.11 Encoded by the *KNOLLE* gene, syntaxin proteins play a critical role in the fusion of Golgi-derived membranes, which is required for normal cytokinesis in most organisms, including *Arabidopsis.* (A) Electron micrograph of a region of an *Arabidopsis* embryo with the *knolle* mutation. The box outlined is 5 mm wide. (B) Higher-magnification photomicrograph showing an incomplete and abnormal cross-wall attached to the parent cell wall. (C) A model for the fusion of vesicles during cell plate formation. A complex of soluble proteins mediates the interaction of synaptobrevin protein with the syntaxin protein (encoded by the *KNOLLE* gene) on the target membrane. (A and B from Lukowitz et al. 1996, courtesy of G. Jürgens; C after Assaad et al. 1996.)

Although cell division is not blocked by the *knolle* mutation, cell plate formation is irregular and often incomplete. As a result, many cells are binucleate, while other cells are only partly separated or are connected by large cytoplasmic bridges. The division planes also are irregular. These irregularities have severe effects on development.

Plants homozygous for the *knolle* mutation go through embryogenesis, but the radial tissue pattern is severely disrupted and an epidermal layer does not form in early embryogenesis. The *knolle* mutation does not prevent formation of the apical–basal axis, and embryogenesis is completed, although the seedlings are very short-lived and die soon after germination. The plants also lack functional meristems.

The conclusion drawn from studies of the *knolle* mutation appears to contradict what we learned from the *ton* (*fass*) mutations. Both the *knolle* and the *ton* mutations disrupt the normal pattern of cell division in embryonic and postembryonic development. But whereas the *knolle* mutations block the establishment of the radial tissue pattern, in the *ton* mutants the pattern is established.

One difference between the *ton* and the *knolle* mutations is that the latter usually prevents the effective separation of daughter cells during cytokinesis because the cell plate is incomplete. Since cell–cell communication is important for pattern formation, it may be necessary for cells to be isolated effectively so that the information exchange can be regulated. Even though the cytosol is continuous between adjacent plant cells through plasmodesmata, complete cellularization is required for normal development. Thus the *ton* mutants are able to perceive positional information correctly, while the *knolle* mutants cannot. For a review of the mechanisms determining the plane of cell division in plant cells, see **Web Essay 16.3**.

MERISTEMS IN PLANT DEVELOPMENT

Meristems are populations of small, isodiametric (having equal dimensions on all sides) cells with embryonic characteristics. Vegetative meristems are self-perpetuating. Not only do they produce the tissues that will form the body of the root or stem, but they also continuously regenerate themselves. A meristem can retain its embryonic character indefinitely, possibly even for thousands of years in the case of trees. The reason for this ability is that some meristematic cells do not become committed to a differentiation pathway, and they retain the capacity for cell division, as long as the meristem remains vegetative.

Undifferentiated cells that retain the capacity for cell division indefinitely are said to be **stem cells**. Although historically called *initial cells* in plants, in function they are very similar, if not identical, to animal stem cells (Weigel and Jürgens 2002). When stem cells divide, on average one of the daughter cells retains the identity of the stem cell, while the other is committed to a particular developmental pathway (Figure 16.12).

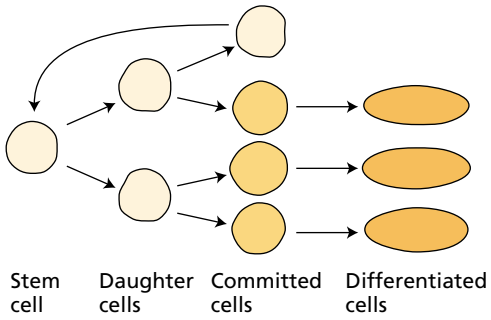


FIGURE 16.12 Stem cells generate daughter cells, some of which remain uncommitted and retain the property of stem cells, while others become committed to differentiate.

Stem cells usually divide slowly. Their committed daughters, however, may enter a period of rapid cell division before they stop dividing and can be recognized as specific cell types. Stem cells represent the ultimate source of all the cells in the meristem and the entire rest of the plant—both roots, leaves, and other organs, as well as stems.

The Shoot Apical Meristem Is a Highly Dynamic Structure

The vegetative shoot apical meristem generates the stem, as well as the lateral organs attached to the stem (leaves and lateral buds). The shoot apical meristem typically contains a few hundred to a thousand cells, although the *Arabidopsis* shoot apical meristem has only about 60 cells.

The shoot apical meristem is located at the extreme tip of the shoot, but it is surrounded and covered by immature leaves. These are the youngest leaves produced by the activity of the meristem. It is useful to distinguish the shoot apex from the meristem proper. The **shoot apex** (plural *apices*) consists of the apical meristem plus the most recently formed leaf primordia. The **shoot apical meristem** is the undifferentiated cell population only and does not include any of the derivative organs.

The shoot apical meristem is a flat or slightly mounded region, 100 to 300 μm in diameter, composed mostly of small, thin-walled cells, with a dense cytoplasm, and lacking large central vacuoles. The shoot apical meristem is a dynamic structure that changes during its cycle of leaf and stem formation. In addition, in many plants it exhibits seasonal activity, as does the entire shoot. Shoot apical meristems may grow rapidly in the spring, enter a period of slower growth during the summer, and become dormant in the fall, with dormancy lasting through the winter. The size and structure of the shoot apical meristem also change with seasonal activity.

Shoots develop and grow at their tips, as is the case with roots, but the developing regions are not as stratified and precisely ordered as they are in the root. Moreover, growth occurs over a much broader region of the shoot than is the case for roots. At any given time, a region containing several internodes, typically 10 to 15 cm long, may be undergoing primary growth.

The Shoot Apical Meristem Contains Different Functional Zones and Layers

The shoot apical meristem consists of different functional regions that can be distinguished by the orientation of the cell division planes and by cell size and activity. The angiosperm vegetative shoot apical meristem usually has a highly stratified appearance, typically with *three distinct layers of cells*. These layers are designated **L1**, **L2**, and **L3**, where L1 is the outermost layer (Figure 16.13). Cell divisions are anticlinal in the L1 and L2 layers; that is, the new cell wall separating the daughter cells is oriented at right angles to the meristem surface. Cell divisions tend to be less regularly oriented in the L3 layer. Each layer has its own stem cells, and all three layers contribute to the formation of the stem and lateral organs.

Active apical meristems also have an organizational pattern called **cytohistological zonation**. Each zone is composed of cells that may be distinguished not only on the basis of their division planes, but also by differences in size and by degrees of vacuolation (see Figure 16.13B). These zones exhibit different patterns of gene expression, reflecting the different functions of each zone (Nishimura et al. 1999; Fletcher and Meyerowitz 2000).

The center of an active meristem contains a cluster of relatively large, highly vacuolate cells called the **central zone**. The central zone is somewhat comparable to the quiescent center of root meristems (which will be discussed later in the chapter). A doughnut-shaped region of smaller cells, called the **peripheral zone**, flanks the central zone. A **rib zone** lies underneath the central cell zone and gives rise to the internal tissues of the stem.

These different zones most likely represent different developmental domains. The peripheral zone is the region in which the first cell divisions leading to the formation of leaf primordia will occur. The rib zone contributes cells that become the stem. The central zone contains the pool of stem cells, some fraction of which remains uncommitted, while others replenish the rib and peripheral zone populations (Bowman and Eshed 2000).

Some Meristems Arise during Postembryonic Development

The root and shoot apical meristems formed during embryogenesis are called **primary meristems**. After germination, the activity of these primary meristems generates the primary tissues and organs that constitute the primary plant body.

Most plants also develop a variety of **secondary meristems** during postembryonic development. Secondary meristems can have a structure similar to that of primary meristems, but some secondary meristems have a quite different structure. These include axillary meristems, inflorescence meristems, floral meristems, intercalary meristems, and lateral meristems (the vascular cambium and cork cambium). (Inflorescence and floral meristems will be discussed in Chapter 24.):

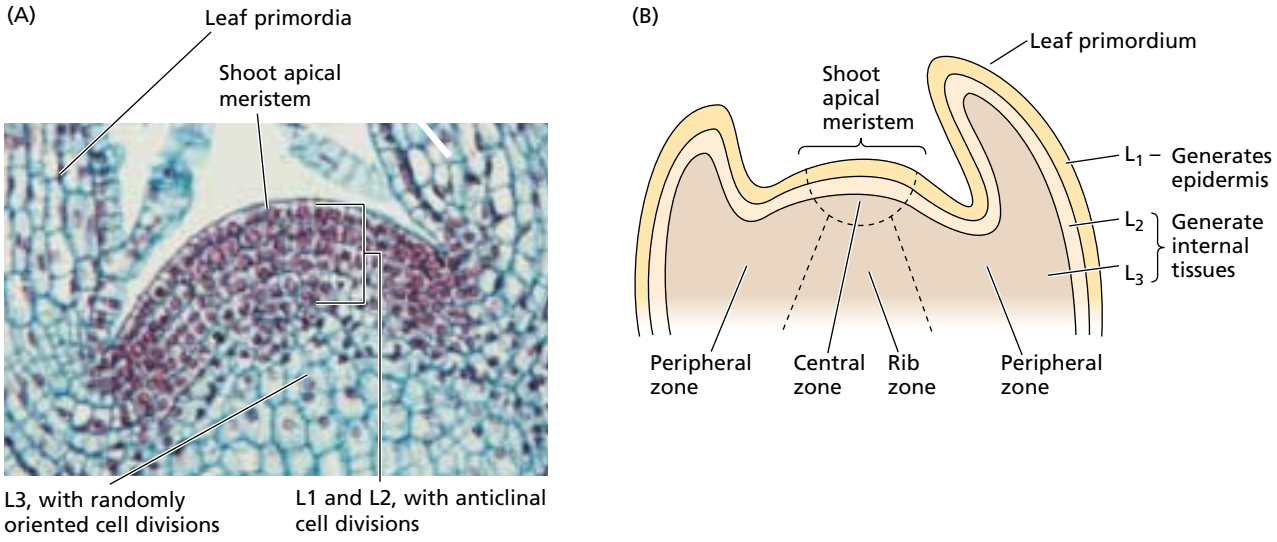


FIGURE 16.13 The shoot apical meristem generates the aerial organs of the plant. (A) This longitudinal section through the center of the shoot apex of *Coleus blumei* shows the layered appearance of the shoot apical meristem. Most cell divisions are anticlinal in the outer L1 and L2 layers, while the planes of cell divisions are more randomly oriented in the L3 layer. The outermost (L1) layer generates the shoot epidermis; the L2 and L3 layers generate internal tissues. (B) The shoot apical meristem also has cytohistological zones, which represent regions with different identities and functions. The central zone contains the stem cells, which divide slowly but are the ultimate source of the tissues that make up the plant body. The peripheral zone, in which cells divide rapidly, surrounds the central zone and produces the leaf primordia. A rib zone lies below the central zone and generates the central tissues of the stem. (A ©J. N. A. Lott/Biological Photo Service.)

- **Axillary meristems** are formed in the axils of leaves and are derived from the shoot apical meristem. The growth and development of axillary meristems produces branches from the main axis of the plant.
- **Intercalary meristems** are found within organs, often near their bases. The intercalary meristems of grass leaves and stems enables them to continue to grow despite mowing or grazing by cows.
- **Branch root meristems** have the structure of the primary root meristem, but they form from pericycle cells in mature regions of the root. Adventitious roots also can be produced from lateral root meristems that develop on stems, as when stem cuttings are rooted to propagate a plant.
- The **vascular cambium** (plural *cambia*) is a secondary meristem that differentiates along with the primary vascular tissue from the procambium within the vascular cylinder. It does not produce lateral organs, but only the woody tissues of stems and roots. The vascular cambium contains two types of meristematic cells: fusiform stem cells and ray stem cells. *Fusiform stem cells* are highly elongated, vacuolate cells that divide longitudinally to regenerate themselves, and whose derivatives differentiate into the conducting cells of the secondary xylem and phloem. *Ray stem cells* are small cells whose derivatives include the radially oriented files of parenchyma cells within wood known as rays.
- The **cork cambium** is a meristematic layer that develops within mature cells of the cortex and the secondary phloem. Derivatives of the cork cambium differentiate as cork cells that make up a protective layer called the *periderm*, or *bark*. The periderm forms the protective outer surface of the secondary plant body, replacing the epidermis in woody stems and roots.

Axillary, Floral, and Inflorescence Shoot Meristems Are Variants of the Vegetative Meristem

Several different types of shoot meristems can be distinguished on the basis of their developmental origin, the types of lateral organs they generate, and whether they are **determinate** (having a genetically programmed limit to their growth) or **indeterminate** (showing no predetermined limit to growth; growth continues so long as resources permit).

The vegetative shoot apical meristem usually is indeterminate in its development. It repetitively forms phytomeres as long as environmental conditions favor growth but do not generate a flowering stimulus. A **phytomere** is a developmental unit consisting of one or more leaves, the node to which the leaves are attached, the internode below the node, and one or more axillary buds (Figure 16.14). **Axillary buds** are secondary meristems; if they are also vegetative meristems, they will have a structure and developmental potential similar to that of the apical meristem.

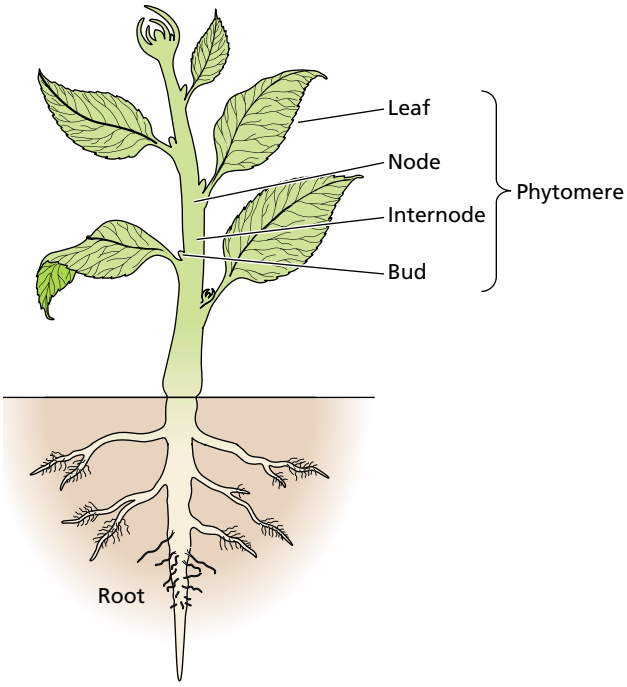


FIGURE 16.14 The shoot apical meristem repetitively forms units known as phytomeres. Each phytomere consists of one or more leaves, the node at which the leaves are attached, the internode immediately below the leaves, and one or more buds in the axils of the leaves.

