Chapter

19

Auxin: The Growth Hormone

THE FORM AND FUNCTION of multicellular organism would not be possible without efficient communication among cells, tissues, and organs. In higher plants, regulation and coordination of metabolism, growth, and morphogenesis often depend on chemical signals from one part of the plant to another. This idea originated in the nineteenth century with the German botanist Julius von Sachs (1832–1897).

Sachs proposed that chemical messengers are responsible for the formation and growth of different plant organs. He also suggested that external factors such as gravity could affect the distribution of these substances within a plant. Although Sachs did not know the identity of these chemical messengers, his ideas led to their eventual discovery.

Many of our current concepts about intercellular communication in plants have been derived from similar studies in animals. In animals the chemical messengers that mediate intercellular communication are called **hormones**. Hormones interact with specific cellular proteins called *receptors*.

Most animal hormones are synthesized and secreted in one part of the body and are transferred to specific target sites in another part of the body via the bloodstream. Animal hormones fall into four general categories: proteins, small peptides, amino acid derivatives, and steroids.

Plants also produce signaling molecules, called *hormones*, that have profound effects on development at vanishingly low concentrations. Until quite recently, plant development was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. However, there is now compelling evidence for the existence of plant steroid hormones, the brassinosteroids, that have a wide range of morphological effects on plant development. (Brassinosteroids as plant hormones are discussed in **Web Essay 19.1**.)

A variety of other signaling molecules that play roles in resistance to pathogens and defense against herbivores have also been identified, including jasmonic acid, salicylic acid, and the polypeptide systemin (see Chapter 13). Thus the number and types of hormones and hormonelike signaling agents in plants keep expanding.

The first plant hormone we will consider is auxin. Auxin deserves pride of place in any discussion of plant hormones because it was the first growth hormone to be discovered in plants, and much of the early physiological work on the mechanism of plant cell expansion was carried out in relation to auxin action.

Moreover, both auxin and cytokinin differ from the other plant hormones and signaling agents in one important respect: They are required for viability. Thus far, no mutants lacking either auxin or cytokinin have been found, suggesting that mutations that eliminate them are lethal. Whereas the other plant hormones seem to act as on/off switches that regulate specific developmental processes, auxin and cytokinin appear to be required at some level more or less continuously.

We begin our discussion of auxins with a brief history of their discovery, followed by a description of their chemical structures and the methods used to detect auxins in plant tissues. A look at the pathways of auxin biosynthesis and the polar nature of auxin transport follows. We will then review the various developmental processes controlled by auxin, such as stem elongation, apical dominance, root initiation, fruit development, and oriented, or *tropic*, growth. Finally, we will examine what is currently known about the mechanism of auxin-induced growth at the cellular and molecular levels.

THE EMERGENCE OF THE AUXIN CONCEPT

During the latter part of the nineteenth century, Charles Darwin and his son Francis studied plant growth phenomena involving tropisms. One of their interests was the bending of plants toward light. This phenomenon, which is caused by differential growth, is called **phototropism**. In some experiments the Darwins used seedlings of canary grass (*Phalaris canariensis*), in which, as in many other grasses, the youngest leaves are sheathed in a protective organ called the **coleoptile** (Figure 19.1).

Coleoptiles are very sensitive to light, especially to blue light (see Chapter 18). If illuminated on one side with a short pulse of dim blue light, they will bend (grow) toward the source of the light pulse within an hour. The Darwins found that the tip of the coleoptile perceived the light, for if they covered the tip with foil, the coleoptile would not bend. But the region of the coleoptile that is responsible for the bending toward the light, called the **growth zone**, is several millimeters below the tip.

Thus they concluded that some sort of signal is produced in the tip, travels to the growth zone, and causes the shaded side to grow faster than the illuminated side. The results of their experiments were published in 1881 in a remarkable book entitled *The Power of Movement in Plants*.

There followed a long period of experimentation by many investigators on the nature of the growth stimulus in coleoptiles. This research culminated in the demonstration in 1926 by Frits Went of the presence of a growth-promoting chemical in the tip of oat (*Avena sativa*) coleoptiles. It was known that if the tip of a coleoptile was removed, coleoptile growth ceased. Previous workers had attempted to isolate and identify the growth-promoting chemical by grinding up coleoptile tips and testing the activity of the extracts. This approach failed because grinding up the tissue released into the extract inhibitory substances that normally were compartmentalized in the cell.

Went's major breakthrough was to avoid grinding by allowing the material to diffuse out of excised coleoptile tips directly into gelatin blocks. If placed asymmetrically on top of a decapitated coleoptile, these blocks could be tested for their ability to cause bending in the absence of a unilateral light source (see Figure 19.1). Because the substance promoted the elongation of the coleoptile sections (Figure 19.2), it was eventually named **auxin** from the Greek *auxein*, meaning "to increase" or "to grow."

BIOSYNTHESIS AND METABOLISM OF AUXIN

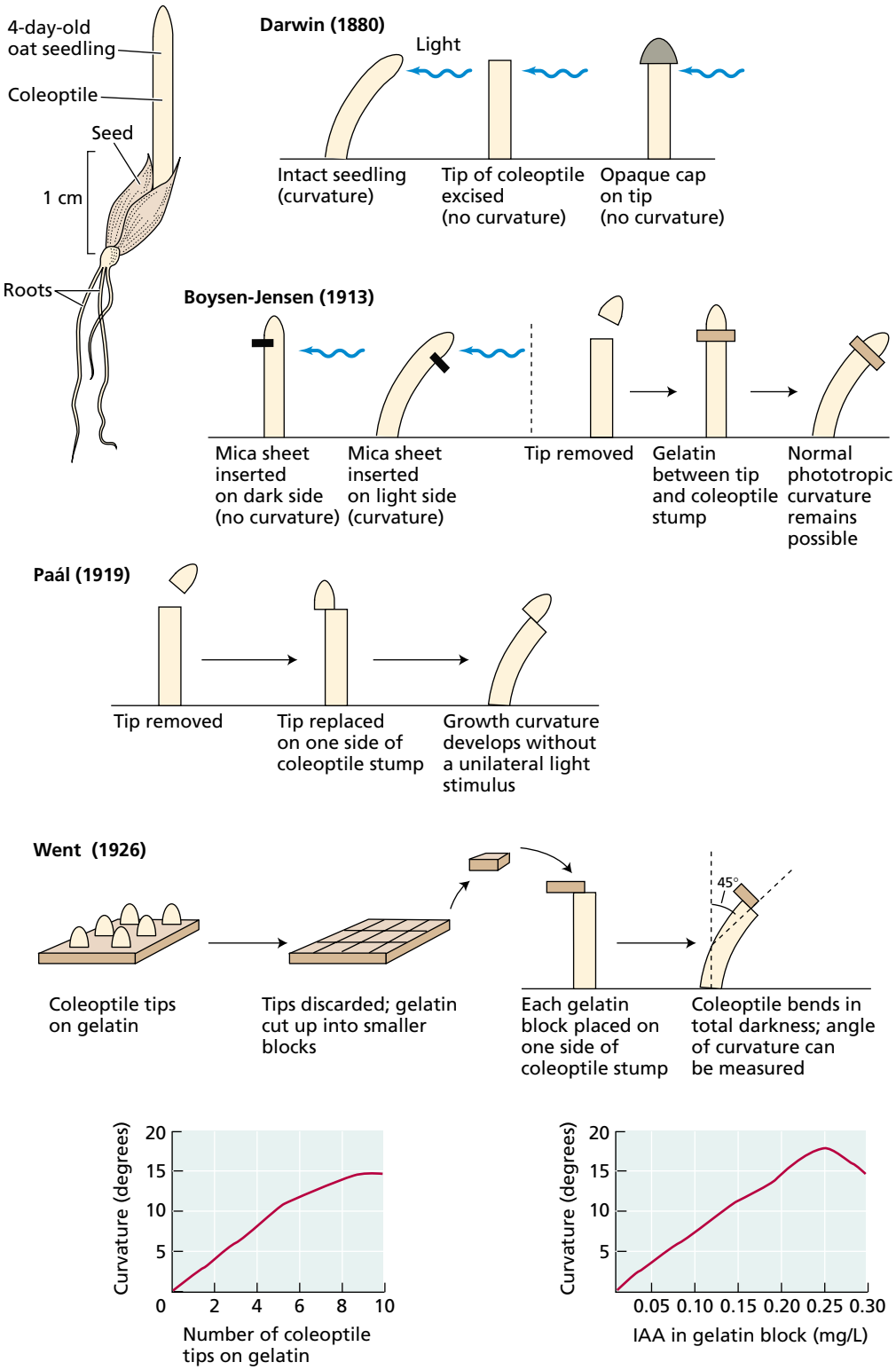
Went's studies with agar blocks demonstrated unequivocally that the growth-promoting "influence" diffusing from the coleoptile tip was a chemical substance. The fact that it was produced at one location and transported in minute amounts to its site of action qualified it as an authentic plant hormone.

In the years that followed, the chemical identity of the "growth substance" was determined, and because of its potential agricultural uses, many related chemical analogs were tested. This testing led to generalizations about the chemical requirements for auxin activity. In parallel with these studies, the agar block diffusion technique was being applied to the problem of auxin transport. Technological advances, especially the use of isotopes as tracers, enabled plant biochemists to unravel the pathways of auxin biosynthesis and breakdown.

Our discussion begins with the chemical nature of auxin and continues with a description of its biosynthesis, transport, and metabolism. Increasingly powerful analytical methods and the application of molecular biological approaches have recently allowed scientists to identify auxin precursors and to study auxin turnover and distribution within the plant.

The Principal Auxin in Higher Plants Is Indole-3-Acetic Acid

In the mid-1930s it was determined that auxin is **indole-3-acetic acid** (**IAA**). Several other auxins in higher plants were discovered later (Figure 19.3), but IAA is by far the most abundant and physiologically relevant. Because the structure of IAA is relatively simple, academic and industrial laboratories were quickly able to synthesize a wide



From experiments on coleoptile phototropism, Darwin concluded in 1880 that a growth stimulus is produced in the coleoptile tip and is transmitted to the growth zone.

In 1913, P. Boysen-Jensen discovered that the growth stimulus passes through gelatin but not through water-impermeable barriers such as mica.

In 1919, A. Paál provided evidence that the growth-promoting stimulus produced in the tip was chemical in nature.

In 1926, F. W. Went showed that the active growth-promoting substance can diffuse into a gelatin block. He also devised a coleoptile-bending assay for quantitative auxin analysis.

FIGURE 19.1 Summary of early experiments in auxin research.

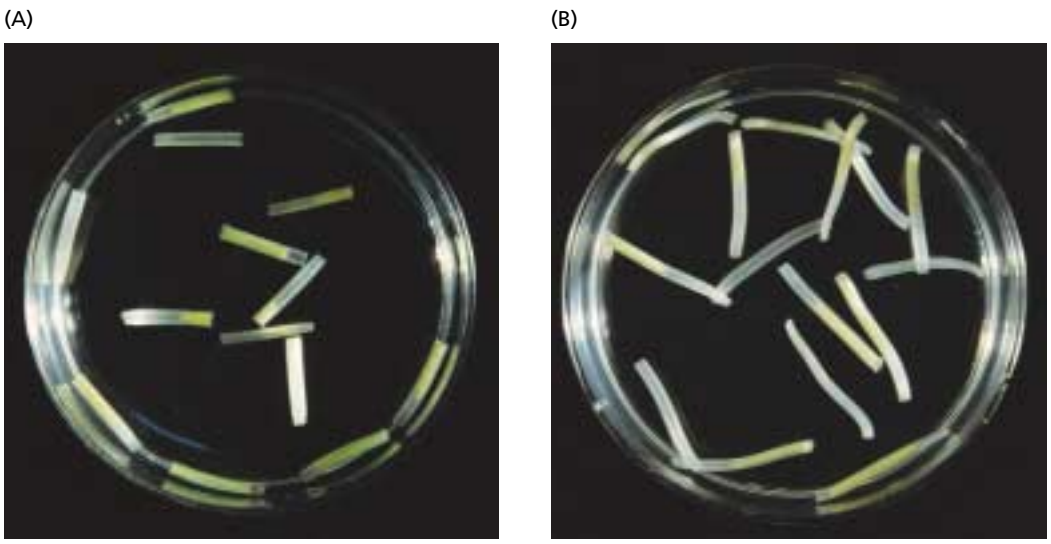


FIGURE 19.2 Auxin stimulates the elongation of oat coleoptile sections. These coleoptile sections were incubated for 18 hours in either water (A) or auxin (B). The yellow tissue inside the translucent coleoptile is the primary leaves. (Photos © M. B. Wilkins.)

array of molecules with auxin activity. Some of these are used as herbicides in horticulture and agriculture (Figure 19.4) (for additional synthetic auxins, see **Web Topic 19.1**).

An early definition of auxins included all natural and synthetic chemical substances that stimulate elongation in coleoptiles and stem sections. However, auxins affect many developmental processes besides cell elongation. Thus auxins can be defined as compounds with biological activities similar to those of IAA, including the ability to promote cell elongation in coleoptile and stem sections, cell division in callus cultures in the presence of cytokinins, formation of adventitious roots on detached leaves and stems, and other developmental phenomena associated with IAA action.

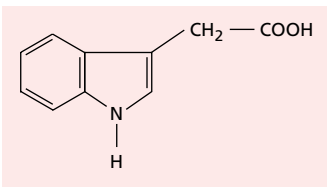
Although they are chemically diverse, a common feature of all active auxins is a molecular distance of about 0.5 nm between a fractional positive charge on the aromatic ring and a negatively charged carboxyl group (see **Web Topic 19.2**).

Auxins in Biological Samples Can Be Quantified

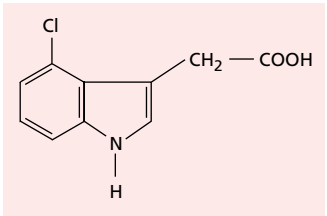
Depending on the information that a researcher needs, the amounts and/or identity of auxins in biological samples can be determined by bioassay, mass spectrometry, or enzyme-linked immunosorbent assay, which is abbreviated as ELISA (see **Web Topic 19.3**).

A **bioassay** is a measurement of the effect of a known or suspected biologically active substance on living material. In his pioneering work more than 60 years ago, Went used *Avena sativa* (oat) coleoptiles in a technique called the ***Avena* coleoptile curvature test** (see Figure 19.1). The coleoptile curved because the increase in auxin on one side stimulated cell elongation, and the decrease in auxin on the other side (due to the absence of the coleoptile tip) caused a decrease in the growth rate—a phenomenon called **differential growth**.

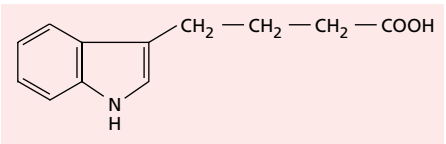
Went found that he could estimate the amount of auxin in a sample by measuring the resulting coleoptile curva-



Indole-3-acetic acid (IAA)

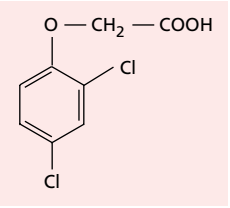


4-Chloroindole-3-acetic acid (4-Cl-IAA)

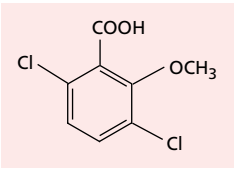


Indole-3-butyric acid (IBA)

FIGURE 19.3 Structure of three natural auxins. Indole-3-acetic acid (IAA) occurs in all plants, but other related compounds in plants have auxin activity. Peas, for example, contain 4-chloroindole-3-acetic acid. Mustards and corn contain indole-3-butyric acid (IBA).



2,4-Dichlorophenoxyacetic acid (2,4-D)



2-Methoxy-3, 6-dichlorobenzoic acid (dicamba)

FIGURE 19.4 Structures of two synthetic auxins. Most synthetic auxins are used as herbicides in horticulture and agriculture.

ture. Auxin bioassays are still used today to detect the presence of auxin activity in a sample. The *Avena* coleoptile curvature assay is a sensitive measure of auxin activity (it is effective for IAA concentrations of about 0.02 to 0.2 mg L^{-1}). Another bioassay measures auxin-induced changes in the straight growth of *Avena* coleoptiles floating in solution (see Figure 19.2). Both of these bioassays can establish the presence of an auxin in a sample, but they cannot be used for precise quantification or identification of the specific compound.

Mass spectrometry is the method of choice when information about both the chemical structure and the amount of IAA is needed. This method is used in conjunction with separation protocols involving gas chromatography. It allows the precise quantification and identification of auxins, and can detect as little as 10^{-12} g (1 picogram, or pg) of IAA, which is well within the range of auxin found in a single pea stem section or a corn kernel. These sophisticated techniques have enabled researchers to accurately analyze auxin precursors, auxin turnover, and auxin distribution within the plant.

IAA Is Synthesized in Meristems, Young Leaves, and Developing Fruits and Seeds

IAA biosynthesis is associated with rapidly dividing and rapidly growing tissues, especially in shoots. Although virtually all plant tissues appear to be capable of producing low levels of IAA, shoot apical meristems, young leaves, and developing fruits and seeds are the primary sites of IAA synthesis (Ljung et al. in press).

In very young leaf primordia of *Arabidopsis*, auxin is synthesized at the tip. During leaf development there is a gradual shift in the site of auxin production basipetally along the margins, and later, in the central region of the lamina. The basipetal shift in auxin production correlates closely with, and is probably causally related to, the basipetal maturation sequence of leaf development and vascular differentiation (Aloni 2001).

By fusing the *GUS* (β-glucuronidase) reporter gene to a promoter containing an auxin response element, and transforming *Arabidopsis* leaves with this construct in a Ti plasmid using *Agrobacterium*, it is possible to visualize the distribution of free auxin in young, developing leaves. Wherever free auxin is produced, *GUS* expression occurs—and can be detected histochemically. By use of this technique, it has recently been demonstrated that auxin is produced by a cluster of cells located at sites where hydathodes will develop (Figure 19.5).

Hydathodes are glandlike modifications of the ground and vascular tissues, typically at the margins of leaves, that allow the release of liquid water (guttation fluid) through pores in the epidermis in the presence of root pressure (see Chapter 4). As shown in Figure 19.5, during early stages of hydathode differentiation a center of high auxin synthesis is evident as a concentrated dark blue GUS stain (arrow) in the lobes of serrated leaves of *Arabidopsis* (Aloni et al. 2002). A diffuse trail of GUS activity leads down to differentiating vessel elements in a developing vascular strand. This remarkable micrograph captures the process of auxin-regulated vascular differentiation in the very act!

We will return to the topic of the control of vascular differentiation later in the chapter.