Vegetative meristems may be converted directly into floral meristems when the plant is induced to flower (see Chapter 24). **Floral meristems** differ from vegetative meristems in that instead of leaves they produce floral organs: sepals, petals, stamens, and carpels. In addition, floral meristems are determinate: All meristematic activity stops after the last floral organs are produced.

In many cases, vegetative meristems are not directly converted to floral meristems. Instead, the vegetative meristem is first transformed into an **inflorescence meristem**. The types of lateral organs produced by an inflorescence meristem are different from the types produced by a floral meristem. The inflorescence meristem produces bracts and floral meristems in the axils of the bracts, instead of the sepals, petals, stamens, and ovules produced by floral meristems. Inflorescence meristems may be determinate or indeterminate, depending on the species.

LEAF DEVELOPMENT

The leaves of most plants are the organs of photosynthesis. This is where light energy is captured and used to drive the chemical reactions that are vital to the life of the plant. Although highly variable in size and shape from species to species, in general leaves are thin, flat structures with dorsiventral polarity. This pattern contrasts with that of the

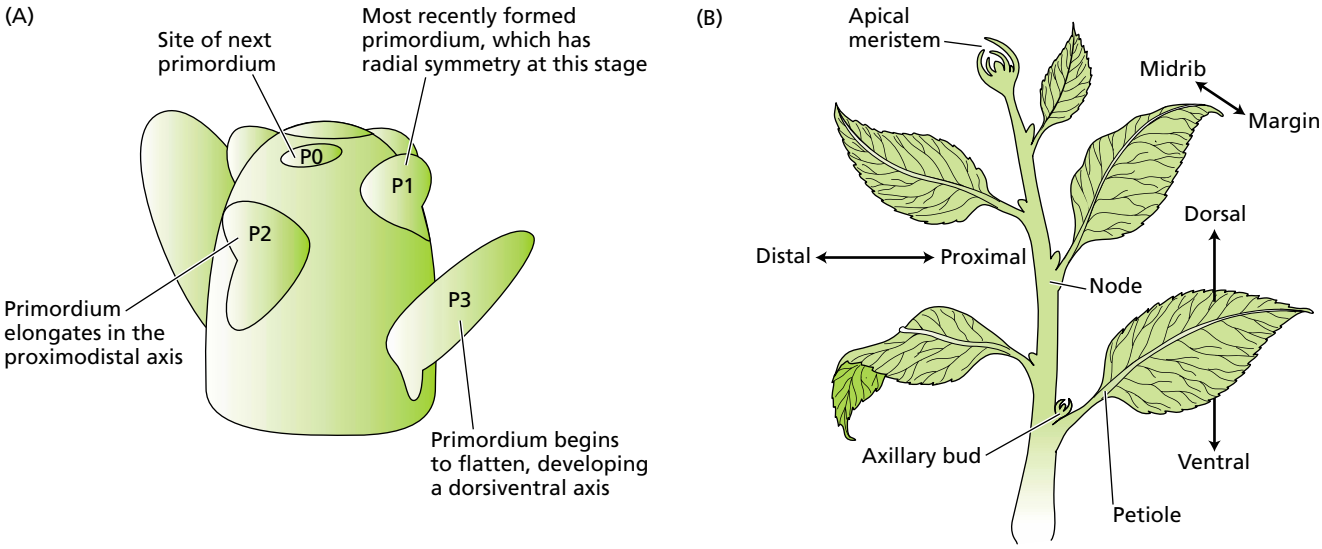


FIGURE 16.15 The origin of leaves at the shoot apex and their axes of symmetry on the stem (A) Leaf primordia in the flanks of the shoot apical meristem. (B) Diagram of a shoot showing the various axes along which development occurs. (After Christensen and Weigel 1998.)

shoot apical meristem and stem, both of which have radial symmetry. Another important difference is that leaf primordia exhibit determinate growth, while the vegetative shoot apical meristem is indeterminate. As described in the sections that follow, several distinct stages can be recognized in leaf development (Sinha 1999).

Stage 1: Organogenesis. A small number of cells in the L1 and L2 layers in the flanks of the apical dome of the shoot apical meristem acquire the **leaf founder cell** identity. These cells divide more rapidly than surrounding cells and produce the outgrowth that represents the **leaf primordium** (plural *primordia*) (Figure 16.15A). These primordia subsequently grow and develop into leaves.

Stage 2: Development of suborgan domains. Different regions of the primordium acquire identity as specific parts of the leaf. This differentiation occurs along three axes: **dorsiventral** (abaxial–adaxial), **proximodistal** (apical–basal), and **lateral** (margin–blade–midrib) (Figure 16.15B). The upper (adaxial) side of the leaf is specialized for light absorption; the lower (abaxial) surface is specialized for gas exchange. Leaf structure and maturation rates also vary along the proximodistal and lateral axes.

Stage 3: Cell and tissue differentiation. As the developing leaf grows, tissues and cells differentiate. Cells derived from the L1 layer differentiate as epidermis (epidermal cells, trichomes, and guard cells), derivatives of the L2 layer differentiate as the photosynthetic mesophyll cells, and vascular elements and bundle sheath cells are derived from the L3 layer. These cells differentiate in a genetically determined pattern that is characteristic of the species but to some degree modified in response to the environment.

The Arrangement of Leaf Primordia Is Genetically Programmed

The timing and pattern with which the primordia form is genetically determined and usually is a characteristic of the species. The number and order in which leaf primordia form is reflected in the subsequent arrangement of leaves around the stem, known as **phyllotaxy** (Figure 16.16). There are five main types of phyllotaxy:

1. ***Alternate phyllotaxy***. A single leaf is initiated at each node (see Figure 16.16A).
2. ***Opposite phyllotaxy***. Leaves are formed in pairs on opposite side of the stem (see Figure 16.16B).
3. ***Decussate phyllotaxy***. Leaves are initiated in a pattern with two opposite leaves per node and with successive leaf pairs oriented at right angles to each other during vegetative development (see Figure 16.16C).
4. ***Whorled phyllotaxy***. More than two leaves arise at each node (see Figure 16.16D).
5. ***Spiral phyllotaxy***. A type of alternate phyllotaxy in which each leaf is initiated at a defined angle to the previous leaf, resulting in a spiral arrangement of leaves around the stem (see Figure 16.16E).

The positioning of leaf primordia must result from the precise spatial regulation of growth within the apex. We know little about how this positioning is regulated, or about the signals that initiate the formation of a primordium. One idea is that inhibitory fields generated by existing primordia influence the spacing of the next primordium.

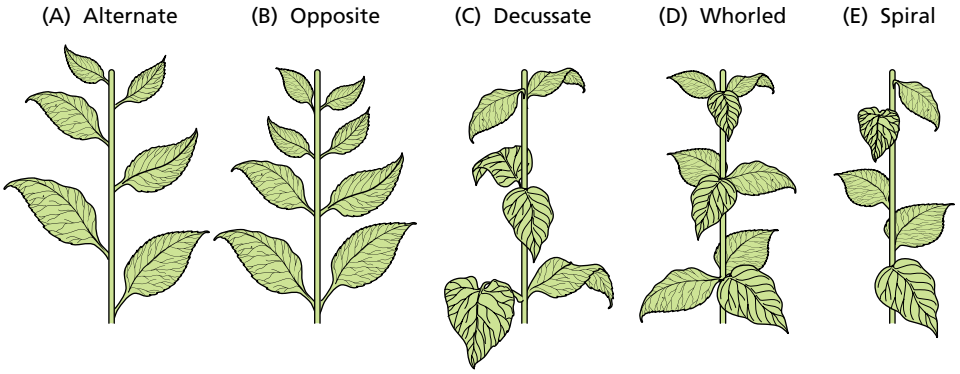


FIGURE 16.16 Five types of leaf arrangements (phyllotactic patterns) along the shoot axis. The same terms also are used for inflorescences and flowers.

ROOT DEVELOPMENT

Roots are adapted for growing through soil and absorbing the water and mineral nutrients in the capillary spaces between soil particles. These functions have placed constraints on the evolution of root structure. For example, lateral appendages would interfere with their penetration through the soil. As a result, roots have a streamlined axis, and no lateral organs are produced by the apical meristem. Branch roots arise internally and form only in mature, nongrowing regions. Absorption of water and minerals is enhanced by fragile root hairs, which also form behind the growth zone. These long, threadlike cells greatly increase the root's absorptive surface area.

In this section we will discuss the origin of root form and structure (*root morphogenesis*), beginning with a description of the four developmental zones of the root tip. We will then turn to the apical meristem. The absence of leaves or buds makes cell lineages easier to follow in roots than in shoots, thus facilitating molecular genetic studies on the role of patterns of cell division in root development.

The Root Tip Has Four Developmental Zones

Roots grow and develop from their distal ends. Although the boundaries are not sharp, four developmental zones can be distinguished in a root tip: the root cap, the meristematic zone, the elongation zone, and the maturation zone (Figure 16.17). These four developmental zones occupy only a little more than a millimeter of the tip of the *Arabidopsis* root. The developing region is larger in other species, but growth is still confined to the tip. With the exception of the root cap, the boundaries of these zones overlap considerably:

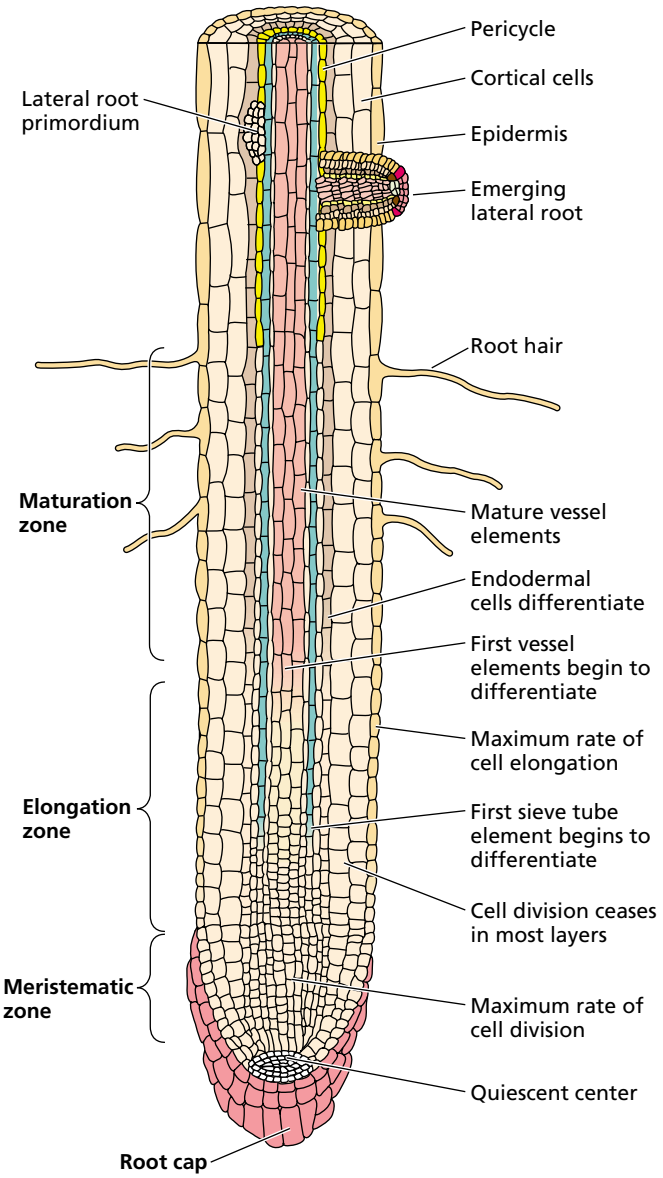


FIGURE 16.17 Simplified diagram of a primary root showing the root cap, the meristematic zone, the elongation zone, and the maturation zone. Cells in the meristematic zone have small vacuoles and expand and divide rapidly, generating many files of cells.

- The **root cap** protects the apical meristem from mechanical injury as the root pushes its way through the soil. Root cap cells form by specialized root cap stem cells. As the root cap stem cells produce new cells, older cells are progressively displaced toward the tip, where they are eventually sloughed off. As root cap cells differentiate, they acquire the ability to perceive gravitational stimuli and secrete mucopolysaccharides (slime) that help the root penetrate the soil.
- The **meristematic zone** lies just under the root cap, and in *Arabidopsis* it is about a quarter of a millimeter long. The root meristem generates only one organ, the primary root. It produces no lateral appendages.
- The **elongation zone**, as its name implies, is the site of rapid and extensive cell elongation. Although some cells may continue to divide while they elongate within this zone, the rate of division decreases progressively to zero with increasing distance from the meristem.
- The **maturation zone** is the region in which cells acquire their differentiated characteristics. Cells enter the maturation zone after division and elongation have ceased. Differentiation may begin much earlier, but cells do not achieve the mature state until they reach this zone. The radial pattern of differentiated tissues becomes obvious in the maturation zone. Later in the chapter we will examine the differentiation and maturation of one of these cell types, the tracheary element.

As discussed earlier, lateral or branch roots arise from the pericycle in mature regions of the root. Cell divisions in the pericycle establish secondary meristems that grow out through the cortex and epidermis, establishing a new growth axis (Figure 16.18). The primary and the secondary root meristems behave similarly in that divisions of the cells in the meristem give rise to progenitors of all the cells of the root.

Root Stem Cells Generate Longitudinal Files of Cells

Meristems are populations of dividing cells, but not all cells in the meristematic region divide at the same rate or with the same frequency. Typically, the central cells divide much more slowly than the surrounding cells. These rarely dividing cells are called the **quiescent center** of the root meristem (see Figure 16.17).

Cells are more sensitive to ionizing radiation when they are dividing. This is the basis of the use of radiation as a treatment for cancer in humans. As a result, the rapidly dividing cells of the meristem can be killed by doses of radiation that nondividing and slowly dividing cells, such as those of the quiescent center, can survive. If the rapidly dividing cells of the root are killed by ionizing radiation, in many cases the root can regenerate from the cells of the quiescent center. This ability suggests that quiescent-center cells are important for the patterning involved in forming a root.

The most striking structural feature of the root tip, when viewed in longitudinal section, is the presence of the long files of clonally related cells. Most cell divisions in the root tip are transverse, or **anticlinal**, with the plane of cytokinesis oriented at right angles to the axis of the root (such divisions tend to increase root length). There are relatively few **periclinal** divisions, in which the plane of division is parallel to the root axis (such divisions tend to increase root diameter).