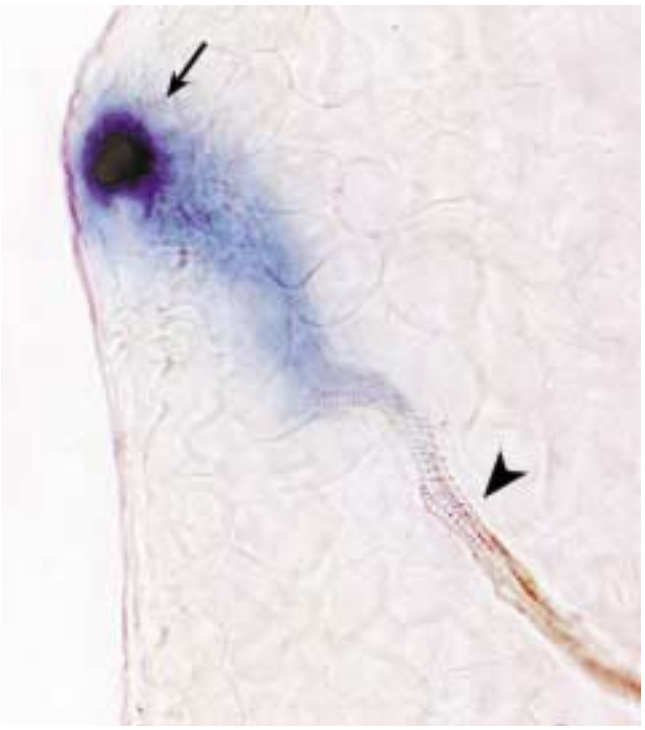


FIGURE 19.5 Detection of sites of auxin synthesis and transport in a young leaf primordium of *DR5 Arabidopsis* by means of a *GUS* reporter gene with an auxin-sensitive promoter. During the early stages of hydathode differentiation, a center of auxin synthesis is evident as a concentrated dark blue *GUS* stain (arrow) in the lobes of the serrated leaf margin. A gradient of diluted GUS activity extends from the margin toward a differentiating vascular strand (arrowhead), which functions as a sink for the auxin flow originating in the lobe. (Courtesy of R. Aloni and C. I. Ullrich.)

Multiple Pathways Exist for the Biosynthesis of IAA

IAA is structurally related to the amino acid tryptophan, and early studies on auxin biosynthesis focused on tryptophan as the probable precursor. However, the incorporation of exogenous labeled tryptophan (e.g., [^{3}H]tryptophan) into IAA by plant tissues has proved difficult to demonstrate. Nevertheless, an enormous body of evidence has now accumulated showing that plants convert tryptophan to IAA by several pathways, which are described in the paragraphs that follow.

The IPA pathway. The **indole-3-pyruvic acid** (**IPA**) pathway (see Figure 19.6C), is probably the most common of the tryptophan-dependent pathways. It involves a deamination reaction to form IPA, followed by a decarboxylation reaction to form indole-3-acetaldehyde (IAld). Indole-3-acetaldehyde is then oxidized to IAA by a specific dehydrogenase.

The TAM pathway. The **tryptamine** (**TAM**) **pathway** (see Figure 19.6D) is similar to the IPA pathway, except that the order of the deamination and decarboxylation reactions is reversed, and different enzymes are involved. Species that do not utilize the IPA pathway possess the TAM pathway. In at least one case (tomato), there is evidence for both the IPA and the TAM pathways (Nonhebel et al. 1993).

The IAN pathway. In the **indole-3-acetonitrile** (**IAN**) pathway (see Figure 19.6B), tryptophan is first converted to indole-3-acetaldoxime and then to indole-3-acetonitrile. The enzyme that converts IAN to IAA is called *nitrilase*. The IAN pathway may be important in only three plant families: the Brassicaceae (mustard family), Poaceae (grass

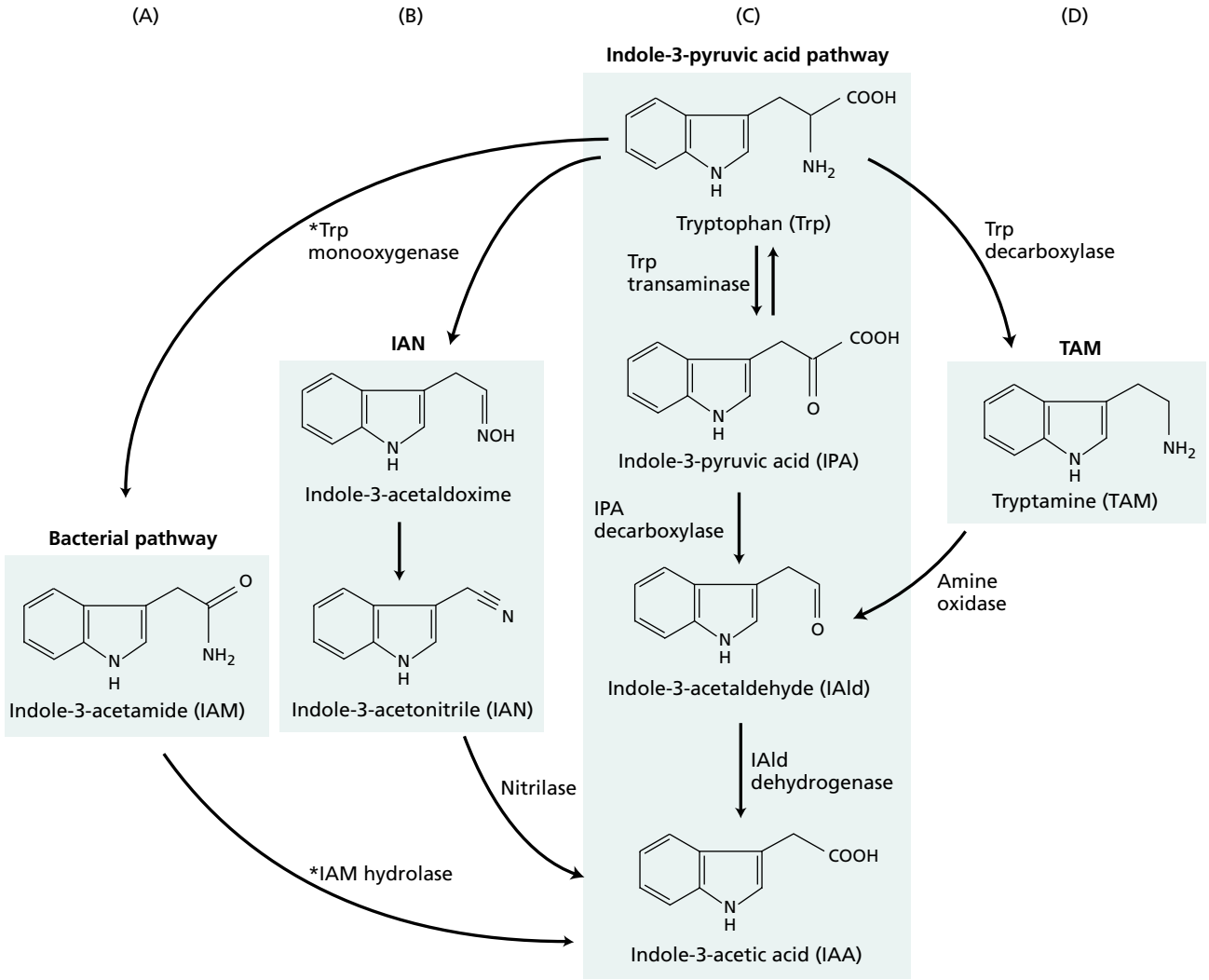


FIGURE 19.6 Tryptophan-dependent pathways of IAA biosynthesis in plants and bacteria. The enzymes that are present only in bacteria are marked with an asterisk. (After Bartel 1997.)

family), and Musaceae (banana family). Nevertheless, nitrilase-like genes or activities have recently been identified in the Cucurbitaceae (squash family), Solanaceae (tobacco family), Fabaceae (legumes), and Rosaceae (rose family).

Four genes (*NIT1* through *NIT4*) that encode nitrilase enzymes have now been cloned from *Arabidopsis*. When *NIT2* was expressed in transgenic tobacco, the resultant plants acquired the ability to respond to IAN as an auxin by hydrolyzing it to IAA (Schmidt et al. 1996).

Another tryptophan-dependent biosynthetic pathway—one that uses **indole-3-acetamide** (**IAM**) as an intermediate (see Figure19.6A)—is used by various pathogenic bacteria, such as *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*. This pathway involves the two enzymes tryptophan monooxygenase and IAM hydrolase. The auxins produced by these bacteria often elicit morphological changes in their plant hosts.

In addition to the tryptophan-dependent pathways, recent genetic studies have provided evidence that plants can synthesize IAA via one or more tryptophan-independent pathways. The existence of multiple pathways for IAA biosynthesis makes it nearly impossible for plants to run out of auxin and is probably a reflection of the essential role of this hormone in plant development.

IAA Is Also Synthesized from Indole or from Indole-3-Glycerol Phosphate

Although a tryptophan-independent pathway of IAA biosynthesis had long been suspected because of the low levels of conversion of radiolabeled tryptophan to IAA, not until genetic approaches were available could the existence of such pathways be confirmed and defined. Perhaps the most striking of these studies in maize involves the *orange pericarp* (*orp*) mutant (Figure 19.7), in which both subunits of the enzyme tryptophan synthase are inactive (Figure 19.8). The *orp* mutant is a true tryptophan auxotroph, requiring exogenous tryptophan to survive. However, neither the *orp* seedlings nor the wild-type seedlings can convert tryptophan to IAA, even when the mutant seedlings are given enough tryptophan to reverse the lethal effects of the mutation.

FIGURE 19.7 The orange pericarp (*orp*) mutant of maize is missing both subunits of tryptophan synthase. As a result, the pericarps surrounding each kernel accumulate glycosides of anthranilic acid and indole. The orange color is due to excess indole. (Courtesy of Jerry D. Cohen.)

Despite the block in tryptophan biosynthesis, the *orp* mutant contains amounts of IAA 50-fold higher than those of a wild-type plant (Wright et al. 1991). Signficantly, when *orp* seedlings were fed [^{15}N]anthranilate (see Figure 19.8), the label subsequently appeared in IAA, but not in tryptophan. These results provided the best experimental evidence for a tryptophan-independent pathway of IAA biosynthesis.

Further studies established that the branch point for IAA biosynthesis is either indole or its precursor, indole-3-glycerol phosphate (see Figure 19.8). IAN and IPA are possible intermediates, but the immediate precursor of IAA in the tryptophan-independent pathway has not yet been identified.

The discovery of the tryptophan-independent pathway has drastically altered our view of IAA biosynthesis, but the relative importance of the two pathways (tryptophan-dependent versus tryptophan-independent) is poorly understood. In several plants it has been found that the type of IAA biosynthesis pathway varies between different tissues, and between different times of development. For example, during embryogenesis in carrot, the tryptophan-dependent pathway is important very early in development, whereas the tryptophan-independent pathway takes over soon after the root–shoot axis is established. (For more evidence of the tryptophan-independent biosynthesis of IAA, see **Web Topic 19.4**.)

Most IAA in the Plant Is in a Covalently Bound Form

Although free IAA is the biologically active form of the hormone, the vast majority of auxin in plants is found in a covalently bound state. These conjugated, or "bound," auxins have been identified in all higher plants and are considered hormonally inactive.

IAA has been found to be conjugated to both high- and low-molecular-weight compounds.

- Low-molecular-weight conjugated auxins include esters of IAA with glucose or *myo*-inositol and amide conjugates such as IAA-*N*-aspartate (Figure 19.9).
- High-molecular-weight IAA conjugates include IAA-glucan (7–50 glucose units per IAA) and IAA-glycoproteins found in cereal seeds.

The compound to which IAA is conjugated and the extent of the conjugation depend on the specific conjugating enzymes. The best-studied reaction is the conjugation of IAA to glucose in *Zea mays*.

The highest concentrations of free auxin in the living plant are in the apical meristems of shoots and in young leaves because these are the primary sites of auxin synthe-

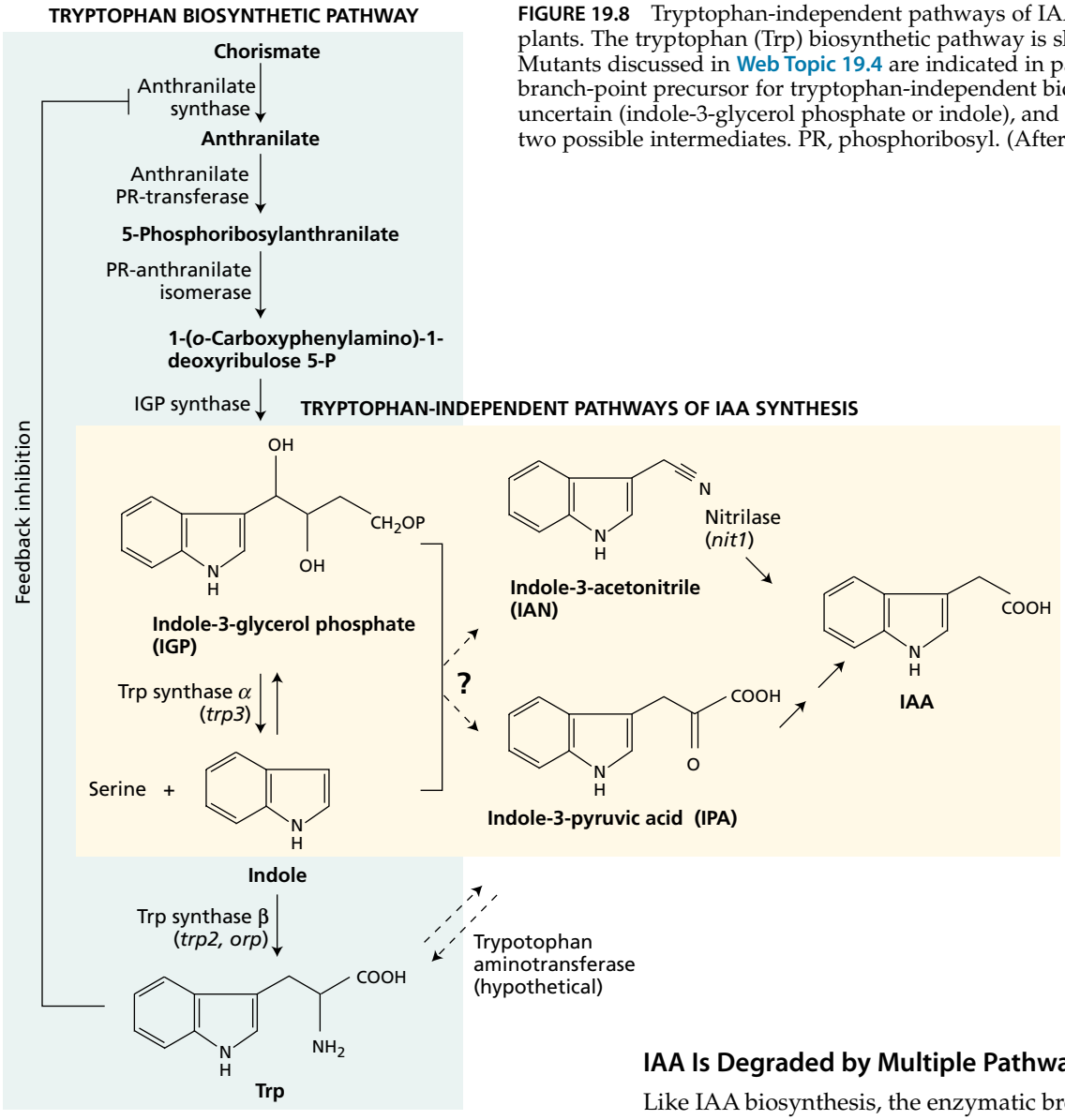


FIGURE 19.8 Tryptophan-independent pathways of IAA biosynthesis in plants. The tryptophan (Trp) biosynthetic pathway is shown on the left. Mutants discussed in **Web Topic 19.4** are indicated in parentheses. The branch-point precursor for tryptophan-independent biosynthesis is uncertain (indole-3-glycerol phosphate or indole), and IAN and IPA are two possible intermediates. PR, phosphoribosyl. (After Bartel 1997.)

sis. However, auxins are widely distributed in the plant. Metabolism of conjugated auxin may be a major contributing factor in the regulation of the levels of free auxin. For example, during the germination of seeds of *Zea mays*, IAA-*myo*-inositol is translocated from the endosperm to the coleoptile via the phloem. At least a portion of the free IAA produced in coleoptile tips of *Zea mays* is believed to be derived from the hydrolysis of IAA-*myo*-inositol.

In addition, environmental stimuli such as light and gravity have been shown to influence both the rate of auxin conjugation (removal of free auxin) and the rate of release of free auxin (hydrolysis of conjugated auxin). The formation of conjugated auxins may serve other functions as well, including storage and protection against oxidative degradation.

IAA Is Degraded by Multiple Pathways

Like IAA biosynthesis, the enzymatic breakdown (oxidation) of IAA may involve more than one pathway. For some time it has been thought that peroxidative enzymes are chiefly responsible for IAA oxidation, primarily because these enzymes are ubiquitous in higher plants and their ability to degrade IAA can be demonstrated in vitro (Figure 19.10A). However, the physiological significance of the peroxidase pathway is unclear. For example, no change in the IAA levels of transgenic plants was observed with either a tenfold increase in peroxidase expression or a tenfold repression of peroxidase activity (Normanly et al. 1995).

On the basis of isotopic labeling and metabolite identification, two other oxidative pathways are more likely to be involved in the controlled degradation of IAA (see Figure 19.10B). The end product of this pathway is oxindole-3-acetic acid (OxIAA), a naturally occurring compound in the endosperm and shoot tissues of *Zea mays*. In one pathway, IAA is oxidized without decarboxylation to OxIAA.

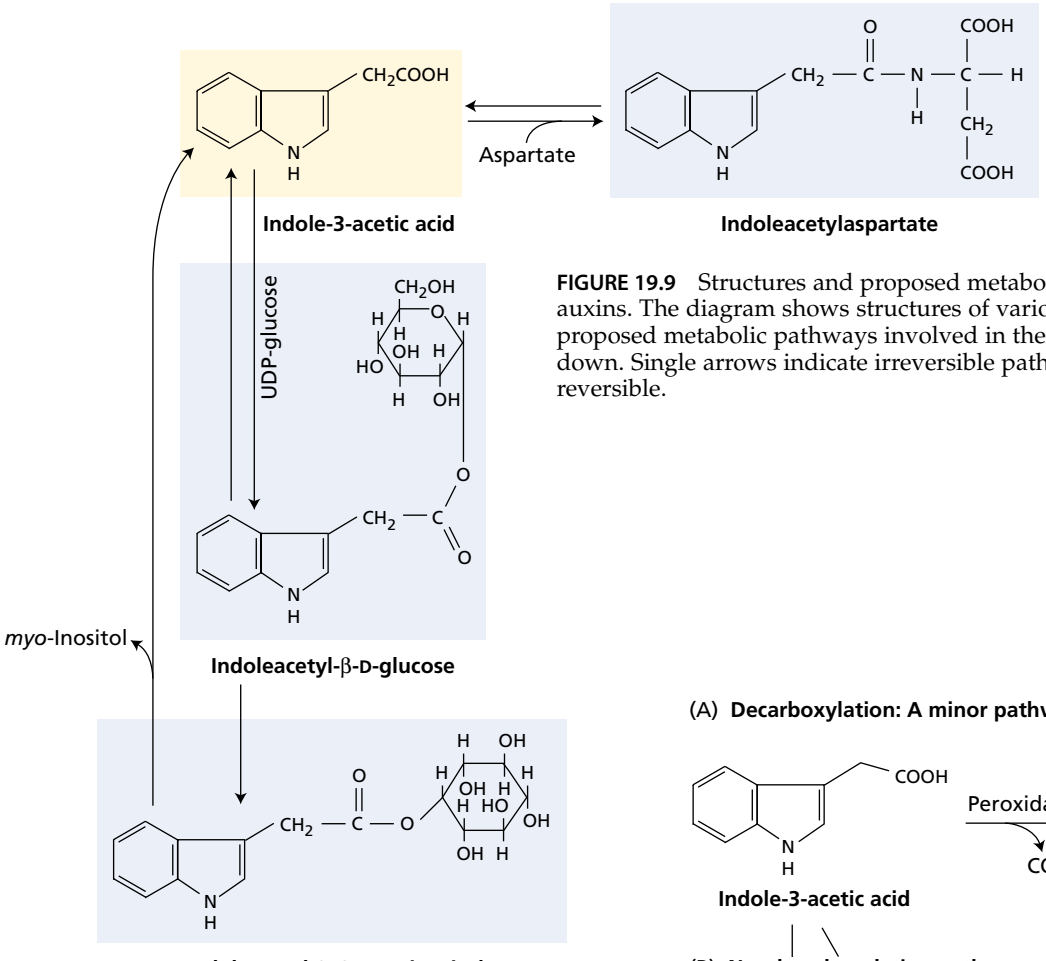


FIGURE 19.9 Structures and proposed metabolic pathways of bound auxins. The diagram shows structures of various IAA conjugates and proposed metabolic pathways involved in their synthesis and breakdown. Single arrows indicate irreversible pathways; double arrows, reversible.

In another pathway, the IAA-aspartate conjugate is oxidized first to the intermediate dioxindole-3-acetylaspartate, and then to OxIAA.

In vitro, IAA can be oxidized nonenzymatically when exposed to high-intensity light, and its photodestruction in vitro can be promoted by plant pigments such as riboflavin. Although the products of auxin photooxidation have been isolated from plants, the role, if any, of the photooxidation pathway in vivo is presumed to be minor.

Two Subcellular Pools of IAA Exist: The Cytosol and the Chloroplasts

The distribution of IAA in the cell appears to be regulated largely by pH. Because IAA^- does not cross membranes unaided, whereas IAAH readily diffuses across membranes,

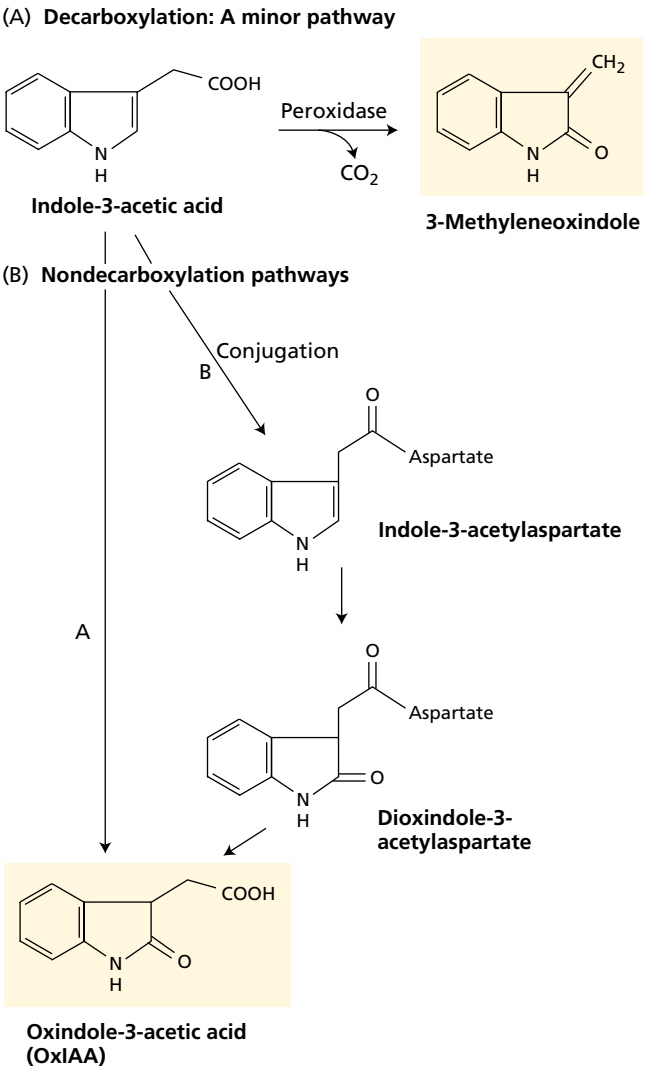


FIGURE 19.10 Biodegradation of IAA. (A) The peroxidase route (decarboxylation pathway) plays a relatively minor role. (B) The two nondecarboxylation routes of IAA oxidative degradation, A and B, are the most common metabolic pathways.

auxin tends to accumulate in the more alkaline compartments of the cell.

The distribution of IAA and its metabolites has been studied in tobacco cells. About one-third of the IAA is found in the chloroplast, and the remainder is located in the cytosol. IAA conjugates are located exclusively in the cytosol. IAA in the cytosol is metabolized either by conjugation or by nondecarboxylative catabolism (see Figure 19.10). The IAA in the chloroplast is protected from these processes, but it is regulated by the amount of IAA in the cytosol, with which it is in equilibrium (Sitbon et al. 1993).

The factors that regulate the steady-state concentration of free auxin in plant cells are diagrammatically summarized in **Web Topic 19.5**.

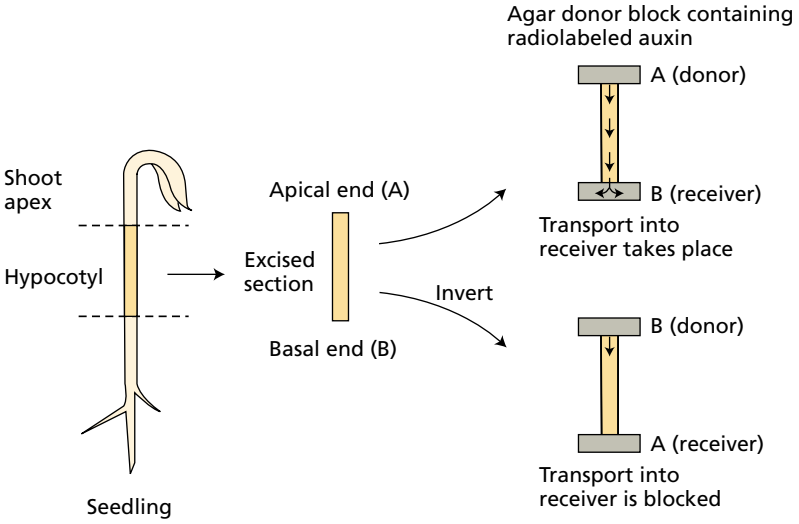


FIGURE 19.11 The standard method for measuring polar auxin transport. The polarity of transport is independent of orientation with respect to gravity.

AUXIN TRANSPORT

The main axes of shoots and roots, along with their branches, exhibit apex–base structural polarity, and this structural polarity has its origin in the polarity of auxin transport. Soon after Went developed the coleoptile curvature test for auxin, it was discovered that IAA moves mainly from the apical to the basal end (*basipetally*) in excised oat coleoptile sections. This type of unidirectional transport is termed **polar transport**. Auxin is the only plant growth hormone known to be transported polarly.

Because the shoot apex serves as the primary source of auxin for the entire plant, polar transport has long been believed to be the principal cause of an auxin gradient extending from the shoot tip to the root tip. The longitudinal gradient of auxin from the shoot to the root affects various developmental processes, including stem elongation, apical dominance, wound healing, and leaf senescence.

Recently it has been recognized that a significant amount of auxin transport also occurs in the phloem, and that the phloem is probably the principal route by which auxin is transported *acropetally* (i.e., toward the tip) in the root. Thus, more than one pathway is responsible for the distribution of auxin in the plant

Polar Transport Requires Energy and Is Gravity Independent

To study polar transport, researchers have employed the *donor–receiver agar block method* (Figure 19.11): An agar block containing radioisotope-labeled auxin (donor block) is placed on one end of a tissue segment, and a receiver block is placed on the other end. The movement of auxin through the tissue into the receiver block can be determined over time by measurement of the radioactivity in the receiver block.

From a multitude of such studies, the general properties of polar IAA transport have emerged. Tissues differ in the degree of polarity of IAA transport. In coleoptiles, vegetative stems, and leaf petioles, basipetal transport predominates. Polar transport is not affected by the orientation of the tissue (at least over short periods of time), so it is independent of gravity.

A simple demonstration of the lack of effect of gravity on polar transport is shown in Figure 19.12. When stem cuttings (in this case bamboo) are placed in a moist chamber, adventitious roots always form at the basal end of the cuttings, even when the cuttings are inverted. Because root differentiation is stimulated by an increase in auxin concentration, auxin must be transported basipetally in the stem even when the cutting is oriented upside down.

Polar transport proceeds in a cell-to-cell fashion, rather than via the symplast. That is, auxin exits the cell through the plasma membrane, diffuses across the compound middle lamella, and enters the cell below through its plasma membrane. The loss of auxin from cells is termed *auxin efflux*; the entry of auxin into cells is called *auxin uptake* or *influx*. The overall process requires metabolic energy, as evidenced by the sensitivity of polar transport to O_2 deprivation and metabolic inhibitors.

The velocity of polar auxin transport is 5 to 20 cm h^{-1}—faster than the rate of diffusion (see **Web Topic 3.2**), but slower than phloem translocation rates (see Chapter 10). Polar transport is also specific for active auxins, both natural and synthetic. Neither inactive auxin analogs nor auxin metabolites are transported polarly, suggesting that polar transport involves specific protein carriers on the plasma membrane that can recognize the hormone and its active analogs.

The major site of basipetal polar auxin transport in stems and leaves is the vascular parenchyma tissue. Coleoptiles appear to be the exception in that basipetal polar transport

FIGURE 19.12 Roots grow from the basal ends of these bamboo sections, even when they are inverted. The roots form at the basal end because polar auxin transport in the shoot is independent of gravity. (Photo ©M. B. Wilkins.)

occurs mainly in the nonvascular tissues. Acropetal polar transport in the root is specifically associated with the xylem parenchyma of the stele (Palme and Gälweiler 1999). However, as we shall see later in the chapter, most of the auxin that reaches the root tip is translocated via the phloem.

A small amount of basipetal auxin transport from the root tip has also been demonstrated. In maize roots, for example, radiolabeled IAA applied to the root tip is transported basipetally about 2 to 8 mm (Young and Evans 1996). Basipetal auxin transport in the root occurs in the epidermal and cortical tissues, and as we shall see, it plays a central role in gravitropism.

A Chemiosmotic Model Has Been Proposed to Explain Polar Transport

The discovery of the chemiosmotic mechanism of solute transport in the late 1960s (see Chapter 6) led to the application of this model to polar auxin transport. According to the now generally accepted **chemiosmotic model** for polar auxin transport, auxin uptake is driven by the proton motive force (ΔE + ΔpH) across the plasma membrane, while auxin efflux is driven by the membrane potential, ΔE. (Proton motive force is described in more detail in **Web Topic 6.3** and Chapter 7.)

A crucial feature of the polar transport model is that the auxin efflux carriers are localized at the basal ends of the conducting cells (Figure 19.13). The evidence for each step in this model is considered separately in the discussion that follows.

Auxin influx. The first step in polar transport is auxin influx. According to the model, auxin can enter plant cells from any direction by either of two mechanisms:

1. Passive diffusion of the protonated (IAAH) form across the phospholipid bilayer
2. Secondary active transport of the dissociated (IAA^-) form via a $2H^+$–IAA^- symporter

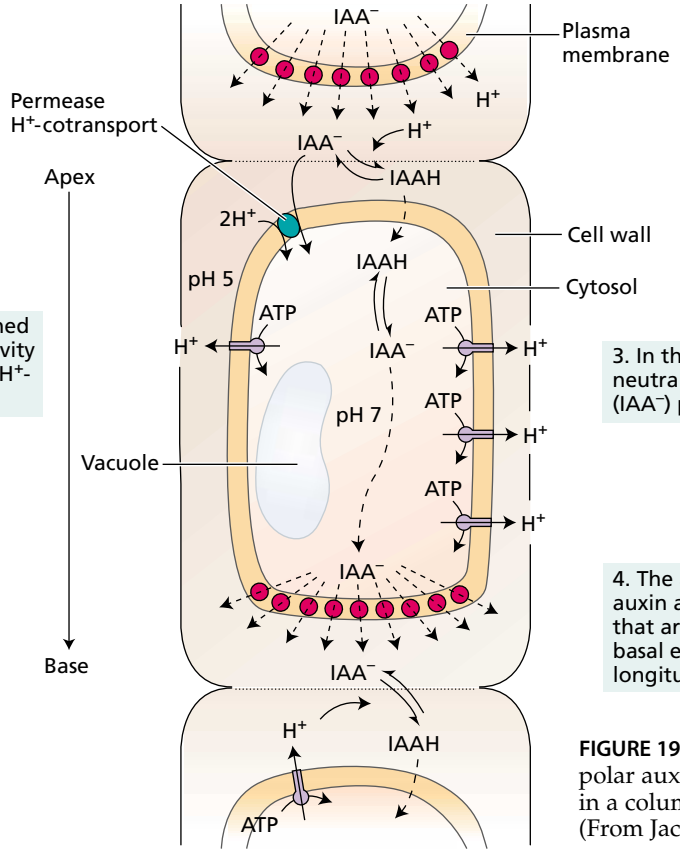
The dual pathway of auxin uptake arises because the passive permeability of the membrane to auxin depends strongly on the apoplastic pH.

The undissociated form of indole-3-acetic acid, in which the carboxyl group is protonated, is lipophilic and readily diffuses across lipid bilayer membranes. In contrast, the dissociated form of auxin is negatively charged and therefore does not cross membranes unaided. Because the plasma membrane H^+-ATPase normally maintains the cell wall solution at about pH 5, about half of the auxin (pK_a = 4.75) in the apoplast will be in the undissociated form and will diffuse passively across the plasma membrane down a concentration gradient. Experimental support for pH-dependent, passive auxin uptake was first provided by the demonstration that IAA uptake by plant cells increases as the extracellular pH is lowered from a neutral to a more acidic value.

A carrier-mediated, secondary active uptake mechanism was shown to be saturable and specific for active auxins (Lomax 1986). In experiments in which the ΔpH and ΔE values of isolated membrane vesicles from zucchini (*Cucurbita pepo*) hypocotyls were manipulated artificially, the uptake of radiolabeled auxin was shown to be stimulated in the presence of a pH gradient, as in passive uptake, but also when the inside of the vesicle was negatively charged relative to the outside.

These and other experiments suggested that an H^+–IAA^- symporter cotransports two protons along with the auxin anion. This secondary active transport of auxin allows for greater auxin accumulation than simple diffusion does because it is driven across the membrane by the proton motive force.

A permease-type auxin uptake carrier, AUX1, related to bacterial amino acid carriers, has been identified in *Arabidopsis* roots (Bennett et al. 1996). The roots of *aux1* mutants are agravitropic, suggesting that auxin influx is a limiting factor for gravitropism in roots. As predicted by the chemiosmotic model, AUX1 appears to be uniformly distributed around cells in the polar transport pathway (Marchant et al. 1999). Thus in general, the polarity of auxin transport is governed by the efflux step rather than the influx step.



1. IAA enters the cell either passively in the undissociated form (IAAH) or by secondary active cotransport in the anionic form (IAA^-).

2. The cell wall is maintained at an acidic pH by the activity of the plasma membrane H^+-ATPase.

3. In the cytosol, which has a neutral pH, the anionic form (IAA^-) predominates.

4. The anions exit the cell via auxin anion efflux carriers that are concentrated at the basal ends of each cell in the longitudinal pathway.

FIGURE 19.13 The chemiosmotic model for polar auxin transport. Shown here is one cell in a column of auxin-transporting cells. (From Jacobs and Gilbert 1983.)

Auxin efflux. Once IAA enters the cytosol, which has a pH of approximately 7.2, nearly all of it will dissociate to the anionic form. Because the membrane is less permeable to IAA^- than to IAAH, IAA^- will tend to accumulate in the cytosol. However, much of the auxin that enters the cell escapes via an *auxin anion efflux carrier*. According to the chemiosmotic model, transport of IAA^- out of the cell is driven by the inside negative membrane potential.

As noted earlier, the central feature of the chemiosmotic model for polar transport is that IAA^- efflux takes place preferentially at the basal end of each cell. The repetition of auxin uptake at the apical end of the cell and preferential release from the base of each cell in the pathway gives rise to the total polar transport effect. A family of putative auxin efflux carriers known as **PIN proteins** (named after the pin-shaped inflorescences formed by the *pin1* mutant of *Arabidopsis*; Figure 19.14A) are localized precisely as the model would predict—that is, at the basal ends of the conducting cells (see Figure 19.14B).

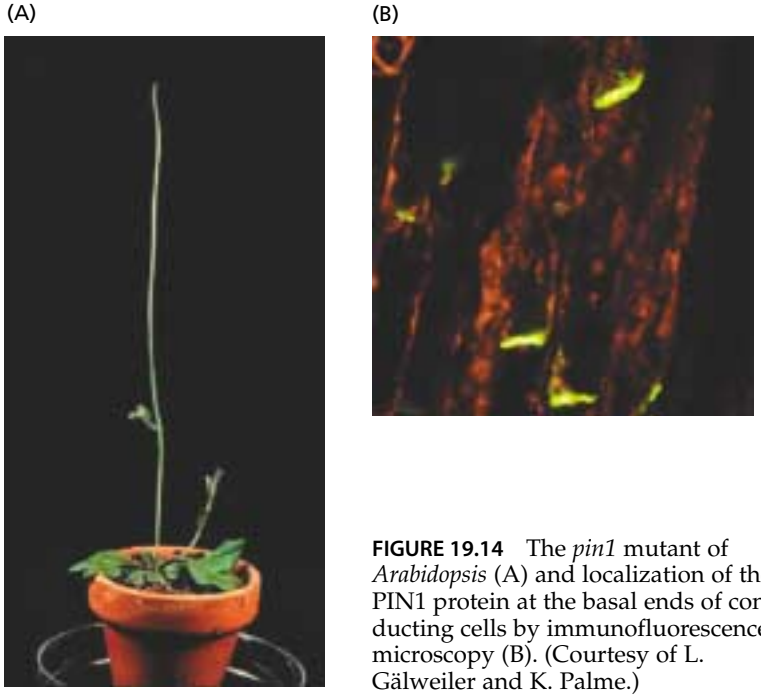


FIGURE 19.14 The *pin1* mutant of *Arabidopsis* (A) and localization of the PIN1 protein at the basal ends of conducting cells by immunofluorescence microscopy (B). (Courtesy of L. Gälweiler and K. Palme.)

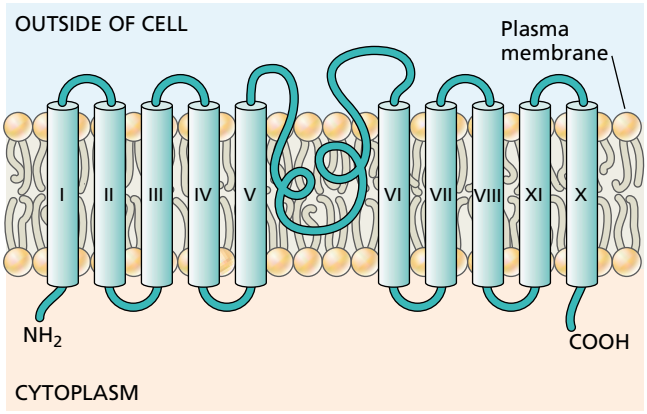


FIGURE 19.15 The topology of the PIN1 protein with ten transmembrane segments and a large hydrophilic loop in the middle. (After Palme and Gälweiler 1999.)