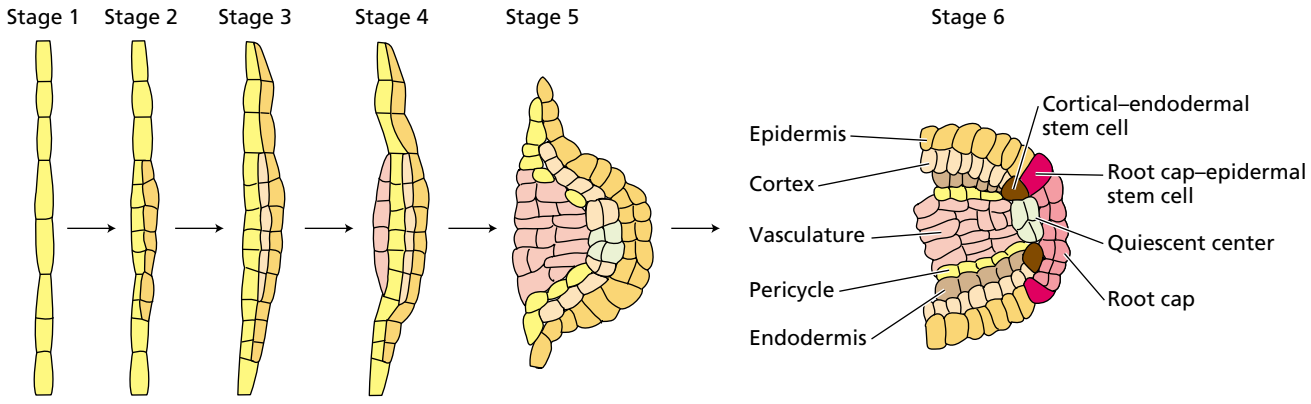


FIGURE 16.18 Model for lateral root formation in *Arabidopsis*. Six major stages are shown in the development of the primordium. The different tissue types are designated by colors. By stage 6, all tissues found in the primary root are present in the typical radial pattern of the branch root. (From Malamy and Benfey 1997.)

Periclinal divisions occur mostly near the root tip and establish new files of cells. As a result, the ultimate origin of any particular mature cell can be traced back to one or a few cells in the meristem. These are the stem cells of a particular file. In *Arabidopsis*, the stem cells surround the quiescent center, but they are not part of the quiescent center. The stem cells ultimately may be derived from quiescent-center cells, but this origin must occur during embryogenesis, since the quiescent-center cells do not divide after germination in normal development. Analysis of the cell division patterns in the roots of the water fern *Azolla* have contributed to our detailed understanding of meristem function. (For a discussion of this work, see **Web Topic 16.3**.)

Root Apical Meristems Contain Several Types of Stem Cells

The patterns of cellular organization found in the root meristems of seed plants are substantially different from those observed in more primitive vascular plants. All seed plants have several stem cells instead of the single stem cell found in plants such as the water fern *Azolla*. However, they are similar to *Azolla* in that it is possible to follow files of cells from the region of maturation into the meristem and, in some cases, to identify the stem cell from which the file was produced.

The *Arabidopsis* root apical meristem has the following structure (Figure 16.19):

- The **quiescent center** is composed of a group of four cells, also known as the center cells in the *Arabidopsis* root meristem. The quiescent-center cells in the *Arabidopsis* root usually do not divide after embryogenesis.
- The **cortical–endodermal stem cells** form a ring of cells that surround the quiescent center. These stem cells generate the cortical and endodermal layers. They undergo one anticlinal division (i.e., perpendicular to the longitudinal axis); then these daughters divide periclinally (i.e., parallel to the longitudinal axis) to establish the files that become the cortex and the endodermis, each of which constitutes only one cell layer in the *Arabidopsis* root (see also Figures 16.2 and 16.8C).
- The **columella stem cells** are the cells immediately above (apical to) the central cells. They divide anticlinally and periclinally to generate a sector of the root cap known as the columella.
- The **root cap–epidermal stem cells** are in the same tier as the columella stem cells but form a ring surrounding them. Anticlinal divisions of the root cap–epidermal stem cells generate the epidermal cell layer. Periclinal divisions of the same stem cells, followed by subsequent anticlinal divisions of the derivatives, produce the lateral root cap.

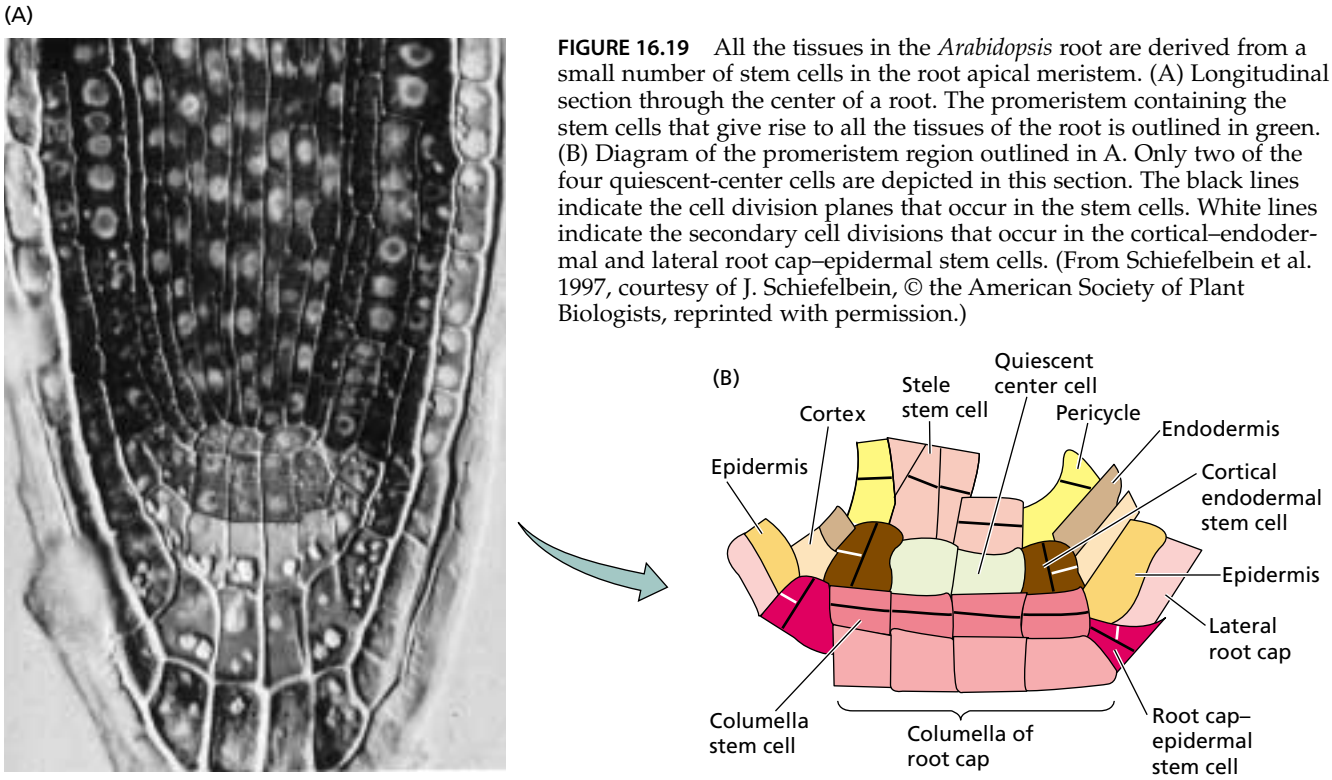


FIGURE 16.19 All the tissues in the *Arabidopsis* root are derived from a small number of stem cells in the root apical meristem. (A) Longitudinal section through the center of a root. The promeristem containing the stem cells that give rise to all the tissues of the root is outlined in green. (B) Diagram of the promeristem region outlined in A. Only two of the four quiescent-center cells are depicted in this section. The black lines indicate the cell division planes that occur in the stem cells. White lines indicate the secondary cell divisions that occur in the cortical–endodermal and lateral root cap–epidermal stem cells. (From Schiefelbein et al. 1997, courtesy of J. Schiefelbein, © the American Society of Plant Biologists, reprinted with permission.)

- The **stele stem cells** are a tier of cells just behind the quiescent-center cells. These cells generate the pericycle and vascular tissues.

The stem cells, together with their immediate derivatives in the apical meristem, are called the *promeristem*.

CELL DIFFERENTIATION

Differentiation is the process by which a cell acquires metabolic, structural, and functional properties that are distinct from those of its progenitor cell. In plants, unlike animals, cell differentiation is frequently reversible, particularly when differentiated cells are removed from the plant and placed in tissue culture. Under these conditions, cells dedifferentiate (i.e., lose their differentiated characteristics), reinitiate cell division, and in some cases, when provided with the appropriate nutrients and hormones, even regenerate whole plants.

This ability to dedifferentiate demonstrates that differentiated plant cells retain all the genetic information required for the development of a complete plant, a property termed **totipotency**. The only exceptions to this rule are cells that lose their nuclei, such as sieve tube elements of phloem, and cells that are dead at maturity, such as vessel elements and tracheids (collectively referred to as tracheary elements) in xylem.

As an example of the process of cell differentiation, we will discuss the formation of tracheary elements. The development of these cells from the meristematic to the fully differentiated state illustrates the types of control that plants exercise over cell specialization and provides an example of the cellular changes that are brought about by differentiation (Fukuda 1996).

A Secondary Cell Wall Forms during Tracheary Element Differentiation

As described in Chapter 4, tracheary elements are the conducting cells in which water and solutes move through the plant. They are dead at maturity, but before their death they are highly active and construct a secondary wall, often with an elaborate pattern, and they may grow extensively. Cell death (discussed later in this chapter) is the genetically programmed finale to tracheary element differentiation.

The formation of secondary walls during tracheary element differentiation involves the deposition of cellulose microfibrils and other noncellulosic polysaccharides at specific sites on the primary or secondary wall, resulting in characteristically patterned wall thickenings (see Chapter 15). The secondary walls of tracheary elements have a higher content of cellulose than primary walls, and they are impregnated with lignin, which is not usually present in primary walls.

In rapidly growing regions, the secondary-wall material is deposited as discrete annular rings, or in a spiral pattern, with the thickenings separated by bands of primary wall (Figure 16.20). As the cell grows, the primary wall extends and the rings or spirals are pulled apart. The tracheary elements that form after elongation stops usually have walls that are thickened. This thickening can be either uniformly or in a reticulate pattern. These cells cannot be stretched by growth.

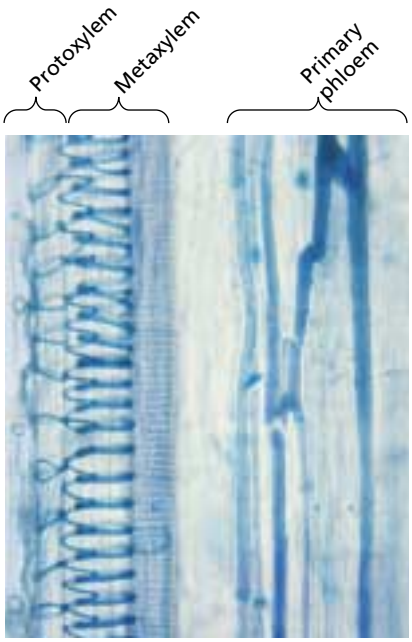


FIGURE 16.20 The formation of primary xylem and primary phloem in a developing strand in a young internode of cucumber (*Cucumis sativus*). The pattern of secondary-wall deposition during vessel element development varies according to the rate of cell elongation. The two first vessels to differentiate—the protoxylem—are observed on the left with secondary-wall thickening in the pattern of "annular rings." Because the first formed vessel was strongly stretched by internode growth, the narrow annular rings are pulled apart. The metaxylem vessels differentiate after the protoxylem and are characterized by spiral thickening. The early formed metaxylem vessel has a stretched helical thickening due to cell elongation, while the later formed vessel shows a dense helical thickening which has not been extended by elongation. The primary phloem sieve tubes are shown on the right, with typical delicate sieve elements. Their sieve plates are stained light blue, while the cytoplasm stains dark blue. (Courtesy of R. Aloni).

Microtubules participate in determining the pattern of secondary-wall deposition. Before any alteration in the pattern of wall deposition is evident, cortical microtubules change from being more or less evenly distributed along the longitudinal walls of the cell to being clustered into bands (Figure 16.21A). Secondary wall is then deposited beneath the microtubule clusters (see Figure 16.21B).

The orientation of the cellulose microfibrils within the secondary-wall thickening is reflected in the alignment of microtubules in the cortical cytoplasm (Hepler 1981). If the microtubules are destroyed with an antimicrotubule agent such as colchicine, cell wall deposition can continue, but the cellulose microfibrils are no longer precisely ordered within the thickening, and the pattern of the secondary wall is disrupted (Figure 16.22).