PIN proteins have 10 to 12 transmembrane regions characteristic of a major superfamily of bacterial and eukaryotic transporters, which include drug resistance proteins and sugar transporters (Figure 19.15). Despite topological similarities to other transporters, recent studies suggest that PIN may require other proteins for activity, and may be part of a larger protein complex.

Inhibitors of Auxin Transport Block Auxin Efflux

Several compounds have been synthesized that can act as **auxin transport inhibitors** (**ATIs**), including NPA (1-*N*-naphthylphthalamic acid) and TIBA (2,3,5-triiodobenzoic acid) (Figure 19.16). These inhibitors block polar transport by preventing auxin efflux. We can demonstrate this phenomenon by incorporating NPA or TIBA into either the donor or the receiver block in an auxin transport experiment. Both compounds inhibit auxin efflux into the receiver block, but they do not affect auxin uptake from the donor block.

Some ATIs, such as TIBA, that have weak auxin activity and are transported polarly, may inhibit polar transport in part by competing with auxin for its binding site on the efflux carrier. Others, such as NPA, are not transported polarly and are believed to interfere with auxin transport by binding to proteins associated in a complex with the efflux carrier. Such NPA-binding proteins are also found at the basal ends of the conducting cells, consistent with the localization of PIN proteins (Jacobs and Gilbert 1983).

Recently another class of ATIs has been identified that inhibits the AUX1 uptake carrier (Parry et al. 2001). For example, 1-naphthoxyacetic acid (1-NOA) (see Figure 19.16) blocks auxin uptake into cells, and when applied to *Arabidopsis* plants it causes root agravitropism similar to that of the *aux1* mutant. Like the *aux1* mutation, neither 1-NOA nor any of the other AUX1-specific inhibitors block polar auxin transport.

PIN Proteins Are Rapidly Cycled to and from the Plasma Membrane

The basal localization of the auxin efflux carriers involves targeted vesicle secretion to the basal ends of the conducting cells. Recently it has been demonstrated that PIN proteins, although stable, do not remain on the plasma membrane permanently, but are rapidly cycled to an unidentified endosomal compartment via endocytotic vesicles, and then recycled back to the plasma membrane (Geldner et al. 2001).

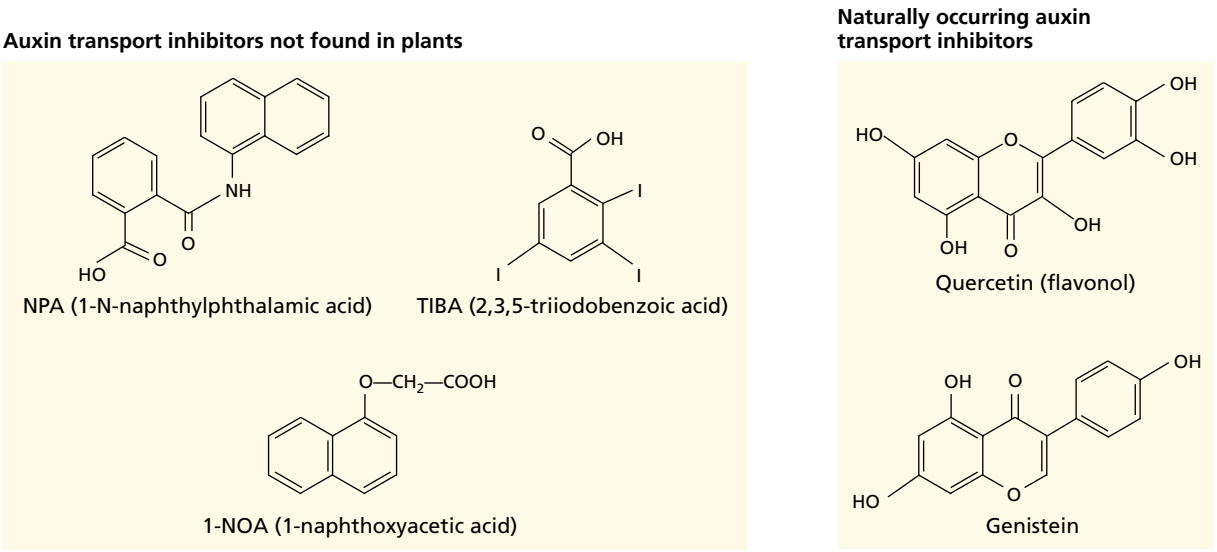


FIGURE 19.16 Structures of auxin transport inhibitors.

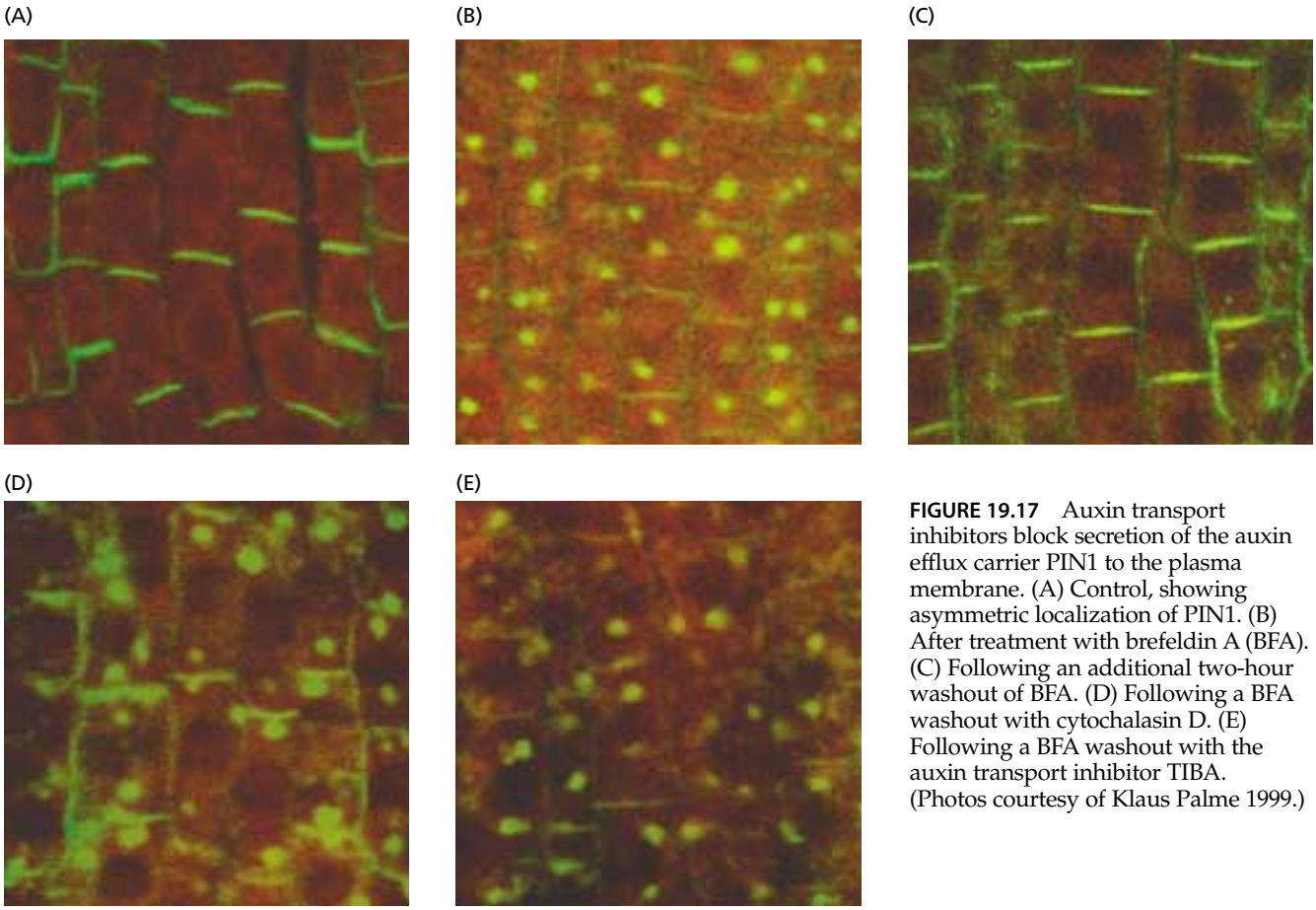


FIGURE 19.17 Auxin transport inhibitors block secretion of the auxin efflux carrier PIN1 to the plasma membrane. (A) Control, showing asymmetric localization of PIN1. (B) After treatment with brefeldin A (BFA). (C) Following an additional two-hour washout of BFA. (D) Following a BFA washout with cytochalasin D. (E) Following a BFA washout with the auxin transport inhibitor TIBA. (Photos courtesy of Klaus Palme 1999.)

Prior to treatment, the PIN1 protein is localized at the basal ends (top) of root cortical parenchyma cells (Figure 19.17A). Treatment of *Arabidopsis* seedlings with brefeldin A (BFA), which causes Golgi vesicles and other endosomal compartments to aggregate near the nucleus, causes PIN to accumulate in these abnormal intracellular compartments (see Figure 19.17B). When the BFA is washed out with buffer, the normal localization on the plasma membrane at the base of the cell is restored (see Figure 19.17C). But when cytochalasin D, an inhibitor of actin polymerization, is included in the buffer washout solution, normal relocalization of PIN to the plasma membrane is prevented (see Figure 19.17D). These results indicate that PIN is rapidly cycled between the plasma membrane at the base of the cell and an unidentified endosomal compartment by an actin-dependent mechanism.

Although they bind different targets, both TIBA and NPA interfere with vesicle traffic to and from the plasma membrane. The best way to demonstrate this phenomenon is to include TIBA in the washout solution after BFA treatment. Under these conditions, TIBA prevents the normal relocalization of PIN on the plasma membrane following the washout treatment (see Figure 19.17E) (Geldner et al. 2001).

The effects of TIBA and NPA on cycling are not specific for PIN proteins, and it has been proposed that ATIs may actually represent general inhibitors of membrane cycling (Geldner et al. 2001). On the other hand, neither TIBA nor NPA alone causes PIN delocalization, even though they block auxin efflux. Therefore, TIBA and NPA must also be able to directly inhibit the transport activity of PIN complexes on the plasma membrane—by binding either to PIN (as TIBA does) or to one or more regulatory proteins (as NPA does).

A simplified model of the effects of TIBA and NPA on PIN cycling and auxin efflux is shown in Figure 19.18. A more complete model that incorporates many of the recent findings is presented in **Web Essay 19.2**.

Flavonoids Serve as Endogenous ATIs

There is mounting evidence that flavonoids (see Chapter 13) can function as endogenous regulators of polar auxin transport. Indeed, naturally occurring aglycone flavonoid compounds (flavonoids without attached sugars) are able to compete with NPA for its binding site on membranes (Jacobs and Rubery 1988) and are typically localized on the plasma membrane at the basal ends of cells where the

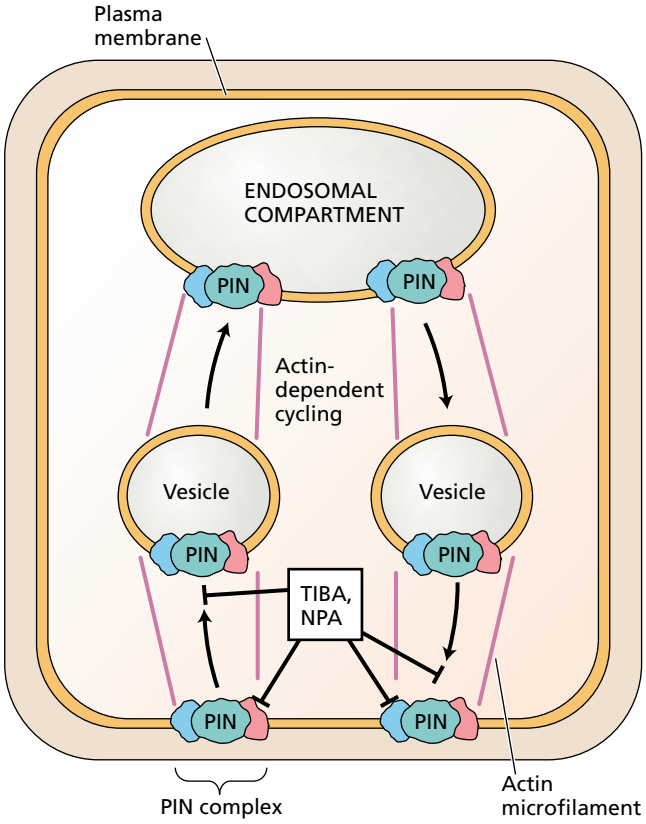


FIGURE 19.18 Actin-dependent PIN cycling between the plasma membrane and an endosomal compartment. Auxin transport inhibitors TIBA and NPA both interfere with relocalization of PIN1 proteins to basal plasma membranes after BFA washout (see Figure 19.17). This suggests that both of these auxin transport inhibitors interfere with PIN1 cycling.

efflux carrier is concentrated (Peer et al. 2001). In addition, recent studies have shown that the cells of flavonoid-deficient *Arabidopsis* mutants are less able to accumulate auxin than wild-type cells, and the mutant seedlings that lack flavonoid have altered auxin distribution profiles (Murphy et al. 1999; Brown et al. 2001).

Many of the flavonoids that displace NPA from its binding site on membranes are also inhibitors of protein kinases and protein phosphatases (Bernasconi 1996). An *Arabidopsis* mutant designated *rcn1* (*r*oots *c*url in *N*PA *1*) was identified on the basis of an enhanced sensitivity to NPA. The *RCN1* gene is closely related to the regulatory subunit of protein phosphatase 2A, a serine/threonine phosphatase (Garbers et al. 1996).

Protein phosphatases are known to play important roles in enzyme regulation, gene expression, and signal transduction by removing regulatory phosphate groups from proteins (see Chapter 14 on the web site). This finding suggests that a signal transduction pathway involving protein kinases and protein phosphatases may be involved in signaling between NPA-binding proteins and the auxin efflux carrier.

Auxin Is Also Transported Nonpolarly in the Phloem

Most of the IAA that is synthesized in mature leaves appears to be transported to the rest of the plant nonpolarly via the phloem. Auxin, along with other components of phloem sap, can move from these leaves up or down the plant at velocities much higher than those of polar transport (see Chapter 10). Auxin translocation in the phloem is largely passive, not requiring energy directly.

Although the overall importance of the phloem pathway versus the polar transport system for the long-distance movement of IAA in plants is still unresolved, the evidence suggests that long-distance auxin transport in the phloem is important for controlling such processes as cambial cell divisions, callose accumulation or removal from sieve tube elements, and branch root formation. Indeed, the phloem appears to represent the principal pathway for long-distance auxin translocation to the root (Aloni 1995; Swarup et al. 2001).

Polar transport and phloem transport are not independent of each other. Recent studies with radiolabeled IAA suggest that in pea, auxin can be transferred from the nonpolar phloem pathway to the polar transport pathway. This transfer takes place mainly in the immature tissues of the shoot apex.

A second example of transfer of auxin from the nonpolar phloem pathway to a polar transport system has recently been documented in *Arabidopsis*. It was shown that the AUX1 permease is asymmetrically localized on the plasma membrane at the upper end of root protophloem cells (i.e., the end distal from the tip) (Figure 19.19).

It has been proposed that the asymmetrically oriented AUX1 permease promotes the acropetal movement of auxin from the phloem to the root apex (Swarup et al. 2001). This type of polar auxin transport based on the asymmetric localization of AUX1 differs from the polar transport that occurs in the shoot and basal region of the root, which is based on the asymmetric distribution of the PIN complex.

Note in Figure 19.19B that AUX1 is also strongly expressed in a cluster of cells in the columella of the root cap, as well as in lateral root cap cells that overlay the cells of the distal elongation zone of the root. These cells form a minor, but physiologically important, basipetal pathway whereby auxin reaching the columella is redirected backward toward the outer tissues of the elongation zone. The importance of this pathway will become apparent when we examine the mechanism of root gravitropism.

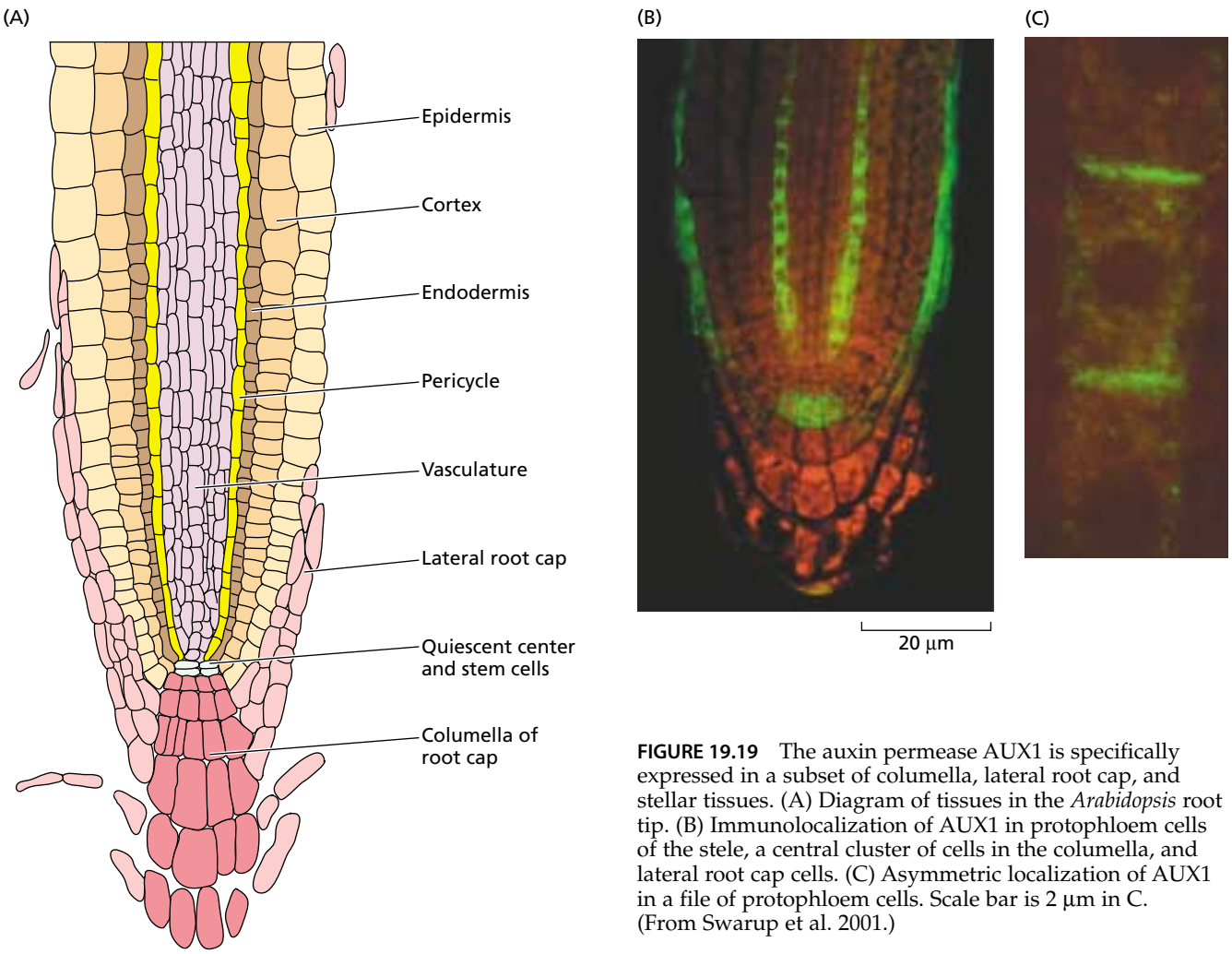


FIGURE 19.19 The auxin permease AUX1 is specifically expressed in a subset of columella, lateral root cap, and stellar tissues. (A) Diagram of tissues in the *Arabidopsis* root tip. (B) Immunolocalization of AUX1 in protophloem cells of the stele, a central cluster of cells in the columella, and lateral root cap cells. (C) Asymmetric localization of AUX1 in a file of protophloem cells. Scale bar is 2 µm in C. (From Swarup et al. 2001.)

PHYSIOLOGICAL EFFECTS OF AUXIN: CELL ELONGATION

Auxin was discovered as the hormone involved in the bending of coleoptiles toward light. The coleoptile bends because of the unequal rates of cell elongation on its shaded versus its illuminated side (see Figure 19.1). The ability of auxin to regulate the rate of cell elongation has long fascinated plant scientists. In this section we will review the physiology of auxin-induced cell elongation, some aspects of which were discussed in Chapter 15.

Auxins Promote Growth in Stems and Coleoptiles, While Inhibiting Growth in Roots

As we have seen, auxin is synthesized in the shoot apex and transported basipetally to the tissues below. The steady supply of auxin arriving at the subapical region of the stem or coleoptile is required for the continued elongation of these cells. Because the level of endogenous auxin in the elongation region of a normal healthy plant is nearly optimal for growth, spraying the plant with exogenous auxin causes only a modest and short-lived stimulation in growth, and may even be inhibitory in the case of dark-grown seedlings, which are more sensitive to supraoptimal auxin concentrations than light-grown plants are.

However, when the endogenous source of auxin is removed by excision of sections containing the elongation zones, the growth rate rapidly decreases to a low basal rate. Such excised sections will often respond dramatically to exogenous auxin by rapidly increasing their growth rate back to the level in the intact plant.

In long-term experiments, treatment of excised sections of coleoptiles (see Figure 19.2) or dicot stems with auxin stimulates the rate of elongation of the section for up to 20 hours (Figure 19.20). The optimal auxin concentration for elongation growth is typically 10^{-6} to 10^{-5} *M* (Figure 19.21).

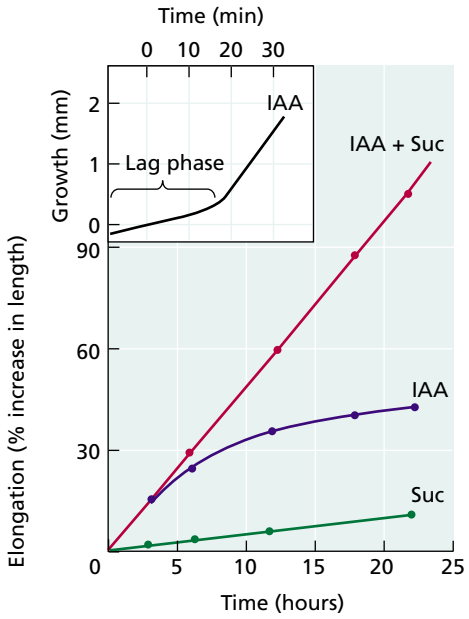


FIGURE 19.20 Time course for auxin-induced growth of *Avena* (oat) coleoptile sections. Growth is plotted as the percent increase in length. Auxin was added at time zero. When sucrose (Suc) is included in the medium, the response can continue for as long as 20 hours. Sucrose prolongs the growth response to auxin mainly by providing osmotically active solute that can be taken up for the maintenance of turgor pressure during cell elongation. KCl can substitute for sucrose. The inset shows a short-term time course plotted with an electronic position-sensing transducer. In this graph, growth is plotted as the absolute length in millimeters versus time. The curve shows a lag time of about 15 minutes for auxin-stimulated growth to begin. (From Cleland 1995.)

The inhibition beyond the optimal concentration is generally attributed to auxin-induced ethylene biosynthesis. As we will see in Chapter 22, the gaseous hormone ethylene inhibits stem elongation in many species.

Auxin control of root elongation growth has been more difficult to demonstrate, perhaps because auxin induces the production of ethylene, a root growth inhibitor. However, even if ethylene biosynthesis is specifically blocked, low concentrations (10^{-10} to 10^{-9} *M*) of auxin promote the growth of intact roots, whereas higher concentrations (10^{-6} *M*) inhibit growth. Thus, roots may require a minimum concentration of auxin to grow, but root growth is strongly inhibited by auxin concentrations that promote elongation in stems and coleoptiles.

The Outer Tissues of Dicot Stems Are the Targets of Auxin Action

Dicot stems are composed of many types of tissues and cells, only some of which may limit the growth rate. This point is illustrated by a simple experiment. When stem sections from growing regions of an etiolated dicot stem, such as pea, are split lengthwise and incubated in buffer, the two halves bend outward.

This result indicates that, in the absence of auxin the central tissues, including the pith, vascular tissues, and inner cortex, elongate at a faster rate than the outer tissues, consisting of the outer cortex and epidermis. Thus the outer tissues must be limiting the extension rate of the stem in the absence of auxin. However, when the split sections are incubated in buffer plus auxin, the two halves now curve inward, demonstrating that the outer tissues of dicot stems are the primary targets of auxin action during cell elongation.

The observation that the outer cell layers are the targets of auxin seems to conflict with the localization of polar transport in the parenchyma cells of the vascular bundles. However, auxin can move laterally from the vascular tissues of dicot stems to the outer tissues of the elongation zone. In coleoptiles, on the other hand, all of the nonvascular tissues (epidermis plus mesophyll) are capable of transporting auxin, as well as responding to it.

The Minimum Lag Time for Auxin-Induced Growth Is Ten Minutes

When a stem or coleoptile section is excised and inserted into a sensitive growth-measuring device, the growth response to auxin can be monitored at very high resolution. Without auxin in the medium, the growth rate declines rapidly. Addition of auxin markedly stimulates the growth rate after a lag period of only 10 to 12 minutes (see the inset in Figure 19.20).

Both *Avena* (oat) coleoptiles and *Glycine max* (soybean) hypocotyls (dicot stem) reach a maximum growth rate after

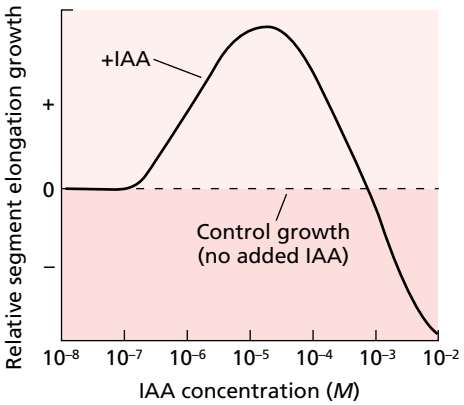


FIGURE 19.21 Typical dose–response curve for IAA-induced growth in pea stem or oat coleoptile sections. Elongation growth of excised sections of coleoptiles or young stems is plotted versus increasing concentrations of exogenous IAA. At higher concentrations (above 10^{-5} M), IAA becomes less and less effective; above about 10^{-4} M it becomes inhibitory, as shown by the fact that the curve falls below the dashed line, which represents growth in the absence of added IAA.

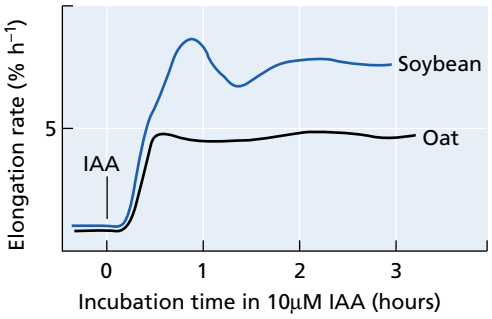


FIGURE 19.22 Comparison of the growth kinetics of oat coleoptile and soybean hypocotyl sections, incubated with 10 μM IAA and 2% sucrose. Growth is plotted as the rate at each time point, rather than the rate of the absolute length. The growth rate of the soybean hypocotyl oscillates after 1 hour, whereas that of the oat coleoptile is constant. (After Cleland 1995.)

30 to 60 minutes of auxin treatment (Figure 19.22). This maximum represents a five- to tenfold increase over the basal rate. Oat coleoptile sections can maintain this maximum rate for up to 18 hours in the presence of osmotically active solutes such as sucrose or KCl.

As might be expected, the stimulation of growth by auxin requires energy, and metabolic inhibitors inhibit the response within minutes. Auxin-induced growth is also sensitive to inhibitors of protein synthesis such as cycloheximide, suggesting that proteins with high turnover rates are involved. Inhibitors of RNA synthesis also inhibit auxin-induced growth, after a slightly longer delay (Cleland 1995).

Although the length of the lag time for auxin-stimulated growth can be increased by lowering of the temperature or by the use of suboptimal auxin concentrations, the lag time cannot be shortened by raising of the temperature, by the use of supraoptimal auxin concentrations, or by abrasion of the waxy cuticle to allow auxin to penetrate the tissue more rapidly. Thus the minimum lag time of 10 minutes is not determined by the time required for auxin to reach its site of action. Rather, the lag time reflects the time needed for the biochemical machinery of the cell to bring about the increase in the growth rate.

Auxin Rapidly Increases the Extensibility of the Cell Wall

How does auxin cause a five- to tenfold increase in the growth rate in only 10 minutes? To understand the mechanism, we must first review the process of cell enlargement in plants (see Chapter 15). Plant cells expand in three steps:

1. Osmotic uptake of water across the plasma membrane is driven by the gradient in water potential ($\Delta\Psi_w$).
2. Turgor pressure builds up because of the rigidity of the cell wall.
3. Biochemical wall loosening occurs, allowing the cell to expand in response to turgor pressure.

The effects of these parameters on the growth rate are encapsulated in the growth rate equation:

$$GR = m\,(\Psi_p - Y)$$

where GR is the growth rate, Ψ_p is the turgor pressure, Y is the yield threshold, and m is the coefficient (*wall extensibility*) that relates the growth rate to the difference between Ψ_p and Y.

In principle, auxin could increase the growth rate by increasing m, increasing Ψ_p, or decreasing Y. Although extensive experiments have shown that auxin does not increase turgor pressure when it stimulates growth, conflicting results have been obtained regarding auxin-induced decreases in Y. However, there is general agreement that auxin causes an increase in the wall extensibility parameter, m.

Auxin-Induced Proton Extrusion Acidifies the Cell Wall and Increases Cell Extension

According to the widely accepted **acid growth hypothesis**, hydrogen ions act as the intermediate between auxin and cell wall loosening. The source of the hydrogen ions is the plasma membrane H^+-ATPase, whose activity is thought to increase in response to auxin. The acid growth hypothesis allows five main predictions:

1. Acid buffers alone should promote short-term growth, provided the cuticle has been abraded to allow the protons access to the cell wall.
2. Auxin should increase the rate of proton extrusion (wall acidification), and the kinetics of proton extrusion should closely match those of auxin-induced growth.
3. Neutral buffers should inhibit auxin-induced growth.
4. Compounds (other than auxin) that promote proton extrusion should stimulate growth.
5. Cell walls should contain a "wall loosening factor" with an acidic pH optimum.

All five of these predictions have been confirmed. Acidic buffers cause a rapid and immediate increase in the growth rate, provided the cuticle has been abraded. Auxin stimulates proton extrusion into the cell wall after 10 to 15 minutes of lag time, consistent with the growth kinetics (Figure 19.23).

Auxin-induced growth has also been shown to be inhibited by neutral buffers, as long as the cuticle has been abraided. **Fusicoccin**, a fungal phytotoxin, stimulates both rapid proton extrusion and transient growth in stem and coleoptile sections (see **Web Topic 19.6**). And finally, wall-loosening proteins called **expansins** have been identified in the cell walls of a wide range of plant species (see Chapter 15). At acidic pH values, expansins loosen cell walls by weakening the hydrogen bonds between the polysaccharide components of the wall.

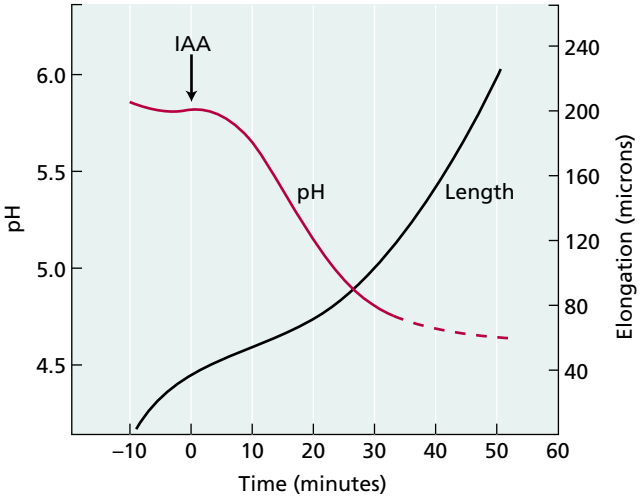


FIGURE 19.23 Kinetics of auxin-induced elongation and cell wall acidification in maize coleoptiles. The pH of the cell wall was measured with a pH microelectrode. Note the similar lag times (10 to 15 minutes) for both cell wall acidification and the increase in the rate of elongation. (From Jacobs and Ray 1976.)

Auxin-Induced Proton Extrusion May Involve Both Activation and Synthesis

In theory, auxin could increase the rate of proton extrusion by two possible mechanisms:

1. Activation of preexisting plasma membrane H^+-ATPases
2. Synthesis of new H^+-ATPases on the plasma membrane

H^+-ATPase activation. When auxin was added directly to isolated plasma membrane vesicles from tobacco cells, a small stimulation (about 20%) of the ATP-driven proton-pumping activity was observed, suggesting that auxin directly activates the H^+-ATPase. A greater stimulation (about 40%) was observed if the living cells were treated with IAA just before the membranes were isolated, suggesting that a cellular factor is also required (Peltier and Rossignol 1996).

Although an auxin receptor has not yet been unequivocally identified (as discussed later in the chapter), various auxin-binding proteins (ABPs) have been isolated and appear to be able to activate the plasma membrane H^+-ATPase in the presence of auxin (Steffens et al. 2001).

Recently an ABP from rice, ABP_{57}, was shown to bind directly to plasma membrane H^+-ATPases and stimulate proton extrusion—but only in the presence of IAA (Kim et al. 2001). When IAA is absent, the activity of the H^+-ATPase is repressed by the C-terminal domain of the enzyme, which can block the catalytic site. ABP_{57} (with bound IAA) interacts with the H^+-ATPase, activating the enzyme. A second auxin-binding site interferes with the action of the first, possibly explaining the bell-shaped curve of auxin action. This hypothetical model for the action of ABP_{57} is shown in Figure 19.24.

H^+-ATPase synthesis. The ability of protein synthesis inhibitors, such as cycloheximide, to rapidly inhibit auxin-induced proton extrusion and growth suggests that auxin might also stimulate proton pumping by increasing the synthesis of the H^+-ATPase. An increase in the amount of plasma membrane ATPase in corn coleoptiles was detected immunologically after only 5 minutes of auxin treatment, and a doubling of the H^+-ATPase was observed after 40 minutes of treatment. A threefold stimulation by auxin of an mRNA for the H^+-ATPase was demonstrated specifically in the nonvascular tissues of the coleoptiles.

In summary, the question of activation versus synthesis is still unresolved, and it is possible that auxin stimulates proton extrusion by both activation and stimulation of synthesis of the H^+-ATPase. Figure 19.25 summarizes

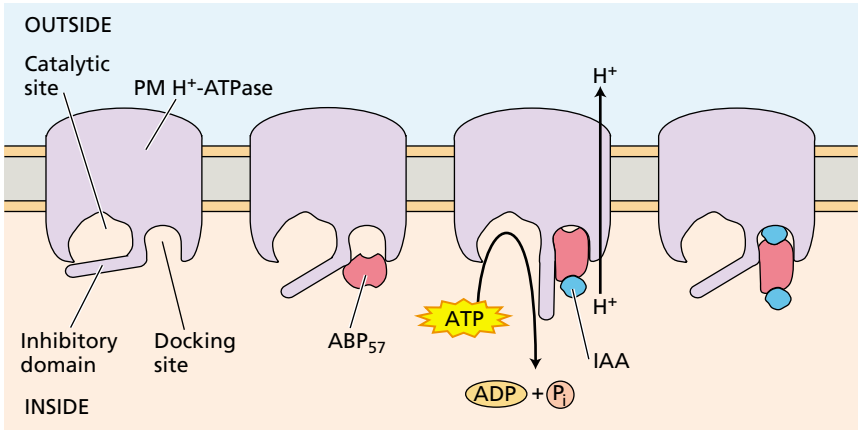


FIGURE 19.24 Model for the activation of the plasma membrane (PM) H^+-ATPase by ABP_{57} and auxin.

Activation hypothesis: Auxin binds to an auxin-binding protein (ABP1) located either on the cell surface or in the cytosol. ABP1-IAA then interacts directly with plasma membrane H^+-ATPase to stimulate proton pumping (step 1). Second messengers, such as calcium or intracellular pH, could also be involved.

Synthesis hypothesis: IAA-induced second messengers activate the expression of genes (step 2) that encode the plasma membrane H^+-ATPase (step 3). The protein is synthesized on the rough endoplasmic reticulum (step 4) and targeted via the secretory pathway to the plasma membrane (steps 5 and 6). The increase in proton extrusion results from an increase in the number of proton pumps on the membrane.

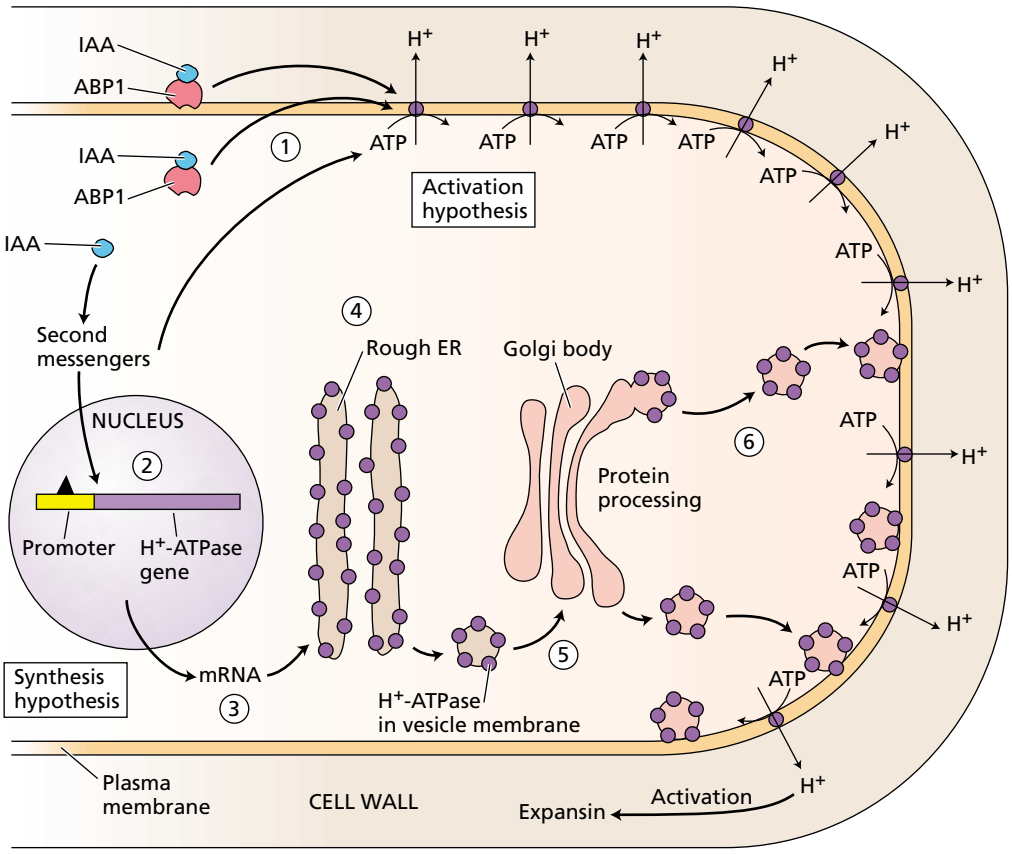


FIGURE 19.25 Current models for IAA-induced H^+ extrusion. In many plants, both of these mechanisms may operate. Regardless of how H^+ pumping is increased, acid-induced wall loosening is thought to be mediated by expansins.

the proposed mechanisms of auxin-induced cell wall loosening via proton extrusion.