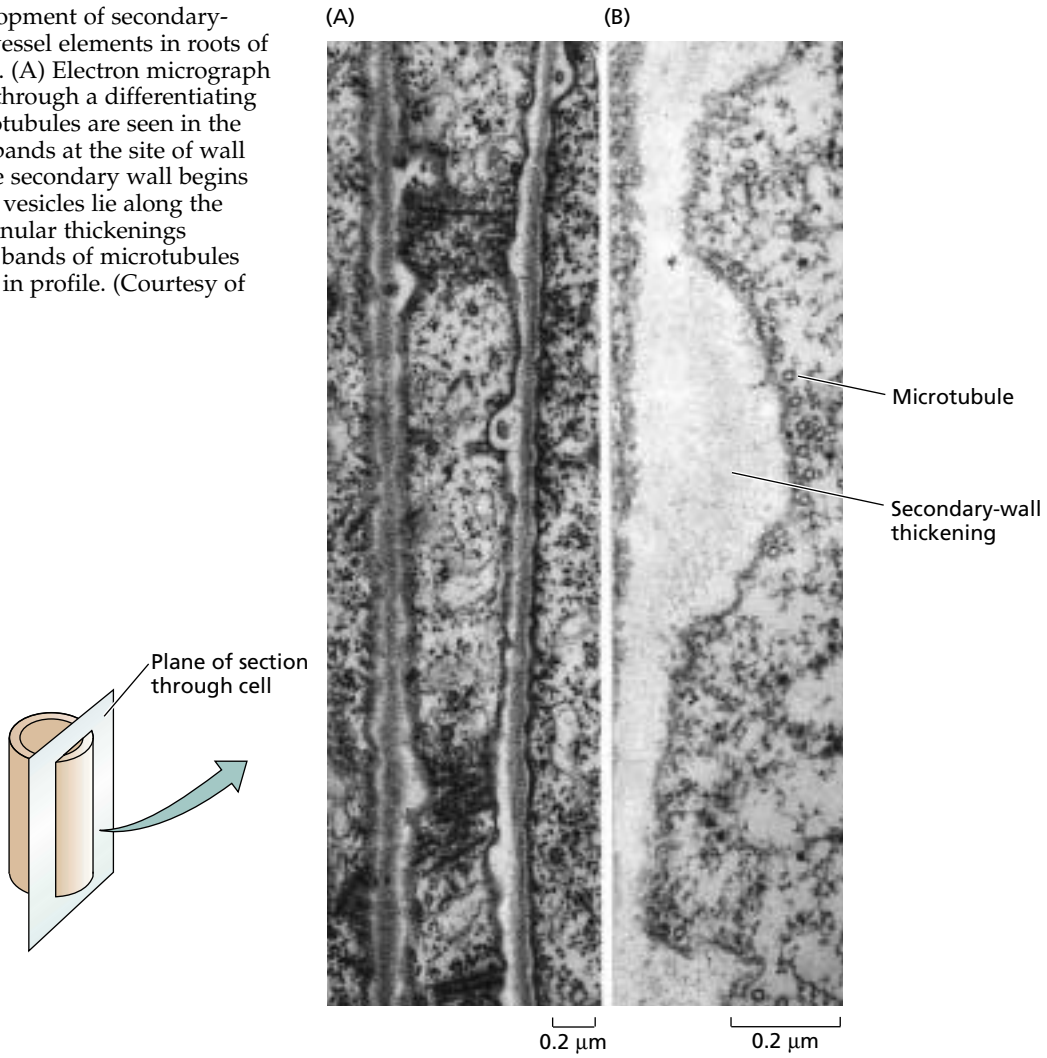


FIGURE 16.21 Development of secondary-wall thickenings in vessel elements in roots of the water fern *Azolla*. (A) Electron micrograph of a grazing section through a differentiating cell. Groups of microtubules are seen in the cell cortex, forming bands at the site of wall thickening before the secondary wall begins to form. Many small vesicles lie along the microtubules. (B) Annular thickenings develop beneath the bands of microtubules and are hemispheric in profile. (Courtesy of A. Hardham.)

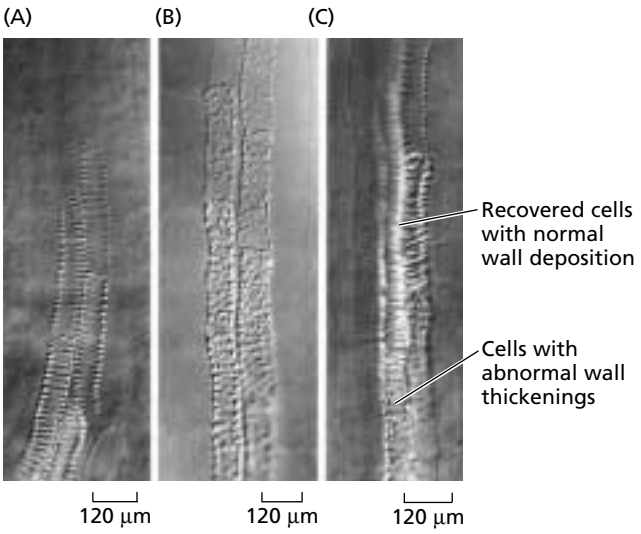


FIGURE 16.22 Colchicine treatments that destroy microtubules also disrupt the normal formation of secondary-wall thickenings in differentiating vessel elements. (A) During normal root growth in Azolla the wall thickenings are spaced evenly along the side walls. (B) In the presence of colchicine, secondary-wall materials are deposited in irregular patterns. (C) Normal growth resumes when the roots are transferred to fresh medium that lacks colchicine, and the newly differentiated vessel elements form with normal annular thickenings. (A from Hardham and Gunning 1979; B and C from Hardham and Gunning 1980.)

INITIATION AND REGULATION OF DEVELOPMENTAL PATHWAYS

Rapid progress has been made in identifying genes that play critical roles in regulating growth, cell differentiation, and pattern formation. This progress is largely a consequence of an intensive, international effort focused on *Arabidopsis*—first to sequence its genome, and subsequently to understand the function of all of its genes. However, many important discoveries have been made as a result of studies with other species, including *Antirrhinum*, maize, petunia, tomato, and tobacco.

In most cases, genes important for development were revealed by elaborate screens of the offspring of mutagenized plants to find mutant individuals with altered development (see the example in Figure 16.8B). These studies often involved heroic efforts to map, clone, and sequence the mutant gene, although now that its genome has been sequenced, the path to identifying any particular mutant gene and what it encodes is now much shorter in *Arabidopsis*.

At this point we have identified some of the players, but the rules of the game and the specific roles of most of the genes are still being worked out. However, many of these developmentally important genes have been found to encode either transcription factors (proteins with the ability to bind to specific DNA sequences and thus control the expression of other genes) or components of signaling pathways. The nature of these genes suggests some possible ways that development might be regulated.

Where these molecular genetic studies have been coupled with clonal analysis, cell biological, physiological, and/or biochemical studies, it has been possible to identify important principles of plant development. Although we are far from a complete understanding, these insights include the following:

- The expression of genes that encode transcription factors determines cell, tissue, and organ identity.
- The fate of a cell is determined by its position and not its clonal history.
- Developmental pathways are controlled by networks of interacting genes.
- Development is regulated by cell-to-cell signaling.

In the following discussion we will first examine the nature of some of the transcription factor and signal transduction component genes that have been shown to play key roles in development. Then we will outline in greater detail each of the developmental principles described here.

[2] The name *MADS* comes from the initials of the first four members of a family of transcription factors: *M*CM1, *A*GAMOUS, *D*EFICIENS, and *S*RF.

Transcription Factor Genes Control Development

With the completion of the sequencing of the *Arabidopsis* genome, it became apparent that approximately 1500 of its nearly 26,000 genes encode transcription factors (Riechmann et al. 2000). **Transcription factors** are proteins that have an affinity for DNA. They are able to turn the expression of genes on or off by binding to specific DNA sequences (see Chapter 14 on the web site).

These 1500 transcription factor genes belong to numerous families. Fewer than half of these families are found only in plants, but the majority are found in all eukaryotes. It is not known, or can even be estimated at this time, how many of these transcription factor genes regulate developmental pathways because only a small percentage of them have been studied. However, many members of two of these families—the MADS box and homeobox genes—have been found to be particularly important in plant development.

MADS box genes are key regulators of important biological functions in plants, animals, and fungi.[2] There are about 30 MADS box genes in the *Arabidopsis* genome, many of which control aspects of development. Specific MADS box genes are important for developmental events in the root, leaf, flower, ovule, and fruit (Riechmann and Meyerowitz 1997). They control the expression of specific sets of target genes, although at this point most of these downstream genes remain to be identified.

Any given MADS box gene is expressed in a specific temporally and spatially restricted manner, with its expression determined by other genes or signaling events. This has been established most clearly in the case of the development of the flower, where interacting sets of MADS box genes have been shown to determine floral organ identity (see Chapter 24).

Homeobox genes encode homeodomain proteins that act as transcription factors. **Homeodomain proteins** play a major role in regulating developmental pathways in all eukaryotes (see Chapter 14 on the web site). As with the MADS box genes, each homeobox gene participates in regulating a unique developmental event by controlling the expression of a unique set of target genes.

Homeodomain proteins belonging to the KNOTTED1 (KN1) class are involved in maintaining the indeterminacy of the shoot apical meristem. The original *knotted* (*kn1*) mutation was found in maize and is a gain-of-function mutation. In **gain-of-function**, or **dominant**, mutations, the phenotype results from the abnormal expression of a gene. In contrast, the phenotypes of **loss-of-function** mutations result from the loss of gene expression, and the mutations are therefore **recessive**.

Plants with the *kn1* mutation have small, irregular, tumorlike knots along the leaf veins. These knots result from abnormal cell divisions within the vascular tissues that distort the veins to form the knots, which protrude from the leaf surface (Figure 16.23) (Hake et al. 1989).

FIGURE 16.23 Inappropriate expression of the *KN1* gene during leaf development causes severe abnormalities around the leaf veins. The gain-of-function mutation *kn1* causes cell proliferation after normal cell division ceases; in addition, the division planes are abnormal, causing gross distortion of the blade surface. (From Sinha et al. 1993a, courtesy of S. Hake.)

Cell differentiation is relatively normal in the leaves of *kn1* mutant plants, except in the vicinity of the knots. The knots are similar to meristems in that they contain undifferentiated cells and continue to divide after cells around them have matured and ceased dividing. This behavior suggests that the *KN1* gene controls meristem function. The mutant phenotype results from the expression of the gene in the wrong tissues, rather than the loss of the normal developmental expression pattern. *KNOTTED1*-like homeobox, or ***KNOX***, genes have been found in several other plant species. *Arabidopsis* has three: *KNAT1*, *KNAT2*, and *SHOOTMERISTEMLESS* (*STM*) (Lincoln et al. 1994; Long et al. 1996).

Tobacco plants that have been transformed with the maize *KN1* gene, driven by a promoter that expresses the gene throughout the plant, develop numerous adventitious shoot meristems along leaf surfaces (Sinha et al. 1993b). These abnormalities are similar to the original gain-of-function *kn1* mutation. We can conclude from this that correct *KN1* gene expression is involved in defining meristem function.

Many Plant Signaling Pathways Utilize Protein Kinases

Protein kinases are ATP-dependent enzymes that add phosphate groups to proteins. Protein phosphorylation is a key regulatory mechanism that is utilized extensively to regulate the activity of enzymes and transcription factors. Although widely utilized by all eukaryotes, plant genomes are especially rich in genes that encode these enzymes. The *Arabidopsis* genome contains over 1200 genes that encode protein kinases. Of these, more than 600 encode *receptor protein kinases* (see Chapter 14 on the web site) (Shiu and Bleecker 2001).

The functions of most of these receptor protein kinases are unknown, but recently some have been shown to play important signaling roles in plant development. *Arabidopsis* has two such genes: *BRI1*, which encodes a receptor kinase that functions in brassinosteroid signaling (see **Web Topic 19.14**) and *CLAVATA1* (*CLV1*), which encodes a receptor kinase that participates in regulating the size of the uncommitted cell population in shoot apical meristem (we'll discuss *CLV1* a little later in the chapter).

Receptor kinases typically are integral membrane proteins. The receptor domain of these kinases resides outside the plasma membrane; the kinase catalytic domain is inside the cell, linked to the receptor domain by a transmembrane domain. The receptor domain has affinity for a signaling molecule, often a small protein or peptide, which is called the **receptor ligand**.

In the absence of the ligand, the kinase enzyme is inactive. The binding of the ligand to the receptor converts the protein to an active kinase (Figure 16.24). In the case of CLV1, ligand binding also triggers the formation of a complex consisting of a related protein, CLAVATA, a kinase-associated protein phosphatase (KAPP), and a rho GTPase-related protein. The ligand for CLV1 most likely is a small protein encoded by a third *CLAVATA* gene, *CLV3* (see Figure 16.24) (Clark et al. 1993; Clark 2001).

The *CLAVATA* genes were first identified as mutations that led to an increase in the size of the vegetative shoot apical meristem and floral meristems. One result was an increase in the number of lateral organs produced by the meristems of these mutants, which is particularly evident in the number of floral organs produced by the mutant meristems. Whereas *CLV1* encodes a typical receptor-like protein kinase, *CLV2* encodes a protein with a receptor domain similar to that of CLV1, but lacking a kinase domain. The protein encoded by the *CLV3* gene is unrelated to either CLV1 or CLV2.

A Cell's Fate Is Determined by Its Position

In both the root and shoot meristem, a small number of stem cells are the ultimate source of any particular tissue, and most of the cells in a given tissue are clonal, having arisen

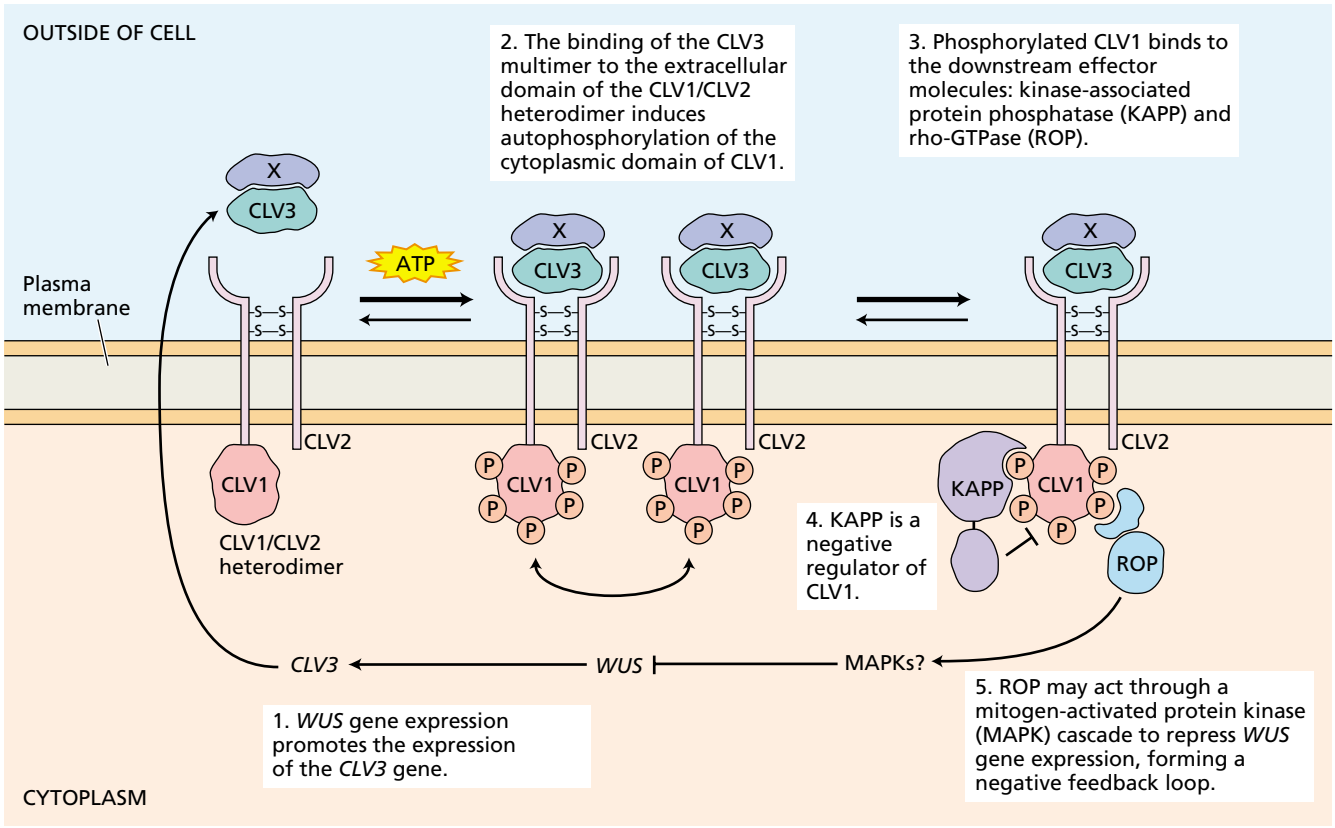


FIGURE 16.24 Model of the CLAVATA1/CLAVATA2 (CLV1/CLV2) receptor kinase signaling cascade, forming a negative feedback loop with the *WUS* gene. See Chapter 14 on the web site for further information about receptor kinase signaling pathways. (After Clark 2001.)

from the same stem cell. However, most evidence supports the view that *cell fate does not depend on cell lineage, but instead is determined by positional information* (Scheres 2001).