PHYSIOLOGICAL EFFECTS OF AUXIN: PHOTOTROPISM AND GRAVITROPISM

Three main guidance systems control the orientation of plant growth:

1. **Phototropism**, or growth with respect to light, is expressed in all shoots and some roots; it ensures that leaves will receive optimal sunlight for photosynthesis.
2. **Gravitropism**, growth in response to gravity, enables roots to grow downward into the soil and shoots to grow upward away from the soil, which is especially critical during the early stages of germination.
3. **Thigmotropism**, or growth with respect to touch, enables roots to grow around rocks and is responsible for the ability of the shoots of climbing plants to wrap around other structures for support.

In this section we will examine the evidence that bending in response to light or gravity results from the lateral redistribution of auxin. We will also consider the cellular mechanisms involved in generating lateral auxin gradients during bending growth. Less is known about the mechanism of thigmotropism, although it, too, probably involves auxin gradients.

Phototropism Is Mediated by the Lateral Redistribution of Auxin

As we saw earlier, Charles and Francis Darwin provided the first clue concerning the mechanism of phototropism by demonstrating that the sites of perception and differential growth (bending) are separate: Light is perceived at the tip, but bending occurs below the tip. The Darwins proposed that some "influence" that was transported from the tip to the growing region brought about the observed asymmetric growth response. This influence was later shown to be indole-3-acetic acid—auxin.

When a shoot is growing vertically, auxin is transported polarly from the growing tip to the elongation zone. The

polarity of auxin transport from tip to base is developmentally determined and is independent of orientation with respect to gravity. However, auxin can also be transported laterally, and this lateral movement of auxin lies at the heart of a model for tropisms originally proposed separately by the Russian plant physiologist, Nicolai Cholodny and Frits Went from the Netherlands in the 1920s.

According to the Cholodny–Went model of phototropism, the tips of grass coleoptiles have three specialized functions:

1. The production of auxin
2. The perception of a unilateral light stimulus
3. The lateral transport of IAA in response to the phototropic stimulus

Thus, in response to a directional light stimulus, the auxin produced at the tip, instead of being transported basipetally, is transported laterally toward the shaded side.

The precise sites of auxin production, light perception, and lateral transport have been difficult to define. In maize coleoptiles, auxin is produced in the upper 1 to 2 mm of the tip. The zones of photosensing and lateral transport extend farther, within the upper 5 mm of the tip. The response is also strongly dependent on the light fluence (see **Web Topic 19.7**).

Two flavoproteins, *phototropins 1* and *2*, are the photoreceptors for the blue-light signaling pathway (see **Web Essay 19.3**) that induces phototropic bending in *Arabidopsis* hypocotyls and oat coleoptiles under both high- and low-fluence conditions (Briggs et al. 2001).

Phototropins are autophosphorylating protein kinases whose activity is stimulated by blue light. The action spectrum for **blue-light** activation of the kinase activity closely matches the action spectrum for phototropism, including the multiple peaks in the blue region. Phototropin 1 displays a lateral gradient in phosphorylation during exposure to low-fluence unilateral blue light.

According to the current hypothesis, the gradient in phototropin phosphorylation induces the movement of auxin to the shaded side of the coleoptile (see **Web Topic 19.7**). Once the auxin reaches the shaded side of the tip, it is transported basipetally to the elongation zone, where it stimulates cell elongation. The acceleration of growth on the shaded side and the slowing of growth on the illuminated side (differential growth) give rise to the curvature toward light (Figure 19.26).

Direct tests of the Cholodny–Went model using the agar block/coleoptile curvature bioassay have supported the model's prediction that auxin in coleoptile tips is transported laterally in response to unilateral light (Figure 19.27). The total amount of auxin diffusing out of the tip (here expressed as the angle of curvature) is the same in the presence of unilateral light as in darkness (compare Figure 19.27A and B). This result indicates that light does not cause the photodestruction of auxin on the illuminated side, as had been proposed by some investigators.

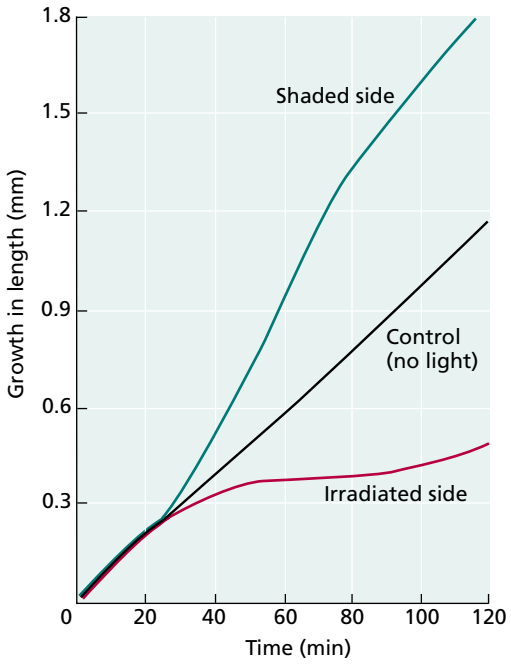


FIGURE 19.26 Time course of growth on the illuminated and shaded sides of a coleoptile responding to a 30-second pulse of unidirectional blue light. Control coleoptiles were not given a light treatment. (After Iino and Briggs 1984.)

Consistent with both the Cholodny–Went hypothesis and the acid growth hypothesis, the apoplastic pH on the shaded side of a phototropically bending stem or coleoptile is more acidic than the side facing the light (Mulkey et al. 1981).

Gravitropism Also Involves Lateral Redistribution of Auxin

When dark-grown *Avena* seedlings are oriented horizontally, the coleoptiles bend upward in response to gravity. According to the Cholodny–Went model, auxin in a horizontally oriented coleoptile tip is transported laterally to the lower side, causing the lower side of the coleoptile to grow faster than the upper side. Early experimental evidence indicated that the tip of the coleoptile can perceive gravity and redistribute auxin to the lower side. For example, if coleoptile tips are oriented horizontally, a greater amount of auxin diffuses from the lower half than the upper half (Figure 19.28).

Tissues below the tip are able to respond to gravity as well. For example, when vertically oriented maize coleoptiles are decapitated by removal of the upper 2 mm of the tip and oriented horizontally, gravitropic bending occurs at a slow rate for several hours even without the tip. Application of IAA to the cut surface restores the rate of bending

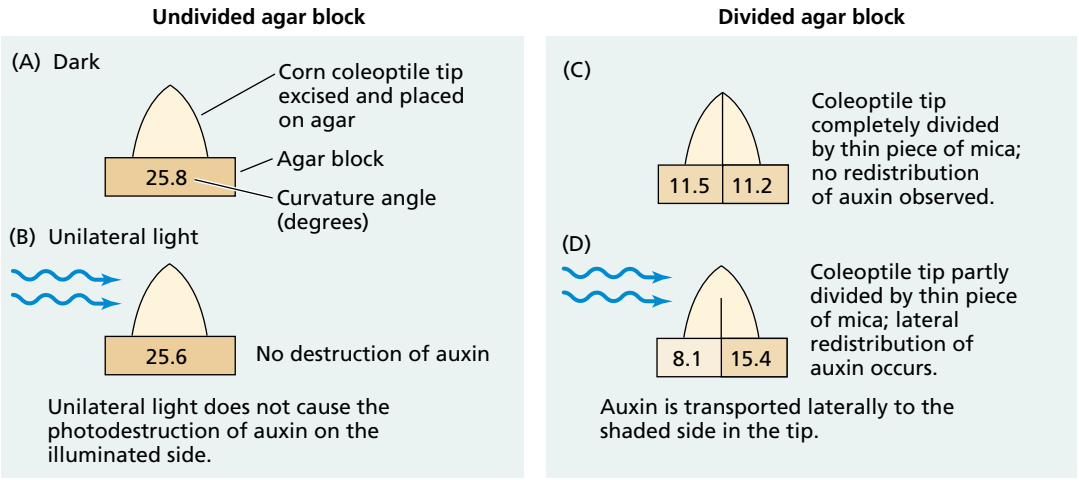


FIGURE 19.27 Evidence that the lateral redistribution of auxin is stimulated by unidirectional light in corn coleoptiles.

to normal levels. This finding indicates that both the perception of the gravitational stimulus and the lateral redistribution of auxin can occur in the tissues below the tip, although the tip is still required for auxin production.

Lateral redistribution of auxin in shoot apical meristems is more difficult to demonstrate than in coleoptiles because of the presence of leaves. In recent years, molecular markers have been widely used as reporter genes to detect lateral auxin gradients in horizontally placed stems and roots.

In soybean hypocotyls, gravitropism leads to a rapid asymmetry in the accumulation of a group of auxin-stimulated mRNAs called **SAURs** (*s*mall *a*uxin *u*p-regulated *R*NAs) (McClure and Guilfoyle 1989). In vertical seedlings, SAUR gene expression is symmetrically distributed. Within 20 minutes after the seedling is oriented horizontally, SAURs begin to accumulate on the lower half of the hypocotyl. Under these conditions, gravitropic bending first becomes evident after 45 minutes, well after the induction of the SAURs (see **Web Topic 19.8**). The existence of a lateral gradient in SAUR gene expression is indirect evidence for the existence of a lateral gradient in auxin detectable within 20 minutes of the gravitropic stimulus.

As will be discussed later in the chapter, the *GH3* gene family is also up-regulated within 5 minutes of auxin treat-

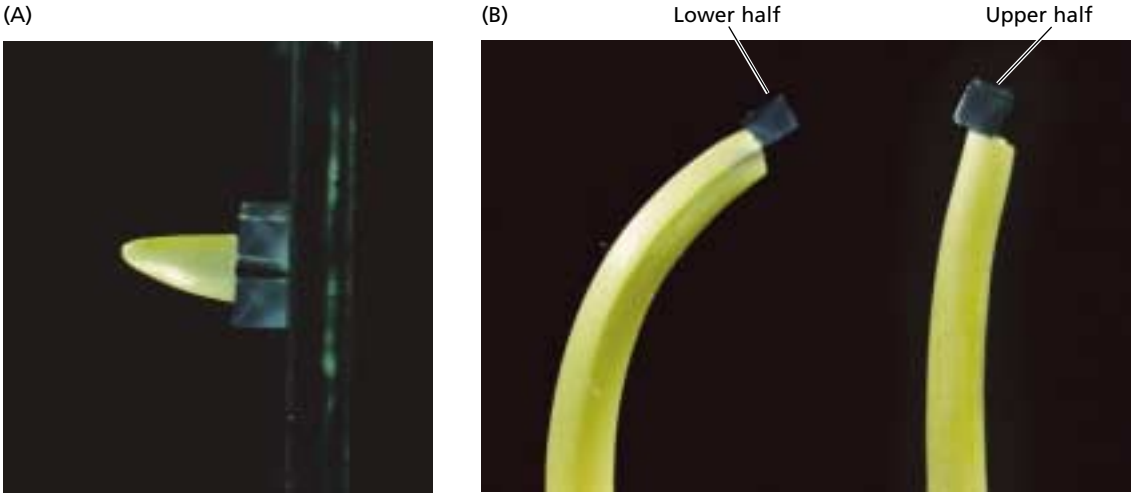


FIGURE 19.28 Auxin is transported to the lower side of a horizontally oriented oat coleoptile tip. (A) Auxin from the upper and lower halves of a horizontal tip is allowed to diffuse into two agar blocks. (B) The agar block from the lower half (left) induces greater curvature in a decapitated coleoptile than the agar block from the upper half (right). (Photo © M. B. Wilkins.)

FIGURE 19.29 Lateral auxin gradients are formed in *Arabidopsis* hypocotyls during the differential growth responses to light (A) and gravity (B). The plants were transformed with the *DR5::GUS* reporter gene. Auxin accumulation on the shaded (A) or lower (B) side of the hypocotyls is indicated by the blue staining shown in the insets. (Photos courtesy of Klaus Palme.)

ment and has been used as a molecular marker for the presence of auxin. By fusing an artificial promoter sequence based on the *GH3* promoter to the *GUS* reporter gene, it is possible to visualize the lateral gradient in auxin concentration that occurs during both photo- and gravitropism (Figure 19.29).

Statoliths Serve as Gravity Sensors in Shoots and Roots

Unlike unilateral light, gravity does not form a gradient between the upper and lower sides of an organ. All parts of the plant experience the gravitational stimulus equally. How do plant cells detect gravity? The only way that gravity can be sensed is through the motion of a falling or sedimenting body.

Obvious candidates for intracellular gravity sensors in plants are the large, dense amyloplasts that are present in many plant cells. These specialized amyloplasts are of sufficiently high density relative to the cytosol that they readily sediment to the bottom of the cell (Figure 19.30). Amyloplasts that function as gravity sensors are called **statoliths**, and the specialized gravity-sensing cells in which they occur are called **statocytes**. Whether the statocyte is able to detect the downward motion of the statolith as it passes through the cytoskeleton or whether the stimulus is perceived only when the statolith comes to rest at the bottom of the cell has not yet been resolved.

Shoots and Coleoptiles. In shoots and coleoptiles, gravity is perceived in the **starch sheath**, a layer of cells that surrounds the vascular tissues of the shoot. The starch sheath is continuous with the endodermis of the root, but unlike the endodermis it contains amyloplasts. *Arabidopsis* mutants lacking amyloplasts in the starch sheath display agravitropic shoot growth but normal gravitropic root growth (Fujihira et al. 2000).

As noted in Chapter 16, the *scarecrow* (*scr*) mutant of *Arabidopsis* is missing both the endodermis and the starch sheath. As a result, the hypocotyl and inflorescence of the *scr* mutant are agravitropic, while the root exhibits a normal gravitropic response. On the basis of the phenotypes of these two mutants, we can conclude the following:

- The starch sheath is required for gravitropism in shoots.
- The root endodermis, which does not contain statoliths, is not required for gravitropism in roots.

Roots. The site of gravity perception in primary roots is the root cap. Large, graviresponsive amyloplasts are located in the statocytes (see Figure 19.30A and B) in the central cylinder, or **columella**, of the root cap. Removal of the root cap from otherwise intact roots abolishes root gravitropism without inhibiting growth.

Precisely how the statocytes sense their falling statoliths is still poorly understood. According to one hypothesis, contact or pressure resulting from the amyloplast resting on the endoplasmic reticulum on the lower side of the cell triggers the response (see Figure 19.30C). The endoplasmic reticulum of columella cells is structurally unique, consisting of five to seven rough-ER sheets attached to a central nodal rod in a whorl, like petals on a flower. This specialized "nodal ER" differs from the more tubular cortical ER cisternae and may be involved in the gravity response (Zheng and Staehelin 2001).

The **starch–statolith hypothesis** of gravity perception in roots is supported by several lines of evidence. Amyloplasts are the only organelles that consistently sediment in the columella cells of different plant species, and the rate of sedimentation correlates closely with the time required to perceive the gravitational stimulus. The gravitropic responses of starch-deficient mutants are generally much slower than those of wild-type plants. Nevertheless, starchless mutants exhibit some gravitropism, suggesting that although starch is required for a normal gravitropic response, starch-independent gravity perception mechanisms may also exist.

Other organelles, such as nuclei, may be dense enough to act as statoliths. It may not even be necessary for a statolith to come to rest at the bottom of the cell. The cytoskeletal network may be able to detect a partial vertical displacement of an organelle.

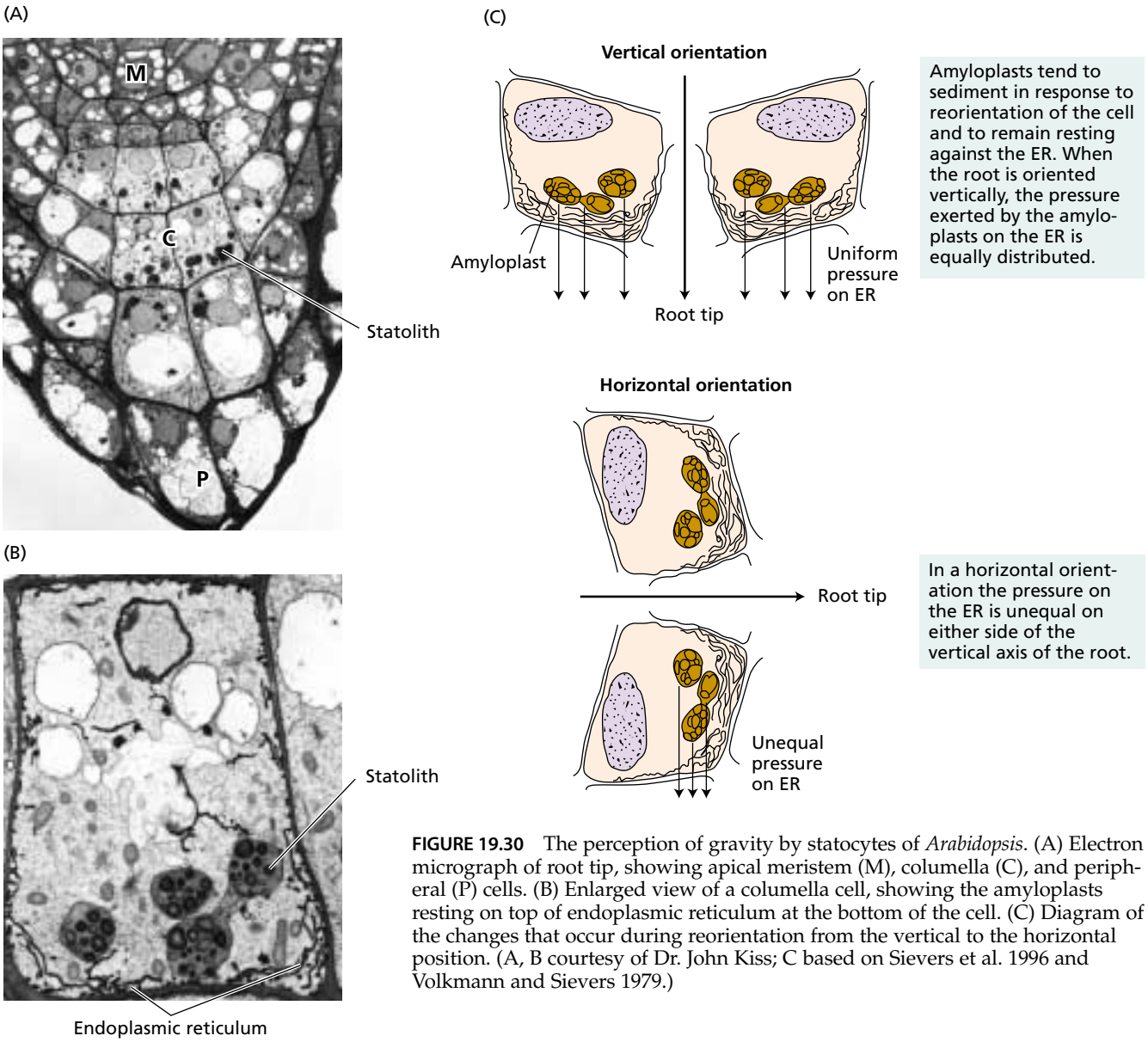


FIGURE 19.30 The perception of gravity by statocytes of *Arabidopsis*. (A) Electron micrograph of root tip, showing apical meristem (M), columella (C), and peripheral (P) cells. (B) Enlarged view of a columella cell, showing the amyloplasts resting on top of endoplasmic reticulum at the bottom of the cell. (C) Diagram of the changes that occur during reorientation from the vertical to the horizontal position. (A, B courtesy of Dr. John Kiss; C based on Sievers et al. 1996 and Volkmann and Sievers 1979.)