In the vast majority of cases, shoot epidermal cells are derived from a small number of stem cells in the L1 layer. However, the derivatives of the L1 layer are committed to become epidermal cells because they occupy the outermost layer and lie on top of the cortical cell layer, not because they were clonally derived from the stem cells in the L1 layer.

The plane in which a cell divides will determine the position of its daughter cells within a tissue, and this positioning in turn plays the most significant role in determining the fate of the daughter cells. The strongest evidence for the importance of position in determining a cell's ultimate fate comes from an examination of the fate of cells that are displaced from their usual position, such that they come to occupy a different layer.

The vast majority of the divisions in the L1 and L2 layers of the meristem are anticlinal, and anticlinal division is responsible for generating the layers in the first place. Nevertheless, occasional periclinal divisions occur, causing one derivative to occupy the adjacent layer. This periclinal division does not alter the composition of the tissue derived from this layer. Instead, the derivatives assume a function that is appropriate for a cell occupying that layer.

Further support for the importance of position in determining cell fate has been obtained through observations of cell differentiation in leaves of English ivy (*Hedera helix*), which have a mixture of mutant and wild-type cells. When a mutation occurs in a stem cell in the shoot apical meristem, all the cells in the plant derived from that stem cell will carry the mutation. Such a plant is said to be a **chimera**, a mixture of cells with a different genetic makeup. The analysis of chimeras is useful for studies on the clonal origin of different tissues.

When the mutation affects the ability of chloroplasts to differentiate, the presence of albino sectors shows that these sectors were derived from the stem cells carrying the mutation. In the ivy plant shown in Figure 16.25, the L2 layer carried a mutation causing albinism, and the L1 and L3 layers had a wild-type copy of the same gene. The L1 layer gives rise to the leaf and stem epidermis, but it is colorless because chloroplasts do not differentiate in most epidermal cells. Mesophyll tissue typically is derived from the L2 layer, so the leaves should be white because the L2 stem cells carried the mutant gene and passed it on to their derivatives.

FIGURE 16.25 Periclinal chimeras demonstrate that the mesophyll tissue has more than a single clonal origin in English ivy (*Hedera helix*). These variegated leaves provide clues on the clonal origins of different tissues. A mutation in a gene essential for chloroplast development occurred in some of the initial cells of the meristem, and cells derived from these mutated stem cells lack chloroplasts and are white, while cells derived from other stem cells have normal chloroplasts and appear green. (Courtesy of S. Poethig.)

Although a few of the leaves are white, or nearly so, most of the leaves show green patches. They are **variegated**. The green tissue in these leaves was derived from the cells originally in the L1 or L3 layer; the colorless regions were derived from the L2 layer. The variegation occurs because occasional periclinal divisions in the L1 or L3 layer early in leaf development establish clones of cells that can differentiate as green mesophyll cells. This is further evidence that cell differentiation is not dependent on cell lineage. The fate of a cell during development is determined by the position it occupies in the plant body.

Developmental Pathways Are Controlled by Networks of Interacting Genes

We have a great deal more to learn about the regulatory networks that control developmental pathways. However, several discoveries point to a model in which local and long-distance signaling events control the expression of genes that encode transcription factors. These transcription factors in turn determine the character or activities of a given tissue or cell. Often these mechanisms involve feedback loops in which two or more genes interact to regulate each other's expression. These interactions are seen most clearly in the case of the shoot apical meristem.

Expression of the *KNOX* gene *STM* (*SHOOTMERISTEMLESS*) is essential for the formation of the shoot apical meristem in the *Arabidopsis* embryo and for meristem function in the growing plant. *STM* is expressed throughout the apical dome of the vegetative meristem, except in the developing leaf primordia. Similarly, *STM* is expressed in the dome of the floral meristem, but it is silenced as floral organs appear. Two additional *KNOX* genes—*KNAT1* and *KNAT2*—also are expressed in the apical meristem of *Arabidopsis* and participate in maintaining the meristem cells in an undifferentiated state.

Because cells actively divide in the early stages of leaf and floral organ primordia development, *STM* is not necessary for cell division. Rather *KN1*, *STM*, and their functional homologs maintain meristem identity by suppressing differentiation. Another gene, *ASYMMETRIC LEAVES1* (*AS1*) promotes leaf development and is expressed in the primordia and young leaves of *Arabidopsis* (Figure 16.26) (Byrne et al. 2000). *STM* represses the expression of *AS1*, and *AS1* in turn represses the expression of *KNAT1* in the developing leaf primordia (Ori et al. 2000):

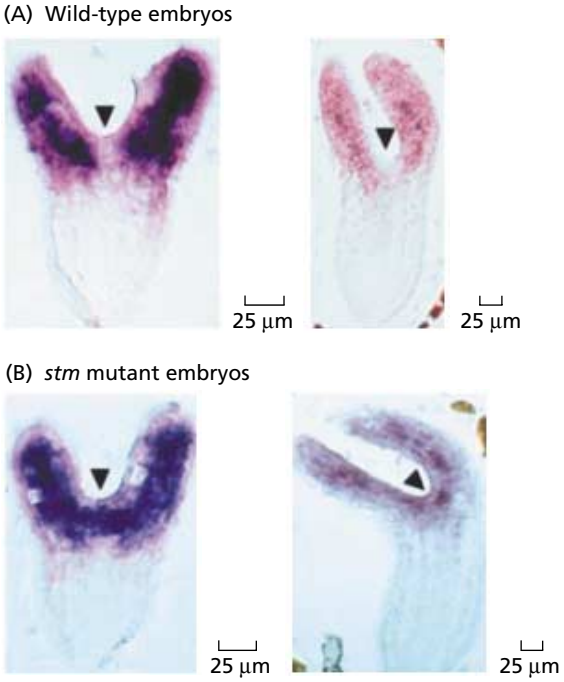
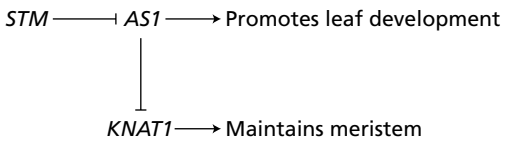


FIGURE 16.26 The meristem identity gene, *STM*, inhibits expression of the *ASYMMETRIC LEAVES1* (*AS1*) gene, which promotes leaf development in *Arabidopsis*. Arrows point to the shoot apical meristem–forming region. (A) Expression of the *STM* gene is normally confined to the shoot apical meristem in the wild type, and it confers meristem identity on the vegetative meristem. In contrast, the *AS1* gene is confined to leaf primordia and developing cotyledons in the wild type, as shown by in situ hybridization in embryos at two stages of development. (B) In *stm* mutants, expression of *AS1* expands into the region that would normally become the shoot apical meristem. As a result, the apical meristem does not form. (From Byrne et al. 2000.)



The *WUSCHEL* (*WUS*) gene, which encodes another homeodomain transcription factor, is a key regulator of stem cell indeterminacy (Laux et. al. 1996). In plants with loss-of-function *wus* mutations, either an apical meristem is lacking entirely, or their stem cells are used up after they have formed a few leaves. The *CLAVATA* genes negatively regulate *WUS* expression. *WUS* expression is expanded in both *clv1* and *clv3* mutants (Figure 16.27). Conversely, *WUS* expression positively regulates *CLV3* gene expression; (see Figure 16.24) (Brand et al. 2000).

Development Is Regulated by Cell-to-Cell Signaling

How do cells know where they are? If a cell's fate is determined by its position and not by clonal lineage, then cells must be able to sense their position relative to other cells, tissues, and organs. Neighboring cells and distant tissues and organs provide positional information. Cells in multicellular plants usually are in close contact with others around them, and the behavior of each cell is carefully coordinated with that of its neighbors throughout the life of the plant. Furthermore, each cell occupies a specific position within the tissue and organ to which it belongs.

Coordination of cellular activity requires cell–cell communication. That is, some developmentally important genes act *nonautonomously*. They do not have to be expressed in a given cell to affect the fate of that cell. A given gene or set of genes can exert an effect on development in neighboring cells or even cells in distant tissues through cell–cell communication, via at least three different mechanisms:

1. Ligand-induced signaling
2. Hormonal signaling
3. Signaling via trafficking of regulatory proteins and/or mRNAs

Ligand-induced signaling. There is evidence that cell wall components, particularly a class of glycoprotein macromolecules known as **arabinogalactan proteins**, or **AGPs**, may communicate positional information that will determine cell fate (see Chapter 15). AGPs would not be involved in signaling over a distance, but rather in telling a given cell who its neighbors were. That information then would program the cell to differentiate, or acquire a fate appropriate to its position.

Because plants have numerous, perhaps hundreds, of receptor kinases, we might expect many signaling events to be initiated by ligand-induced protein phosphorylation. At present, however, relatively few of the ligands activating protein kinases are known. But there is good evidence that the small protein encoded by the *CLV3* gene is the ligand that activates the CLV1 protein kinase.

The CLV3 protein contains fewer than 100 amino acids and contains a leader sequence suggesting that it would be excreted from the cells that produce it (Fletcher et al. 1999). Because of its small size and water solubility, it could freely diffuse through the extracellular space, or apoplast.

The **apoplast** consists mostly of the space occupied by the cell walls. Cell wall macromolecules are largely hydrophilic, and the wall contains passages between the macromolecules with an apparent pore size of 3.5 to 5 nm. This means that molecules with a mass of less than approximately 15 kDa can diffuse freely through the apoplast. With a molecular weight of approximately 11 kDa, the CLV3 protein easily could diffuse through the apoplast.

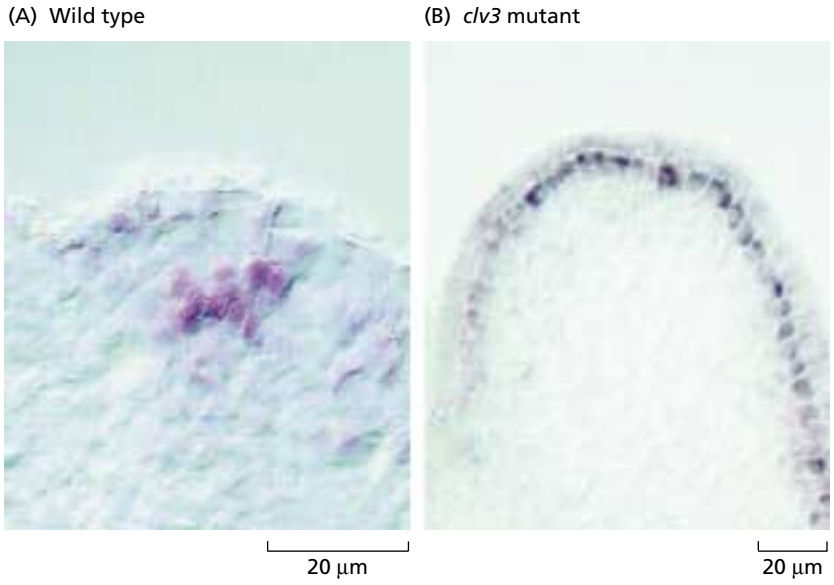


FIGURE 16.27 *WUS* gene expression in the shoot apical meristem of the wild type and the *clv3* mutant. The localization of *WUS* mRNA was detected by an in situ hybridization procedure. (A) In the wild type, *WUS* expression is confined to a small cluster of cells. (B) In the *clv3* mutant, *WUS* expression expands both apically and laterally, and the apical meristem itself is enlarged. (Brand et al. 2000.)

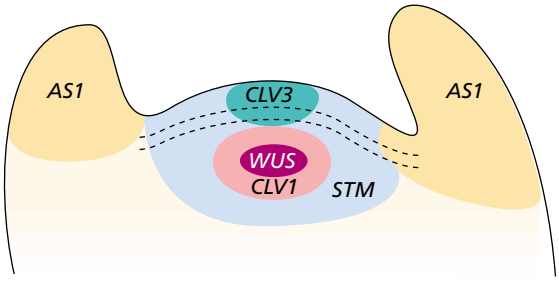


FIGURE 16.28 Patterns of expression of some developmentally important genes in the *Arabidopsis* shoot apical meristem. (From Clark 2001.)

The *CLV3* gene is expressed in cells of the L1 and L2 layers in the central zone of the shoot apical meristem, but not within the L3 layer or in the peripheral zone. In contrast, *CLV1* is expressed in deeper layers within the central zone in the L3 layer, as is the *WUS* gene. However, *CLV1* is expressed within a somewhat larger domain than *WUS* (Figure 16.28). Although *WUS* gene expression is required to maintain stem cell identity, *WUS* is expressed in only a small number of cells in the L3 layer of the meristem. It functions nonautonomously, acting on cells a short distance from the cells that express the gene.

The CLV3 protein controls the size of the stem cell population in the shoot apex by negatively regulating the expression of *WUS* in the L3 layer. The *CLV3* gene is expressed in cells in the central zone of the meristem, within the L1 and L2 layers. When *CLV1* or *CLV3* is knocked out by mutation, *WUS* gene expression spreads, and the number of undifferentiated stem cells expands (Brand et al. 2000). Because this expansion requires *CLV1*, it is likely that CLV3 protein diffuses from the L1 cells and binds to the receptor domain of CLV1 to activate its kinase domain to initiate a signal that represses *WUS* gene transcription.

WUS expression promotes *CLV3* expression, which in turn represses *WUS* expression. Thus the meristem has a sensitive feedback mechanism for controlling the size of the stem cell population.

Hormonal signaling. The plant hormones—auxin, ethylene, gibberellins, abscisic acid, cytokinins, and brassinosteroids—all play roles in regulating development. These roles will be presented in some detail in the chapters and sections devoted to these topics. In this discussion, however, we will focus on auxin signaling as an example of the types of mechanisms these roles might entail. This topic will be discussed in greater detail in Chapter 19.

Auxin signaling is essential for the development of axial polarity and the development of vascular tissue. Auxin has long been known to be the signal for the initiation of vascular tissue differentiation (see Chapter 19). This conclusion, however, is based largely on studies of the effects of applied auxins and auxin transport inhibitors. More recently, two *Arabidopsis* genes—*GNOM* and *MONOPTEROS*—known to be essential for the development of axial polarity and tissue differentiation during embryogenesis and adult plant development, have been found to be involved in auxin signaling. As presented earlier, the *Arabidopsis GNOM* gene was identified because embryos homozygous for mutations in this gene lack both roots and cotyledons and fail to develop axial polarity (see Figure 16.7A) (Mayer et al. 1993).

The *GNOM* gene product is required for correct localization of the auxin efflux carrier protein PIN1 (Figure 16.29).

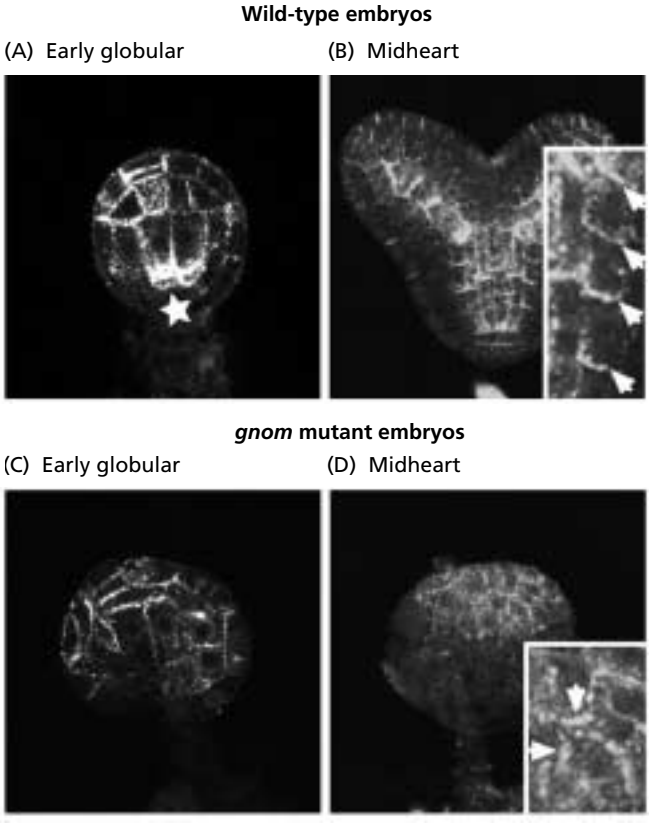


FIGURE 16.29 Comparison of the distribution patterns of the auxin efflux protein PIN1 in wild-type and *gnom* mutant *Arabidopsis* embryos. (A) Wild-type, early globular; PIN1 is localized in the provascular tissue early in the early globular stage, where the protein accumulates at the basal boundary of the four inner cells that will give rise to the provascular tissue. (B) Wild-type, midheart; in the heart stage, the provascular cells have accumulated PIN1 protein at their basal ends (see insert). (C) *gnom* mutant, early globular; PIN1 does not accumulate in the region where the provascular tissue will form in the early globular stage of the *gnom* mutant. (From Steinmann et al. 1999). (D) *gnom* mutant, midheart; formation of provascular tissue is blocked in the *gnom* mutant, and normal development is disrupted. PIN1 is still inserted in membranes in the mutant, but the localization is disorganized (see insert). (From Steinmann et al. 1999.)

GNOM encodes a guanine nucleotide exchange factor that is a component of the cellular machinery that establishes cell polarity. This machinery, and the GNOM protein in particular, are required for the correct localization of the auxin efflux carrier protein PIN1 at the basal end of the procambium cells during the globular stage of embryogenesis and subsequently in vascular cells throughout development (Steinmann et al. 1999; Grebe et al. 2000).

As we have seen, mutations in the *MONOPTEROS* (*MP*) gene result in seedlings that lack both a hypocotyl and root, although they do produce an apical region. The apical structures in the *mp* mutant embryos are not structurally normal, however, and the tissues of the cotyledons are disorganized (see Figure 16.7B) (Berleth and Jürgens 1993). Embryos of *mp* mutants first show abnormalities at the octant stage, and they do not form a procambium in the lower part of the globular embryo, the part that should give rise to the hypocotyl and root. Later some vascular tissue does form in the cotyledons, but the strands are improperly connected.

The *MP* gene encodes a protein related to the transcription factor known as **ARF** (***a*uxin *r*esponse *f*actor**) (Hardtke and Berleth 1998). Both ARF and MONOPTEROS bind to auxin response elements in the promoters of certain genes that are transcribed in the presence of auxin. Apparently the *MP* gene is required for expression of genes involved in vascular tissue differentiation.

Other evidence in support of auxin signaling during embryogenesis includes the finding that the putative auxin receptor protein, **ABP1**, is required for organized cell elongation and division in embryogenesis. *Arabidopsis* mutants homozygous for *abp1* do not form mature embryos, although they develop normally up to the early globular stage. These mutants cannot make the transition to bilateral symmetry, and cells fail to elongate (Chen et al. 2001).

Auxin signaling also participates in organogenesis from the shoot apical meristem and in the formation of lateral roots. *Arabidopsis* plants with mutations in the auxin efflux carrier gene *PIN1* develop a pinlike inflorescence that is devoid of lateral organs (Figure 16.30). In wild-type plants, *PIN1* gene expression is up-regulated in the early stages of primordium formation, before the primordia begin to bulge. The shoot apical meristem at the tip of the pinlike inflorescence in the *pin1* mutant plants has a normal structure, except that no organs are generated in the peripheral zone and the shoot produced lacks lateral appendages (Vernoux et al. 2000). Thus, auxin is likely to be required for signaling early events necessary for organogenesis from the shoot apical meristem.

This hypothesis is supported by work with tomato. When tomato apical meristems are cultured on medium containing the auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA), they continue to grow, but they develop into pinlike shoots lacking lateral appendages. When these NPA-induced pin meristems were treated with auxin at their tips, leaf initiation was restored (Reinhardt et al. 2000).

Other signaling mechanisms remain to be discovered. The mechanism by which cells communicate has not been established in other cases, although it is clear that positional information is exchanged between cells in different tissues. As presented earlier, the *SHR* and *SCR* genes are important for the establishment of the radial tissue patterns in roots. They encode rather similar transcription factors, but these two genes are expressed and function in different tissues.

SCR is required for the asymmetric cell division that forms the epidermis and cortex, and it also determines the endodermis cell fate. *SCR* is expressed in the stem cell that will give rise to the ground tissue before it divides asymmetrically to form the precursors of endodermis and cortex (Figure 16.31A). *SCR* continues to be expressed in the endodermis after the stem cell divides (Figure 16.31B).

SCR gene expression requires *SHR* expression, but the *SHR* gene is not expressed in either the cortex or the endodermis. Rather, *SHR* is expressed in the pericycle and the vascular cylinder (Figure 16.31C) (Helariutta et al. 2000). This implies that *SHR* gene expression generates a signal

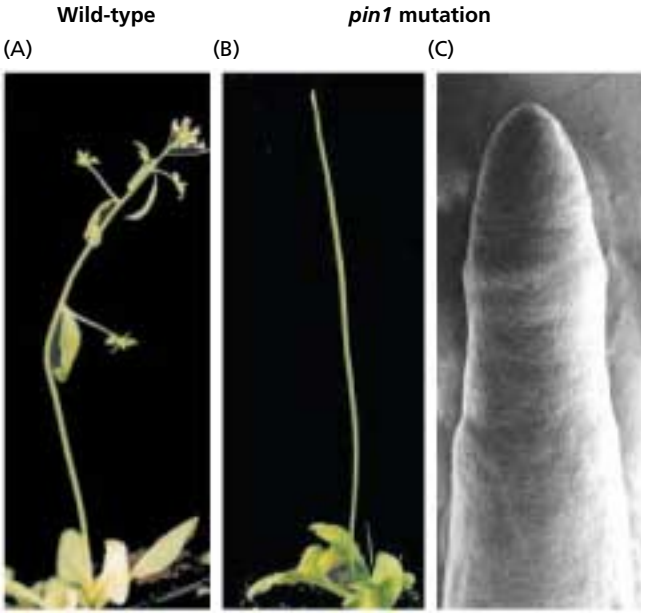


FIGURE 16.30 The *PIN1* gene is essential for the formation of lateral organs from the inflorescence meristem in *Arabidopsis*. (A) The inflorescence meristem generates a stem bearing cauline leaves and numerous floral buds in the wild type. (B) Plants with *pin1* mutations produce an inflorescence meristem, but it fails to generate lateral organs. (C) The inflorescence meristem produces only axial tissues, similar to the root apical meristem, as shown in this scanning electron micrograph. (From Vernoux et al. 2000.)

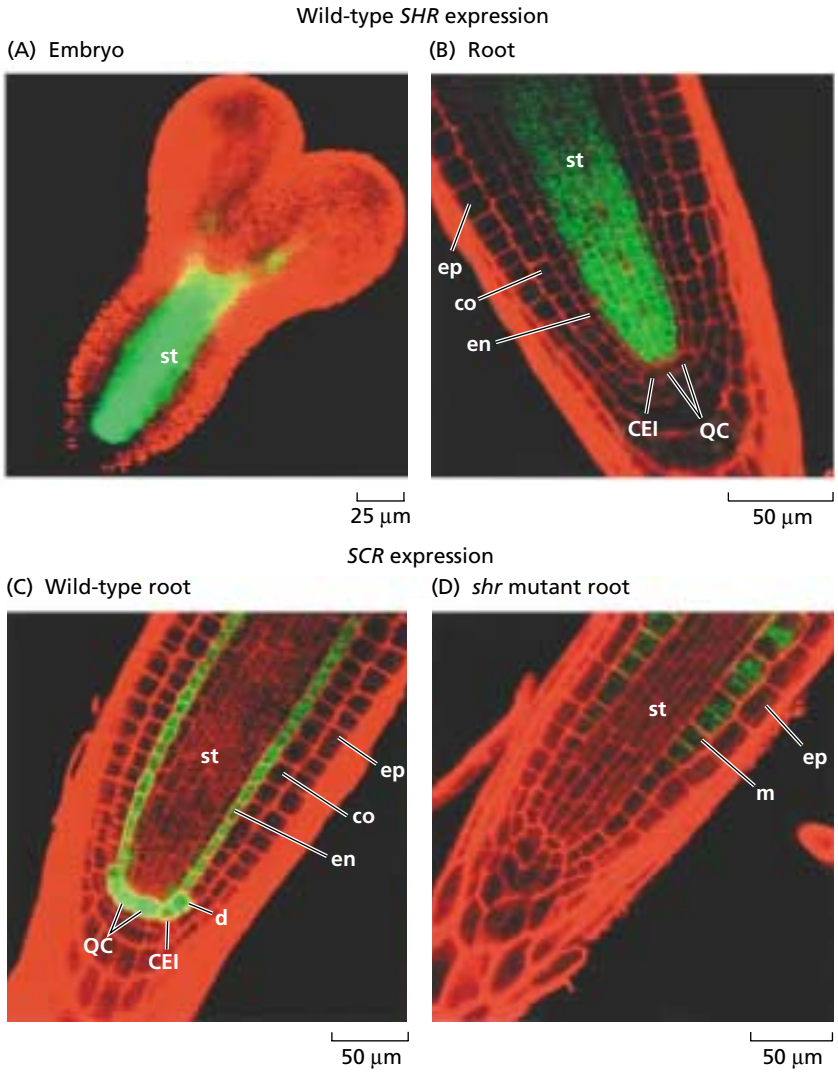


FIGURE 16.31 The *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*) genes in *Arabidopsis* control tissue patterning during root development. The SHR or SCR proteins have been localized by confocal laser scanning microscopy after being tagged with green fluorescent protein (GFP), which has a greenish-yellow color. (A) During embryogenesis in wild-type *Arabidopsis*, the SHR protein is localized in the provascular tissues. (B) The SHR protein continues to be localized in the vascular cylinder throughout growth of the primary root. (C) In wild-type roots, SCR protein is localized in the quiescent center, endodermis, and cortical–endodermal stem cell (CEI). It is not present in the cortex, vascular cylinder, or epidermis. (D) The expression of *SCR* is markedly reduced in the *shr* mutant root, and now appears only in the mutant cell layer that has characteristics of both endodermis and cortex. CEI = cortical–endodermal stem cell; co = cortex; d = daughter cells; en = endodermis; ep = epidermis; m = mutant cell layer; QC = quiescent center; st = vascular cylinder. (From Helariutta et al. 2000.)

that is received by the ground tissue stem cells and causes the expression of the *SCR* gene in these cells. This illustrates again the potential importance of cell-to-cell signaling in cell fate determination and in plant development. At present it is not known how this communication takes place.

Signaling via trafficking of regulatory proteins and/or mRNAs. Symplastic communication between plant cells occurs via the plasmodesmatal connections through their cell walls (see Chapter 1). Most living cells in a plant are connected symplastically to their neighbors by plasmodesmata that pass through the adjoining cell walls and provide some degree of cytosolic continuity between them. There is increasing evidence that the signals exchanged through plasmodesmata include both regulatory proteins and mRNAs (Zambryski and Crawford 2000).

The importance of plasmodesmata for cell–cell communication during development became apparent with the discovery that the mRNA of the maize meristem identity gene *KN1* cannot be detected in the L1 layer of the maize vegetative shoot apical meristem. The *KN1* gene is expressed only in cells of the L2 layer. The KN1 protein, however, is detected in all regions of the shoot apical meristem, including the L1 layer. Since the KN1 protein is not synthesized in the L1 layer, it must be transported into the L1 layer from the L2 layer, through the plasmodesmata joining them (Figure 16.32) (Lucas et al. 1995).