Recently Andrew Staehelin and colleagues proposed a new model for gravitropism, called the **tensegrity model** (Yoder et al. 2001). *Tensegrity* is an architectural term—a contraction of *tensional integrity*—coined by the innovative architect R. Buckminster Fuller. In essence, *tensegrity* refers to structural integrity created by interactive tension between the structural components. In this case the structural components consist of the meshwork of actin microfilaments that form part of the cytoskeleton of the central columella cells of the root cap. The actin network is assumed to be anchored to stretch-activated receptors on the plasma membrane. Stretch receptors in animal cells are typically mechanosensitive ion channels, and stretch-activated calcium channels have been demonstrated in plants.

According to the tensegrity model, sedimentation of the statoliths through the cytosol locally disrupts the actin meshwork, changing the distribution of tension transmitted to calcium channels on the plasma membrane, thus altering their activities. Yoder and colleagues (2001) have further proposed that the nodal ER, which is also connected to channels via actin microfilaments, may protect the cytoskeleton from being disrupted by the statoliths in specific regions, thus providing a signal for the directionality of the stimulus.

Gravity perception without statoliths? An alternative mechanism of gravity perception that does not involve statoliths has been proposed for the giant-celled freshwater alga *Chara*. See **Web Topic 19.8** for details.

Auxin Is Redistribution Laterally in the Root Cap

In addition to functioning to protect the sensitive cells of the apical meristem as the tip penetrates the soil, the root cap is the site of gravity perception. Because the cap is some distance away from the elongation zone where bending occurs, a chemical messenger is presumed to be involved in communication between the cap and the elongation zone. Microsurgery experiments in which half of the

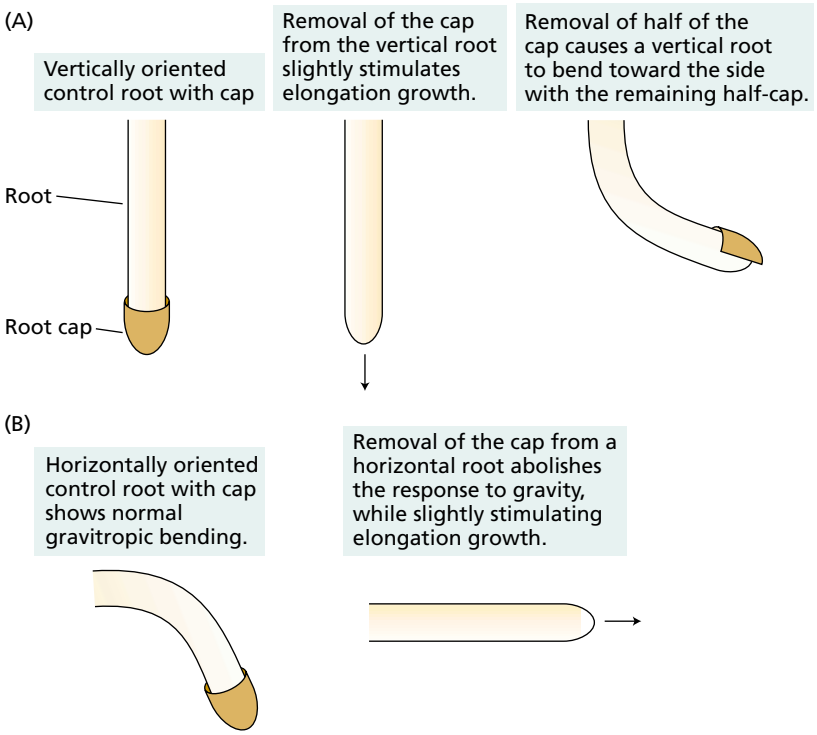


FIGURE 19.31 Microsurgery experiments demonstrating that the root cap produces an inhibitor that regulates root gravitropism. (After Shaw and Wilkins 1973.)

cap was removed showed that the cap produces a root growth inhibitor (Figure 19.31). This finding suggests that the cap supplies an inhibitor to the lower side of the root during gravitropic bending.

Although root caps contain small amounts of IAA and abscisic acid (ABA) (see Chapter 23), IAA is more inhibitory to root growth than ABA when applied directly to the elongation zone, suggesting that IAA is the root cap inhibitor. Consistent with this conclusion, ABA-deficient *Arabidopsis* mutants have normal root gravitropism, whereas the roots of mutants defective in auxin transport, such as *aux1* and *agr1*, are agravitropic (Palme and Gälweiler 1999). The *agr* mutant lacks an auxin efflux carrier related to the PIN proteins (Chen et al. 1998; Müller et al. 1998; Utsuno et al. 1998). The AGR1 protein is localized at the basal (distal) end of cortical cells near the root tip in *Arabidopsis*.

How do we reconcile the fact that the shoot apical meristem is the primary source of auxin to the root with the role of the root cap as the source of the inhibitory auxin during gravitropism? As discussed earlier in the chapter, auxin from the shoot is translocated from the stele to the root tip via protophloem cells. Asymmetrically localized AUX1 permeases on the protophloem parenchyma cells direct the acropetal transport of auxin from the phloem to a cluster of cells in the columella of the cap. Auxin is then transported radially to the lateral root cap cells, where AUX1 is also strongly expressed (see Figure 19.19).

The lateral root cap cells overlay the distal elongation zone (DEZ) of the root—the first region that responds to gravity. The auxin from the cap is taken up by the cortical parenchyma of the DEZ and transported basipetally through the elongation zone of the root. This basipetal transport, which is limited to the elongation zone, is facilitated by auxin anion carriers related to the PIN family (called AGR1), which are localized at the basal ends of the cortical parenchyma cells.

The basipetally transported auxin accumulates in the elongation zone and does not pass beyond this region. Flavonoids capable of inhibiting auxin efflux are synthesized in this region of the root and probably promote auxin retention by these cells (Figure 19.32) (Murphy et al. 2000).

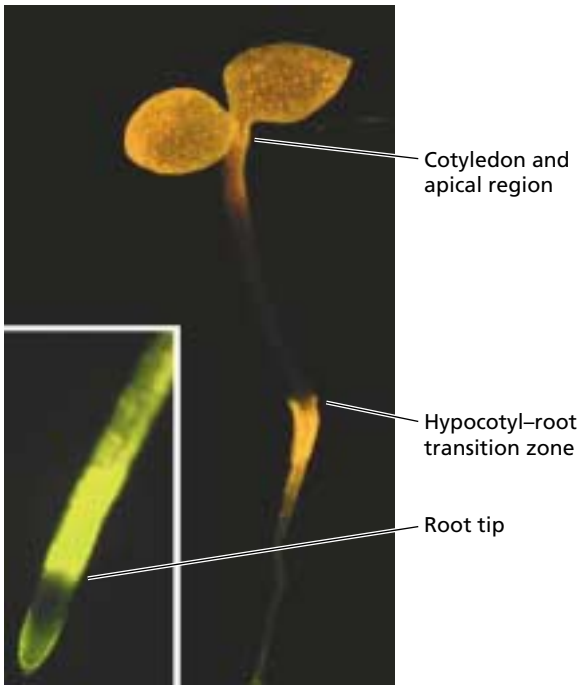


FIGURE 19.32 Flavonoid localization in a 6-day-old *Arabidopsis* seedling. The staining procedure used causes the flavonoids to fluoresce. Flavonoids are concentrated in three regions: the cotyledon and apical region, the hypocotyl–root transition zone, and the root tip area (inset). In the root tip, flavonoids are localized specifically in the elongation zone and the cap, the tissues involved in basipetal auxin transport. (From Murphy et al. 2000.)

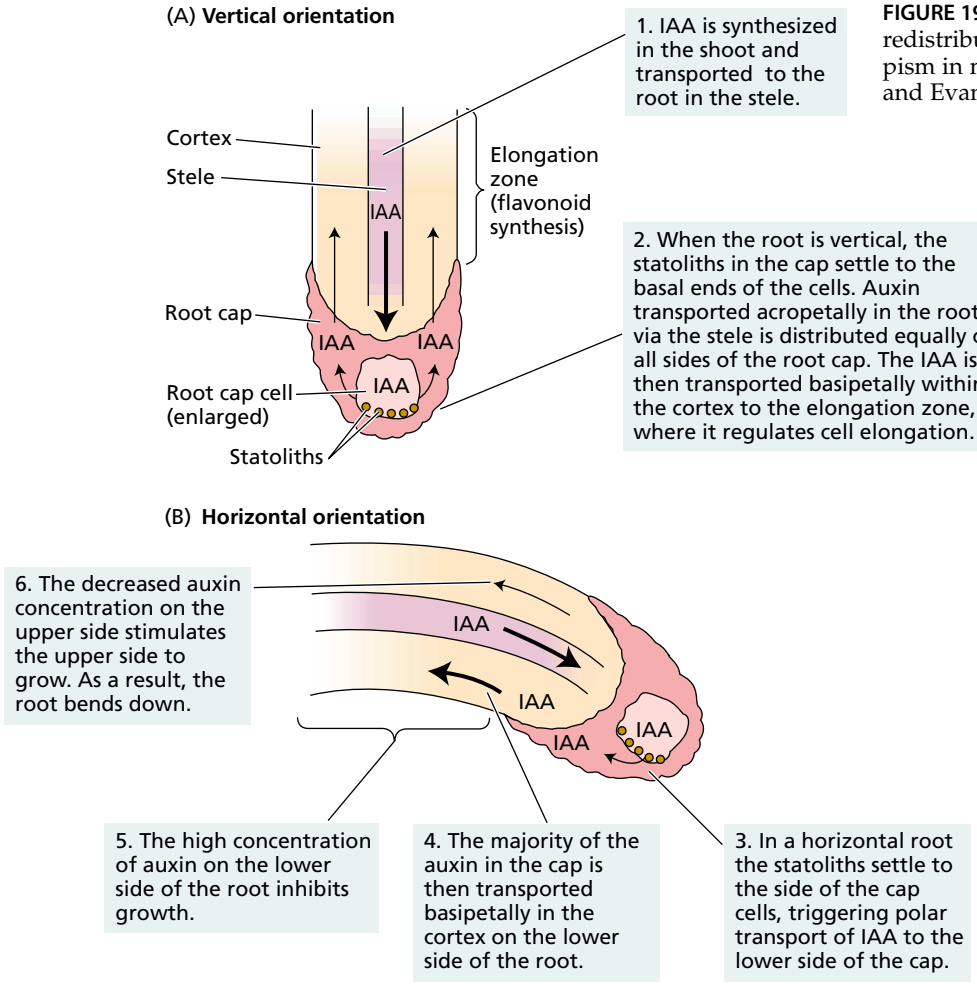


FIGURE 19.33 Proposed model for the redistribution of auxin during gravitropism in maize roots. (After Hasenstein and Evans 1988.)

According to the model, basipetal auxin transport in a vertically oriented root is equal on all sides (Figure 19.33A). When the root is oriented horizontally, however, the cap redirects the bulk of the auxin to the lower side, thus inhibiting the growth of that lower side (see Figure 19.33B). Consistent with this idea, the transport of [^{3}H]IAA across a horizontally oriented root cap is polar, with a preferential downward movement (Young et al. 1990).

PIN3 Is Relocated Laterally to the Lower Side of Root Columella Cells

Recently the mechanism of lateral auxin redistribution in the root cap has new been elucidated (Friml et al. 2002). One of the members of the PIN protein family of auxin efflux carriers, PIN3, is not only required for both photo- and gravitropism in *Arabidopsis*, but it has been shown to be relocalized to the lower side of the columella cells during root gravitropism (Figure 19.34).

As noted previously, PIN proteins are constantly being cycled between the plasma membrane and intracellular secretory compartments. This cycling allows some PIN proteins to be targeted to specific sides of the cell in response to a directional stimulus. In a vertically oriented root, PIN3 is uniformly distributed around the columella cell (see Figure 19.34A). But when the root is placed on its side, PIN3 is preferentially targeted to the lower side of the cell (see Figure 19.34B). As a result, auxin is transported polarly to the lower half of the cap.

Gravity Sensing May Involve Calcium and pH as Second Messengers

A variety of experiments have suggested that calcium–calmodulin is required for root gravitropism in maize. Some of these experiments involve EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid), a compound that can chelate (form a complex with) calcium ions, thus preventing calcium uptake by cells. EGTA inhibits both root gravitropism and the asymmetric distribution of auxin in response to gravity (Young and Evans 1994).

Placing a block of agar that contains calcium ions on the side of the cap of a vertically oriented corn root induces the root to grow toward the side with the agar block (Figure 19.35). As in the case of [$^+$H]IAA, $^{45}Ca^{2+}$ is polarly transported to the lower half of the cap of a root stimulated by

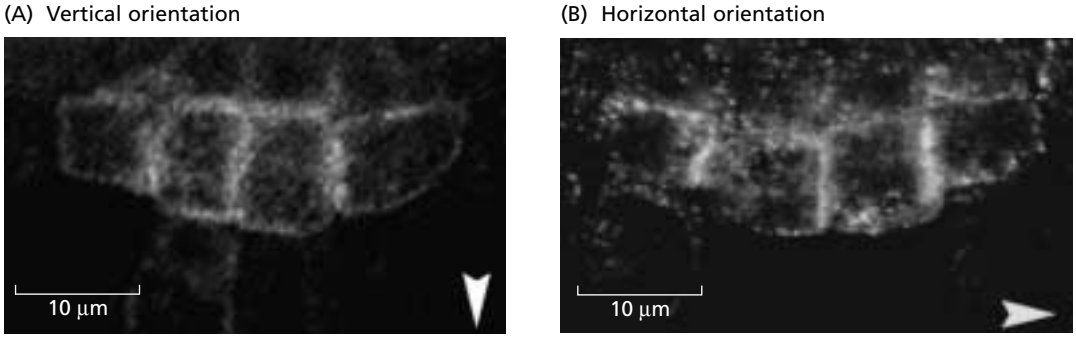


FIGURE 19.34 Relocalization of the auxin efflux carrier PIN3 during root gravitropism in *Arabidopsis*. (A) In a vertically oriented root, PIN3 is uniformly distributed around the columella cells. (B) After being oriented horizontally for 10 minutes, PIN3 has been relocalized to the lower side of the columella cells. The photo in (B) has been reorientated so that the lower side is on the right. (The direction of gravity is indicated by the arrows.) (From Friml et al. 2002, courtesy of Klaus Palme.)

gravity. However, thus far no changes in the distribution of intracellular calcium have been detected in columella cells in response to a gravitational stimulus.

Recent evidence suggests that a change in intracellular pH is the earliest detectable change in columella cells responding to gravity. Fasano et al. (2001) used pH-sensitive dyes to monitor both intracellular and extracellular pH in *Arabidopsis* roots after they were placed in a horizontal position. Within 2 minutes of gravistimulation, the cytoplasmic pH of the columella cells of the root cap increased from 7.2 to 7.6, and the apoplastic pH declined from 5.5 to 4.5. These changes preceded any detectable tropic curvature by about 10 minutes.

The alkalinization of the cytosol combined with the acidification of the apoplast suggests that an activation of the plasma membrane H^+-ATPase is one of the initial events that mediate root gravity perception or signal transduction.

FIGURE 19.35 A corn root bending toward an agar block containing calcium placed on the cap. (Courtesy of Michael L. Evans.)

DEVELOPMENTAL EFFECTS OF AUXIN

Although originally discovered in relation to growth, auxin influences nearly every stage of a plant's life cycle from germination to senescence. Because the effect that auxin produces depends on the identity of the target tissue, the response of a tissue to auxin is governed by its developmentally determined genetic program and is further influenced by the presence or absence of other signaling molecules. As we will see in this and subsequent chapters, interaction between two or more hormones is a recurring theme in plant development.

In this section we will examine some additional developmental processes regulated by auxin, including apical dominance, leaf abscission, lateral-root formation, and vascular differentiation. Throughout this discussion we assume that the primary mechanism of auxin action is comparable in all cases, involving similar receptors and signal transduction pathways. The current state of our knowledge of auxin signaling pathways will be considered at the end of the chapter.

Auxin Regulates Apical Dominance

In most higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds—a phenomenon called

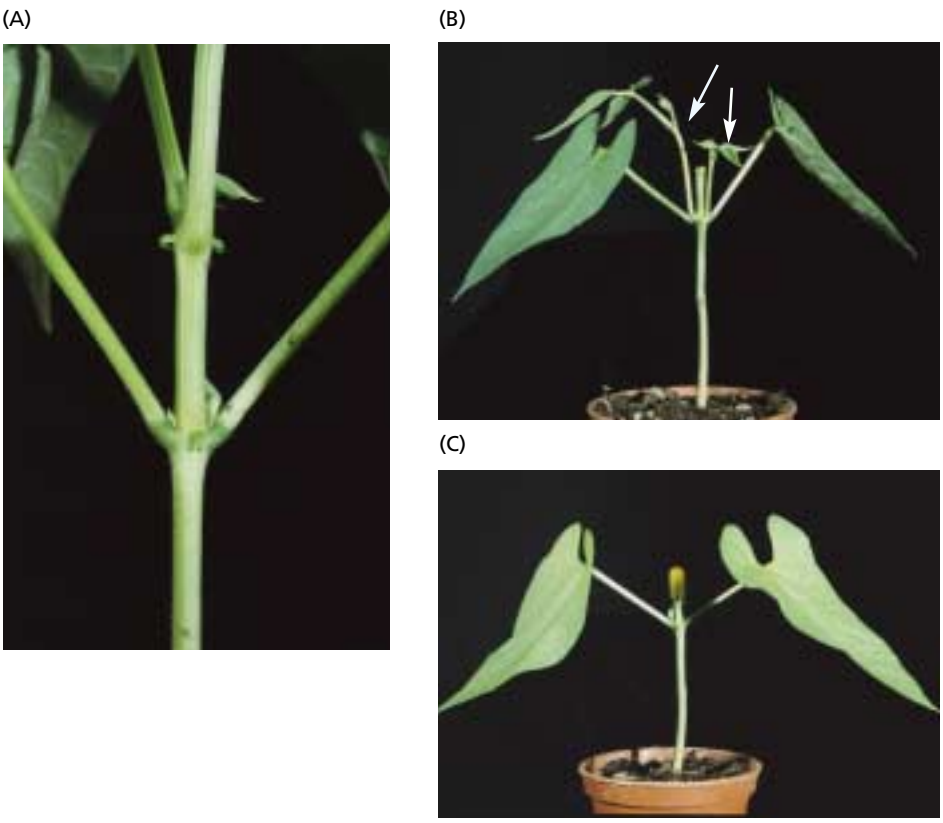


FIGURE 19.36 Auxin suppresses the growth of axillary buds in bean (*Phaseolus vulgaris*) plants. (A) The axillary buds are suppressed in the intact plant because of apical dominance. (B) Removal of the terminal bud releases the axillary buds from apical dominance (arrows). (C) Applying IAA in lanolin paste (contained in the gelatin capsule) to the cut surface prevents the outgrowth of the axillary buds. (Photos ©M. B. Wilkins.)

apical dominance. Removal of the shoot apex (decapitation) usually results in the growth of one or more of the lateral buds. Not long after the discovery of auxin, it was found that IAA could substitute for the apical bud in maintaining the inhibition of lateral buds of bean (*Phaseolus vulgaris*) plants. This classic experiment is illustrated in Figure 19.36.