In *Antirrhinum*, expression of the *FLO* gene in the L1 layer activates expression of the floral organ identity genes in all cell layers of the meristem (Carpenter and Coen 1995). Although many explanations for this relationship are possible, one is that the FLO protein, by passing through the plasmodesmata, moves into these other layers from the cells in which it is synthesized.

Viruses invade plants and spread from cell to cell by passing through plasmodesmata. Their genomes encode proteins designated **movement proteins** that can facilitate the movement of the viral RNA genome through plasmodesmata. It is likely that viruses have hijacked a mechanism that evolved for cell–cell communication. At present it isn't clear why information exchange would be organized in this manner, but this type of communication may be a fairly general phenomenon in plant development.

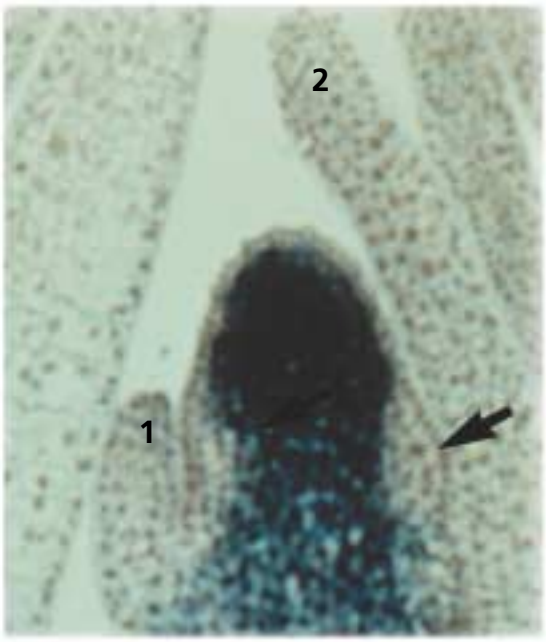


FIGURE 16.32 The *KN1* gene is expressed throughout the maize shoot apical meristem, but it is not expressed in the L1 layer or in leaf primordia. The *KN1* mRNA was localized here in a longitudinal section through the meristem by a hybridization procedure. The arrow points to the predicted site of the next leaf primordium (P0); the numbers 1 and 2 identify the P1 and P2 leaf primordia, respectively. (After Jackson et al. 1994.)

THE ANALYSIS OF PLANT GROWTH

How do plants grow? This deceptively simple question has challenged plant scientists for more than 150 years. New cells form continually in the apical meristems. Cells enlarge slowly in the apical meristem and more rapidly in the subapical regions. The resulting increase in cell volume can range from severalfold to 100-fold, depending on the species and environmental conditions. Classically, plant growth has been analyzed in terms of cell number or overall size (or mass). However, these measures tell only part of the story.

Tissue growth is neither uniform nor random. The derivatives of the apical meristems expand in predictable and site-specific ways, and the expansion patterns in these subapical regions largely determine the size and shape of the primary plant body. The total growth of the plant can be thought of as the sum of the local patterns of cell expansion.

The analysis of the motions of cells or "tissue elements" (and the related problem of cell expansion) is called *kinematics*. In this section we will discuss both the classical definitions of growth and the more modern, kinematic approach. As we will see, the advantage of the kinematic approach is that it allows one to describe the growth patterns of organs mathematically in terms of the expansion patterns of their component cells.

Plant Growth Can Be Measured in Different Ways

Growth in plants is defined as an irreversible increase in volume. The largest component of plant growth is cell expansion driven by turgor pressure. During this process, cells increase in volume manyfold and become highly vacuolate. However, size is only one criterion that may be used to measure growth.

Growth also can be measured in terms of change in fresh weight—that is, the weight of the living tissue—over a particular period of time. However, the fresh weight of plants growing in soil fluctuates in response to changes in the water status, so this criterion may be a poor indicator of actual growth. In these situations, measurements of dry weight are often more appropriate.

Cell number is a common and convenient parameter by which to measure the growth of unicellular organisms, such as the green alga *Chlamydomonas* (Figure 16.33). In multicellular plants, however, cell number can be a misleading growth measurement because cells can divide without increasing in volume.

For example, during the early stages of embryogenesis, the zygote subdivides into progressively smaller cells with no net increase in the size of the embryo. Only after it

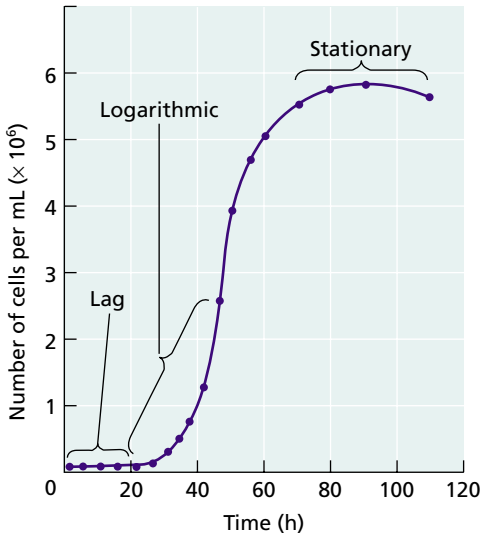


FIGURE 16.33 Growth of the unicellular green alga *Chlamydomonas*. Growth is assessed by a count of the number of cells per milliliter at increasing times after the cells are placed in fresh growth medium. Temperature, light, and nutrients provided are optimal for growth. An initial lag period during which cells may synthesize enzymes required for rapid growth is followed by a period in which cell number increases exponentially. This period of rapid growth is followed by a period of slowing growth in which the cell number increases linearly. Then comes the stationary phase, in which the cell number remains constant or even declines as nutrients are exhausted from the medium.

reaches the eight-cell stage does the increase in volume begin to mirror the increase in cell number. Because the zygote is an especially large cell, this lack of correspondence between an increase in cell number and growth may be unusual, but it points out the potential problem in equating an increase in cell number with growth.

Although cell number may not always be a reliable measure of plant growth, under most circumstances dividing cells, particularly in meristems, double in volume during their cell cycle. Therefore, an increase in cell number, such as the increase brought about by the activity of the apical meristems, does contribute to plant growth. However, the largest component of plant growth is the rapid cell expansion that occurs in the subapical region after cell division ceases.

Because all the cells of the plant axis elongate under normal conditions, the greater the number of cells produced by the apical meristem, the longer the axis will be. For example, when *Arabidopsis* plants are transformed with a gene that encodes cyclin, a key component of the cell cycle regulatory machinery (see Chapter 1), the cells of the apical meristem progress through their cell cycles more rapidly, so more cells form per unit time. As a result, the roots of these transgenic plants have more cells and are substantially longer than the roots of wild-type plants grown under similar conditions (Doerner et al. 1996).

New cells form continually in the apical meristems. With each new round of cell division and associated cell expansion, the older derivatives are displaced a small distance from the apex. As the cells recede farther from the apex, the rate of displacement is greatly accelerated. By viewing plant growth as a process of cell displacement from the apex, we can apply the principles of kinematics.

The Production of Cells by the Meristem Is Comparable to a Fountain

Moving fluids such as waterfalls, fountains, and the wakes of boats can generate specific forms. The study of the motion of fluid particles and the shape changes that the fluids undergo is called **kinematics**. The ideas and numerical methods used to study these fluid forms are useful for characterizing meristematic growth. In both cases, an unchanging form is produced, even though it is composed of moving and changing elements.

An example of an unchanging form composed of changing and displaced elements in plants is the hypocotyl hook of a dicot such as the common bean (Figure 16.34). As the bean seedling emerges from the seed coat, the apical end of the hypocotyl bends back on itself to form a hook. The hook is thought to protect the seedling apex from damage during growth through the soil. During seedling growth (in soil or dim light) the hook migrates up the stem, from the hypocotyl into the epicotyl and then to the first and second internodes, but the form of the hook remains constant.

If we mark a specific epidermal cell on the seedling stem located close to the seedling apex, we can watch it as it flows into the hook summit, then down into the straight region below the hook (see Figure 16.34). The mark is not crawling over the plant surface, of course; plant cells are cemented together and do not experience much relative motion during development. The change in position of the mark relative to the hook implies that the hook is composed of a procession of tissue elements, each of which first curves and then straightens as it is displaced from the plant apex during growth. The steady form is produced by a parade of changing cells.

A root tip is another example of a steady form composed of changing tissue elements. Here, too, the form is observed to be steady only when distance is measured from the root tip. A region of cell division occupies perhaps 2 mm of the root tip. The elongation zone extends for about 10 mm behind the root tip. Phloem differentiation is first observed beginning at 3 mm from the tip, and functional xylem elements may be seen at about 12 mm from the tip. A marked cell near the tip will seem to flow first through the region of cell division, then through the elongation zone and into the region of xylem differentiation, and so on. This shifting implies that developing tissue elements first divide and elongate, and then differentiate.

In an analogous fashion, the shoot bears a succession of leaves of different developmental stages. During a period of 24 hours, a leaf may grow to the same size, shape, and biochemical composition that its neighbor had a day earlier. Thus, shoot form is also produced by a parade of changing elements that can be analyzed with kinematics. Such an analysis is not merely descriptive; it permits calculations of the growth and biosynthetic rates of individual tissue elements (cells) within a dynamic structure.

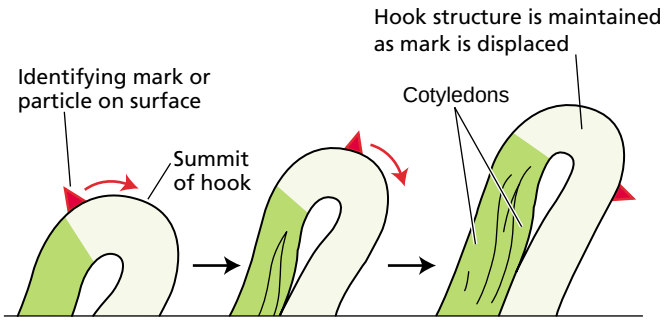


FIGURE 16.34 The dicot hypocotyl hook is an example of a constant form composed of changing elements. The hooked form is maintained over time, while different tissues first curve and then straighten as they are displaced from the seedling apex during growth. If a mark is placed at a fixed point on the surface, it will be displaced (indicated by the arrow), appearing to flow through the hook over time. (After Silk 1994.)

Tissue Elements Are Displaced during Expansion

As we have seen, growth in shoots and roots is localized in regions at the tips of these organs. Regions with expanding tissue are called **growth zones**. With time, meristems move away from the plant base by the growth of the cells in the growth zone.

If successive marks are placed on the stem or root, the distance between the marks will change, depending on where they are within the growth zone. In addition, all of these marks will move away from the tip of the root or shoot, but their rate of movement will differ depending on their distance from the tip.

From another perspective, if you were to stand at the tip of a root that had marks placed at intervals along the axis, you would see that all marks would move farther away from you with time. The reason is that discrete regions on the plant axis experience displacement as well as expansion during growth and development.

As Regions Move Away from the Apex, Their Growth Rate Increases

As a given region of the plant axis moves away from the apex, its growth velocity increases (the rate of elongation accelerates) until a constant limiting velocity is reached equal to the overall organ extension rate. The reason for this increase in growth velocity is that with time, progressively more tissue is located between the moving particle and the apex, and progressively more cells are expanding, so the particle is displaced more and more rapidly. In a rapidly growing maize root, a tissue element takes about 8 hours to move from 2 mm (the end of the meristematic zone) to 12 mm (the end of the elongation zone).

Beyond the growth zone, elements do not separate; neighboring elements have the same velocity (expressed as the change in distance from the tip per unit of time), and the rate at which particles are displaced from the tip is the same as the rate at which the tip moves through the soil. The root tip of maize is pushed through the soil at 3 mm h^{-1}. This is also the rate at which the nongrowing region recedes from the apex, and it is equal to the final slope of the growth trajectory.

The Growth Velocity Profile Is a Spatial Description of Growth

The velocities of different tissue elements are plotted against their distance from the apex to give the spatial pattern of growth velocity, or **growth velocity profile** (Figure 16.35A). Growth velocity increases with position in the growth zone. A constant value is obtained at the base of the growth zone. The final growth velocity is the final, constant slope of the growth trajectory equal to the elongation rate of the organ, as discussed in the previous section. In the rapidly growing maize root, the growth velocity is 1 mm h^{-1} at 4 mm, and it reaches its final value of nearly 3 mm h^{-1} at 12 mm.

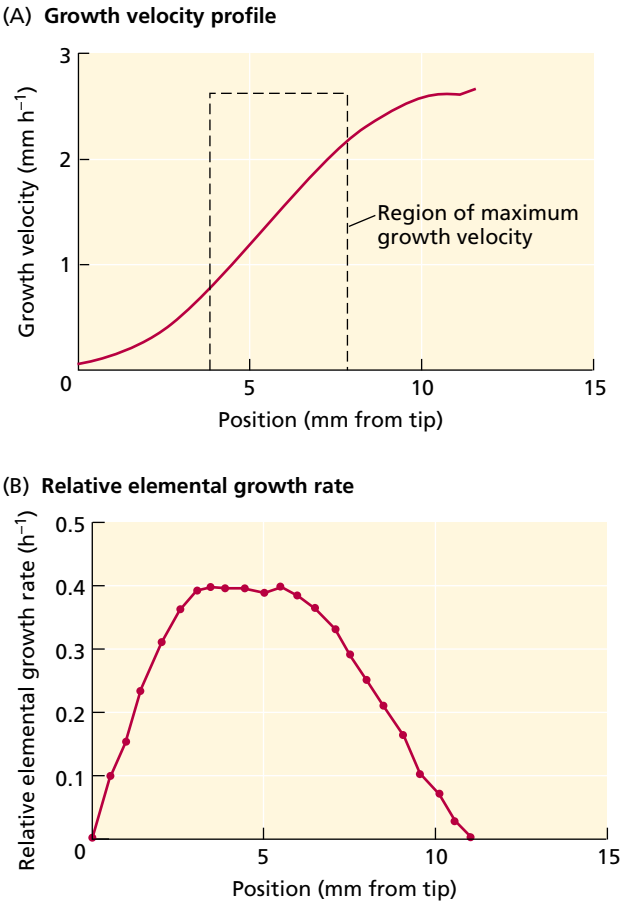


FIGURE 16.35 The growth of the primary root of *Zea mays* (maize) can be represented kinematically by two related growth curves. (A) The growth velocity profile plots the velocity of movement away from the tip of points at different distances from the tip. This tells us that growth velocity increases with distance from the tip until it reaches a uniform velocity equal to the rate of elongation of the root. (B) The relative elemental growth rate tells us the rate of expansion of any particular point on the root. It is the most useful measure for the physiologist because it tells us where the most rapidly expanding regions are located. (From Silk 1994.)

If the growth velocity is known, the **relative elemental growth rate**, which represents the fractional change in length per unit time, can be calculated (see **Web Topic 16.4**). The relative elemental growth rate shows the location and magnitude of the extension rate and can be used to quantify the effects of environmental variation on the growth pattern (Figure 16.35B).

SENESCENCE AND PROGRAMMED CELL DEATH

Every autumn, people who live in temperate regions can enjoy the beautiful color changes that precede the loss of leaves from deciduous trees. The leaves change color

because changing day length and cooling temperatures trigger developmental processes that lead to leaf senescence and death. Senescence is distinct from necrosis, although both senescence and necrosis lead to death. **Necrosis** is death brought about by physical damage, poisons, or other external injury. In contrast, **senescence** is a normal, energy-dependent developmental process that is controlled by the plant's own genetic program. Leaves are genetically programmed to die, and their senescence can be initiated by environmental cues.

As new leaves are initiated from the shoot apical meristem, older leaves often are shaded and lose the ability to function efficiently in photosynthesis. Senescence recovers a portion of the valuable resources that the plant invested in leaf formation. During senescence, hydrolytic enzymes break down many cellular proteins, carbohydrates, and nucleic acids. The component sugars, nucleosides, and amino acids are then transported back into the plant via the phloem, where they will be reused for synthetic processes. Many minerals also are transported out of senescing organs, back into the main body of the plant.

Senescence of plant organs is frequently associated with **abscission**, a process whereby specific cells in the petiole differentiate to form an abscission layer, allowing the senescent organ to separate from the plant. In Chapter 22 we will have more to say about the control of abscission by ethylene.

In this section we will examine the roles that senescence and programmed cell death play in plant development. We will see that there are many types of senescence, each with its own genetic program. Then, in Chapters 21 and 22, we will describe how cytokinins and ethylene can act as signaling agents that regulate plant senescence.

FIGURE 16.36 Monocarpic senescence in soybeans (*Glycine max*). The entire plant on the left underwent senescence after flowering and producing fruit (pods). The plant on the right remained remained green and vegetative because its flowers were continually removed. (Courtesy of L. Noodén.)

Plants Exhibit Various Types of Senescence

Senescence occurs in a variety of organs and in response to many different cues. Many annual plants, including major crop plants such as wheat, maize, and soybeans, abruptly yellow and die following fruit production, even under optimal growing conditions. Senescence of the entire plant after a single reproductive cycle is called **monocarpic senescence** (Figure 16.36).

Other types of senescence include the following:

- Senescence of aerial shoots in herbaceous perennials
- Seasonal leaf senescence (as in deciduous trees)
- Sequential leaf senescence (in which the leaves die when they reach a certain age)
- Senescence (ripening) of fleshy fruits; senescence of dry fruits
- Senescence of storage cotyledons and floral organs (Figure 16.37)
- Senescence of specialized cell types (e.g., trichomes, tracheids, and vessel elements)

The triggers for the various types of senescence are different and can be internal, as in monocarpic senescence, or external, such as day length and temperature in the autumnal leaf senescence of deciduous trees. Regardless of the initial stimulus, the different senescence patterns may share common internal programs in which a regulatory senescence gene initiates a cascade of secondary gene expression that eventually brings about senescence and death.

Senescence Is an Ordered Series of Cytological and Biochemical Events

Because it is genetically encoded, senescence follows a predictable course of cellular events. On the cytological level, some organelles are destroyed while others remain active. The chloroplast is the first organelle to deteriorate during the onset of leaf senescence, with the destruction of thylakoid protein components and stromal enzymes.

In contrast to the rapid deterioration of chloroplasts, nuclei remain structurally and functionally intact until the late stages of senescence. Senescing tissues carry out cata-

FIGURE 16.37 Stages of flower senescence in morning glory (*Ipomoea acuminata*). (Courtesy of S. L. Taiz.)

bolic processes that require the de novo synthesis of various hydrolytic enzymes, such as proteases, nucleases, lipases, and chlorophyll-degrading enzymes. The synthesis of these senescence-specific enzymes involves the activation of specific genes.

Not surprisingly, the levels of most leaf mRNAs decline significantly during the senescence phase, but the abundance of certain specific mRNA transcripts increases. Genes whose expression decreases during senescence are called **senescence down-regulated genes** (**SDGs**). SDGs include genes that encode proteins involved in photosynthesis. However, senescence involves much more than the simple switching off of photosynthesis genes.

Genes whose expression is induced during senescence are called **senescence-associated genes** (**SAGs**). SAGs include genes that encode hydrolytic enzymes, such as proteases, ribonucleases, and lipases, as well as enzymes involved in the biosynthesis of ethylene, such as ACC (l-aminocyclopropane-l-carboxylic acid) synthase and ACC oxidase. SAGs of another class have secondary functions in senescence. These genes encode enzymes involved in the conversion or remobilization of breakdown products, such as glutamine synthetase, which catalyzes the conversion of ammonium to glutamine (see Chapter 12) and is responsible for nitrogen recycling from senescing tissues.

Programmed Cell Death Is a Specialized Type of Senescence

Senescence can occur at the level of the whole plant, as in monocarpic senescence; at the organ level, as in leaf senescence; and at the cellular level, as in tracheary element differentiation. The process whereby individual cells activate an intrinsic senescence program is called **programmed cell death** (**PCD**). PCD plays an important part in animal development, in which the molecular mechanism has been studied extensively. PCD can be initiated by specific signals, such as errors in DNA replication during division, and involves the expression of a characteristic set of genes. The expression of these genes results in cell death. Much less is known about PCD in plants (Pennell and Lamb 1997).

PCD in animals is usually accompanied by a distinct set of morphological and biochemical changes called **apoptosis** (plural *apoptoses*) (from a Greek word meaning "falling off," as in autumn leaves). During apoptosis, the cell nucleus condenses and the nuclear DNA fragments in a specific pattern caused by degradation of the DNA between nucleosomes (see Chapter 2 on the web site).

Some plant cells, particularly in senescing tissues, exhibit similar cytological changes. PCD also appears to occur during the differentiation of xylem tracheary elements, during which the nuclei and chromatin degrade and the cytoplasm disappears. These changes result from the activation of genes that encode nucleases and proteases.

One of the important functions of PCD in plants is protection against pathogenic organisms. When a pathogenic organism infects a plant, signals from the pathogen cause the plant cells at the site of the infection to quickly accumulate high concentrations of toxic phenolic compounds and die. The dead cells form a small circular island of cell death called a **necrotic lesion.**

The necrotic lesion isolates and prevents the infection from spreading to surrounding healthy tissues by surrounding the pathogen with a toxic and nutritionally depleted environment.This rapid, localized cell death due to pathogen attack is called the **hypersensitive response** (see Chapter 13).

The existence of *Arabidopsis* mutants that can mimic the effect of infection and trigger the entire cascade of events leading to the formation of necrotic lesions, even in the absence of the pathogen, has demonstrated that the hypersensitive response is a genetically programmed process rather than simple necrosis.

SUMMARY

The basic body plan of the mature plant is established during embryogenesis; in this process, tissues are arranged radially: an outer epidermal layer surrounding a cylinder of vascular tissue that is embedded within cortical or ground tissues. The apical–basal axial pattern of the mature plant, with root and shoot polar axes, also is established during embryogenesis, as are the primary meristems that will generate the adult plant.

One common type of angiosperm embryonic development, exemplified by *Arabidopsis thaliana*, is characterized by precise patterns of cell divisions, forming successive stages: the globular, heart, torpedo, and maturation stages. The axial body pattern is established during the first division of the zygote, and mutant genes eliminate part of the embryo. The radial tissue pattern is established during the globular stage, apparently as a result of the expression of genes that control cell identity. The *SHOOTMERISTEMLESS* (*STM*) gene is expressed in the region that gives rise to the shoot apical meristem during the heart stage of embryogenesis, and its continued expression suppresses differentiation of the cells of the shoot apical meristem. The *GNOM* gene is required for the establishment of axial polarity, and the *MONOPTEROS* gene is required for formation of the embryonic primary root as well as vascular development.

A complete explanation of the mechanisms responsible for establishing and maintaining these patterns is not possible at present, but there is evidence that an association of microtubules and microfilaments known as the preprophase band is important in determining the plane of cell division. Cell differentiation does not depend on cell lineage; however, the division of the stem cell is essential for this process. Expression of the *SCR* (*SCARECROW*) gene, which has been cloned and encodes a novel protein, is necessary for the division of the stem cell, and the *SHR* (*SHORTROOT*) gene must be expressed for the establishment of endodermal cell identity.

Meristems are populations of small, isodiametric cells that have "embryonic" characteristics. Vegetative meristems generate specific portions of the plant body, and they regenerate themselves. In many plants, the root and shoot apical meristems are capable of indefinite growth.

The vegetative shoot apical meristem repetitively generates lateral organs (leaves and lateral buds), as well as segments of the stem. Shoot apical meristems in angiosperms typically are organized into three distinct layers, designated L1, L2, and L3.

The root and shoot apical meristems are primary meristems formed during embryogenesis. Secondary meristems are initiated during postembryonic development and include the vascular cambium, cork cambium, axillary meristems, and secondary root meristems.

The repetitive activity of the vegetative shoot apical meristem generates a succession of developmental units, called phytomeres, each consisting of one or more leaves, the node, the internode, and one or more axillary buds. The vegetative shoot apical meristem is indeterminate in its activity in that it may function indefinitely, but it gives rise to leaf primordia that are determinate in their growth.

Leaves form in a characteristic pattern, with three stages: (1) organogenesis, (2) development of suborgan domains, (3) cell and tissue differentiation. The number and order in which leaf primordia form is reflected in the subsequent phyllotaxy (alternate, opposite, decussate, whorled, or spiral). The leaf primordia must be positioned as a result of the precise spatial regulation of cell division within the apex, but the factors controlling this activity are not known.

Roots grow from their distal ends. The root apical meristem is subterminal and covered by a root cap. Cell divisions in the root apex generate files of cells that subsequently elongate and differentiate to acquire specialized function. Four developmental zones are recognized in the root: root cap, meristematic zone, elongation zone, and maturation zone. In *Arabidopsis*, files of mature cells can be traced to stem cells within the meristem cell population. The *Arabidopsis* root apical meristem consists of a quiescent center, cortical–endodermal stem cells, columella stem cells, root cap–epidermal stem cells, and stele stem cells.

Differentiation is the process by which cells acquire metabolic, structural, and functional properties distinct from those of their progenitors. Tracheary element differentiation is an example of plant cell differentiation. Microtubules participate in determining the pattern in which the cellulose microfibrils are deposited in the secondary walls of tracheary elements.

MADS box genes are key regulators of important biological functions in plants, animals, and fungi. Homeobox genes encode homeodomain proteins that act as transcription factors. These transcription factors control the expression of other genes whose products transform and characterize the differentiated cell.

In the determination of a cell's fate, the cell's position is more important than its lineage. Plant cell fate is relatively plastic and can be changed when the positional signals necessary for its maintenance are altered.

The expression of homeobox genes similar to the maize genes *KNOTTED1* and *SHOOTMERISTEMLESS* is necessary for the continued indeterminate character of the shoot apical meristem, but the *WUSCHEL* gene determines stem cell identity. Loss of expression of *KNOX* genes in the leaf primordia appears to be important in the shift to determinate growth in these structures.

Cell position is communicated via cell–cell signaling, which may involve ligand-induced signaling, hormone signaling or trafficking of regulatory proteins and/or mRNAs through plasmodesmata. Molecules ranging in size up to about 1.6 nm (700–1000 Da) can pass from cell to cell through plasmodesmata connecting leaf epidermal cells. Plasmodesmata are, to some extent, gated so that passage through them can be regulated, and their size exclusion

limit can be modified to permit the passage of much larger molecules, such as viruses.

Growth in plants is defined as an irreversible increase in volume. Plant growth can be quantitatively analyzed with kinematics, the study of particle movement and shape change.

Plant growth can be described in both spatial and material terms. Spatial descriptions focus on the patterns generated by all the cells located at different positions in the growth zones. Material analyses focus on the fate of the individual cells or tissue elements at various stages of development. A growth trajectory shows the distance of a tissue element from the apex over time, and is therefore a material description of growth. The growth velocity is the speed at which the tissue elements are being displaced from the apex. The relative elemental growth rate is a measure of the fractional increase in length of the axis per unit time and represents the magnitude of growth at a particular location.

Senescence and programmed cell death are essential aspects of plant development. Plants exhibit a variety of different senescence phenomena. Leaves are genetically programmed to senesce and die. Senescence is an active developmental process that is controlled by the plant's genetic program and initiated by specific environmental or developmental cues.

Senescence is an ordered series of cytological and biochemical events. The expression of most genes is reduced during senescence, but the expression of some genes (senescence-associated genes, or SAGs) is initiated. The newly active genes encode various hydrolytic enzymes, such as proteases, ribonucleases, lipases, and enzymes involved in the biosynthesis of ethylene, which carry out the degradative processes as the tissues die.

Programmed cell death (PCD) is a specialized type of senescence. One important function of PCD in plants is protection against pathogenic organisms in what is called the hypersensitive response, which has been demonstrated to be a genetically programmed process.

Web Material

Web Topics

16.1 Polarity of *Fucus* Zygotes
A wide variety of external gradients can polarize growth of cells that are initially apolar.

16.2 The Preprophase Band of Microtubules
Ultrastructural studies have elucidated the structure of the preprophase band of micro-tubules and its role in orienting the plane of cell division.

16.3 *Azolla* Root Development
Anatomical studies of the root of the aquatic fern, *Azolla*, have provided insights into cell fate during root development.

16.4 The Relative Elemental Growth Rate
The relative elemental growth rate at various points along a root can be evaluated by differentiation of the growth velocity with respect to position.

Web Essay

16.1 Plant Meristems: An Historical Overview
Scientists have used many approaches to unravel the secrets of plant meristems.

16.2 The Mermaids Wineglass
The giant marine green alga, *Acetabularia acetabulum*, holds a classic place in the history of biology.

16.3 Division Plane Determination in Plant Cells
Plant cells appear to utilize mechanisms different from those used by other eukaryotes to control their division planes.

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