This result was soon confirmed for numerous other plant species, leading to the hypothesis that the outgrowth of the axillary bud is inhibited by auxin that is transported basipetally from the apical bud. In support of this idea, a ring of the auxin transport inhibitor TIBA in lanolin paste (as a carrier) placed below the shoot apex released the axillary buds from inhibition.

How does auxin from the shoot apex inhibit the growth of lateral buds? Kenneth V. Thimann and Folke Skoog originally proposed that auxin from the shoot apex inhibits the growth of the axillary bud directly—the so-called *direct-inhibition model*. According to the model, the optimal auxin concentration for bud growth is low, much lower than the auxin concentration normally found in the stem. The level of auxin normally present in the stem was thought to inhibit the growth of lateral buds.

If the direct-inhibition model of apical dominance is correct, the concentration of auxin in the axillary bud should decrease following decapitation of the shoot apex. However, the reverse appears to be true. This was demonstrated with transgenic plants that contained the reporter genes for bacterial luciferase (*LUXA* and *LUXB*) under the control of an auxin-responsive promoter (Langridge et al. 1989). These reporter genes allowed researchers to study the level of auxin in different tissues by monitoring the amount of light emitted by the luciferase-catalyzed reaction.

When these transgenic plants were decapitated, the expression of the *LUX* genes increased in and around the axillary buds within 12 hours. This experiment indicated that after decapitation, the auxin content of the axillary buds *increased* rather than decreased.

Direct physical measurements of auxin levels in buds have also shown an increase in the auxin of the axillary buds after decapitation. The IAA concentration in the axillary bud of *Phaseolus vulgaris* (kidney bean) increased fivefold within 4 hours after decapitation (Gocal et al. 1991). These and other similar results make it unlikely that auxin from the shoot apex inhibits the axillary bud directly.

Other hormones, such as cytokinins and ABA, may be involved. Direct application of cytokinins to axillary buds stimulates bud growth in many species, overriding the inhibitory effect of the shoot apex. Auxin makes the shoot apex a sink for cytokinin synthesized in the root, and this may be one of the factors involved in apical dominance (see **Web Topic 19.10**).

Finally, ABA has been found in dormant lateral buds in intact plants. When the shoot apex is removed, the ABA levels in the lateral buds decrease. High levels of IAA in the shoot may help keep ABA levels high in the lateral buds. Removing the apex removes a major source of IAA, which

may allow the levels of bud growth inhibitor to fall (see **Web Topic 19.11**).

Auxin Promotes the Formation of Lateral and Adventitious Roots

Although elongation of the primary root is inhibited by auxin concentrations greater than 10^{-8} *M*, initiation of lateral (branch) roots and adventitious roots is stimulated by high auxin levels. Lateral roots are commonly found above the elongation and root hair zone and originate from small groups of cells in the pericycle (see Chapter 16). Auxin stimulates these pericycle cells to divide. The dividing cells gradually form into a root apex, and the lateral root grows through the root cortex and epidermis.

Adventitious roots (roots originating from non-root tissue) can arise in a variety of tissue locations from clusters of mature cells that renew their cell division activity. These dividing cells develop into a root apical meristem in a manner somewhat analogous to the formation of lateral roots. In horticulture, the stimulatory effect of auxin on the formation of adventitious roots has been very useful for the vegetative propagation of plants by cuttings.

A series of *Arabidopsis* mutants, named *alf* (*a*berrant *l*ateral root *f*ormation), have provided some insights into the role of auxin in the initiation of lateral roots. The *alf1* mutant exhibits extreme proliferation of adventitious and lateral roots, coupled with a 17-fold increase in endogenous auxin (Figure 19.37).

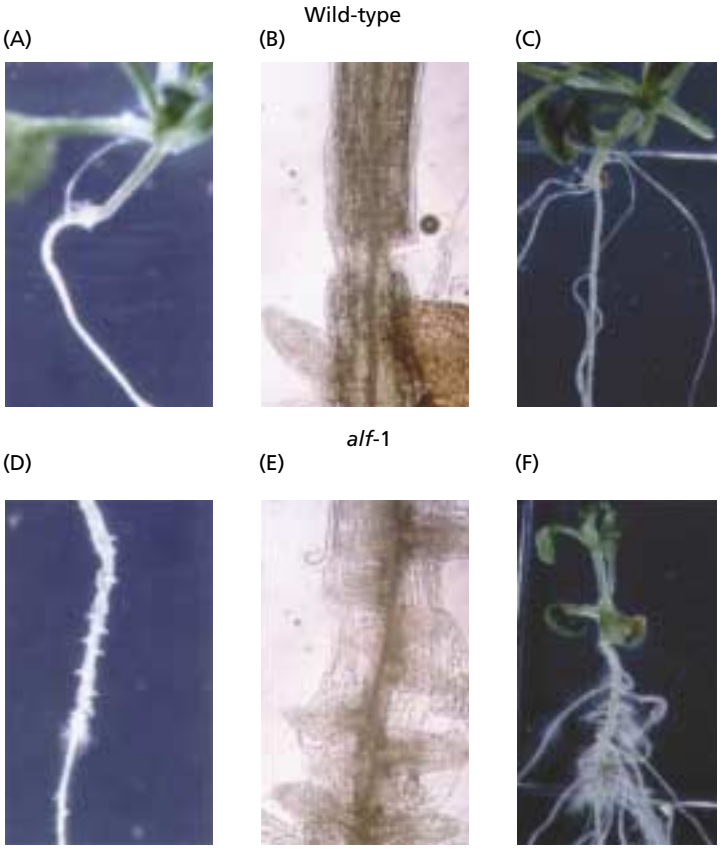


FIGURE 19.37 Root morphology of *Arabidopsis* (A–C) wild-type and *alf1* seedlings (D–F) on hormone-free medium. Note the proliferation of root primoridia growing from the pericycle in the *alf1* seedlings (D and E). (From Celenza et al. 1995, courtesy of J. Celenza.)

Another mutant, *alf4*, has the opposite phenotype: It is completely devoid of lateral roots. Microscopic analysis of *alf4* roots indicates that lateral-root primordia are absent. The *alf4* phenotype cannot be reversed by application of exogenous IAA.

Yet another mutant, *alf3*, is defective in the development of lateral-root primordia into mature lateral roots. The primary root is covered with arrested lateral-root primordia that grow until they protrude through the epidermal cell layer and then stop growing. The arrested growth can be alleviated by application of exogenous IAA.

On the basis of the phenotypes of the *alf* mutants, a model in which IAA is required for at least two steps in the formation of lateral roots has been proposed (Figure 19.38) (Celenza et al. 1995):

1. IAA transported acropetally (toward the tip) in the stele is required to initiate cell division in the pericycle.
2. IAA is required to promote cell division and maintain cell viability in the developing lateral root.

Auxin Delays the Onset of Leaf Abscission

The shedding of leaves, flowers, and fruits from the living plant is known as **abscission**. These parts abscise in a region called the **abscission zone**, which is located near the base of the petiole of leaves. In most plants, leaf abscission is preceded by the differentiation of a distinct layer of cells, the **abscission layer**, within the abscission zone. During leaf senescence, the walls of the cells in the abscission layer are digested, which causes them to become soft and weak. The leaf eventually breaks off at the abscission layer as a result of stress on the weakened cell walls.

Auxin levels are high in young leaves, progressively decrease in maturing leaves, and are relatively low in senescing leaves when the abscission process begins. The role of auxin in leaf abscission can be readily demonstrated by excision of the blade from a mature leaf, leaving the petiole intact on the stem. Whereas removal of the leaf blade accelerates the formation of the abscission layer in the petiole, application of IAA in lanolin paste to the cut surface of the petiole prevents the formation of the abscission layer. (Lanolin paste alone does not prevent abscission.)

These results suggest the following:

- Auxin transported from the blade normally prevents abscission.
- Abscission is triggered during leaf senescence, when auxin is no longer being produced.

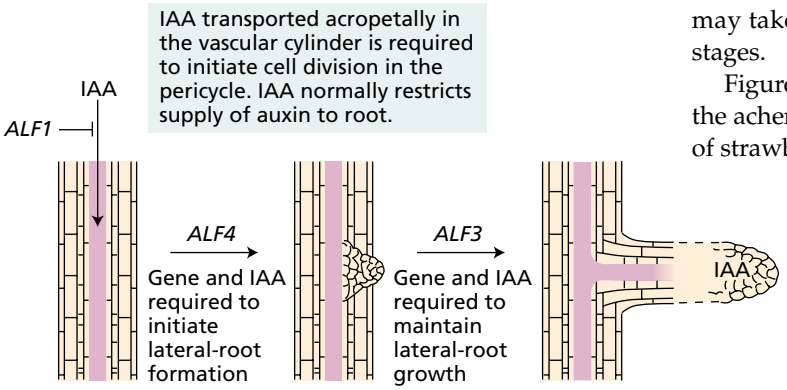


FIGURE 19.38 A model for the formation of lateral roots, based on the *alf* mutants of *Arabidopsis*. (After Celenza et al. 1995.)

However, as will be discussed in Chapter 22, ethylene also plays a crucial role as a positive regulator of abscission.

Auxin Transport Regulates Floral Bud Development

Treating *Arabidopsis* plants with the auxin transport inhibitor NPA causes abnormal floral development, suggesting that polar auxin transport in the inflorescence meristem is required for normal floral development. In *Arabidopsis*, the "pin-formed" mutant *pin1*, which lacks an auxin efflux carrier in shoot tissues, has abnormal flowers similar to those of NPA-treated plants (see Figure 19.14A). Apparently the developing floral meristem depends on auxin being transported to it from subapical tissues. In the absence of the efflux carriers, the meristem is starved for auxin, and normal phyllotaxis and floral development are disrupted (Kuhlemeier and Reinhardt 2001).

Auxin Promotes Fruit Development

Much evidence suggests that auxin is involved in the regulation of fruit development. Auxin is produced in pollen and in the endosperm and the embryo of developing seeds, and the initial stimulus for fruit growth may result from pollination.

Successful pollination initiates ovule growth, which is known as **fruit set**. After fertilization, fruit growth may depend on auxin produced in developing seeds. The endosperm may contribute auxin during the initial stage of fruit growth, and the developing embryo may take over as the main auxin source during the later stages.

Figure 19.39 shows the influence of auxin produced by the achenes of strawberry on the growth of the receptacle of strawberry.

Auxin Induces Vascular Differentiation

New vascular tissues differentiate directly below developing buds and young growing leaves (see Figure 19.5), and removal of the young leaves prevents vascular differentiation (Aloni 1995). The ability of an apical bud to stimulate vascular differentiation can be demonstrated in tissue culture. When the apical bud is grafted onto a clump of undifferentiated cells, or *callus*, xylem and phloem differentiate beneath the graft.

The relative amounts of xylem and phloem formed are regulated by the auxin concentration: High auxin concentrations induce the differentiation of xylem and phloem, but only phloem differentiates at low auxin concentrations. Similarly, experiments on stem tissues have shown that low auxin concentrations induce phloem differentiation, whereas higher IAA levels induce xylem (Aloni 1995).

The regeneration of vascular tissue following wounding is also controlled by auxin produced by the young leaf directly above the wound site (Figure 19.40). Removal of the leaf prevents the regeneration of vascular tissue, and applied auxin can substitute for the leaf in stimulating regeneration.

Vascular differentiation is polar and occurs from leaves to roots. In woody perennials, auxin produced by growing buds in the spring stimulates activation of the cambium in

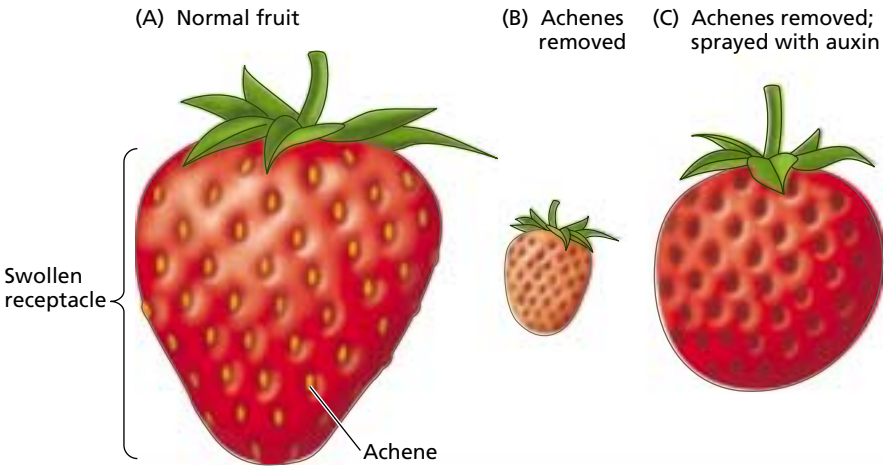


FIGURE 19.39 (A) The strawberry "fruit" is actually a swollen receptacle whose growth is regulated by auxin produced by the "seeds," which are actually achenes–the true fruits. (B) When the achenes are removed, the receptacle fails to develop normally. (C) Spraying the achene-less receptacle with IAA restores normal growth and development. (After A. Galston 1994.)

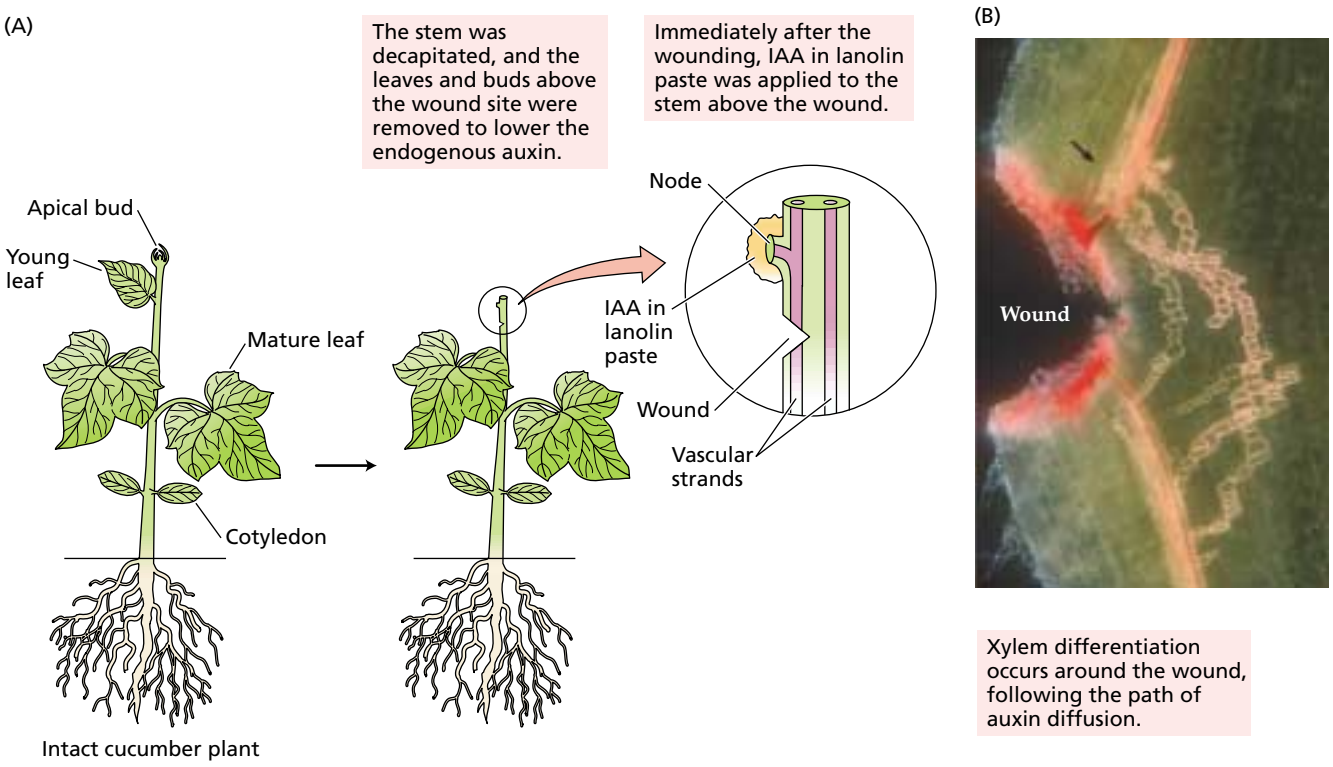


FIGURE 19.40 IAA-induced xylem regeneration around the wound in cucumber (*Cucumis sativus*) stem tissue. (A) Method for carrying out the wound regeneration experiment. (B) Fluorescence micrograph showing regenerating vascular tissue around the wound. (B courtesy of R. Aloni.)

a basipetal direction. The new round of secondary growth begins at the smallest twigs and progresses downward toward the root tip.

Further evidence for the role of auxin in vascular differentiation comes from studies in which the auxin concentration is manipulated by the transformation of plants with auxin biosynthesis genes through use of the Ti plasmid of *Agrobacterium*. When an auxin biosynthesis gene was overexpressed in petunia plants, the number of xylem tracheary elements increased. In contrast, when the level of free IAA in tobacco plants was decreased by transformation with a gene coding for an enzyme that conjugated IAA to the amino acid lysine, the number of vessel elements decreased and their sizes increased (Romano et al. 1991). Thus the level of free auxin appears to regulate the number of tracheary elements, as well as their size.

In *Zinnia elegans* mesophyll cell cultures, auxin is required for tracheary cell differentiation, but cytokinins also participate, perhaps by increasing the sensitivity of the cells to auxin. Whereas auxin is produced in the shoot and transported downward to the root, cytokinins are produced by the root tips and transported upward into the shoot. Both hormones are probably involved in the regulation of cambium activation and vascular differentiation (see Chapter 21).

Synthetic Auxins Have a Variety of Commercial Uses

Auxins have been used commercially in agriculture and horticulture for more than 50 years. The early commercial uses included prevention of fruit and leaf drop, promotion of flowering in pineapple, induction of parthenocarpic fruit, thinning of fruit, and rooting of cuttings for plant propagation. Rooting is enhanced if the excised leaf or stem cutting is dipped in an auxin solution, which increases the initiation of adventitious roots at the cut end. This is the basis of commercial rooting compounds, which consist mainly of a synthetic auxin mixed with talcum powder.

In some plant species, seedless fruits may be produced naturally, or they may be induced by treatment of the unpollinated flowers with auxin. The production of such seedless fruits is called **parthenocarpy**. In stimulating the formation of parthenocarpic fruits, auxin may act primarily to induce fruit set, which in turn may trigger the endogenous production of auxin by certain fruit tissues to complete the developmental process.

Ethylene is also involved in fruit development, and some of the effects of auxin on fruiting may result from the promotion of ethylene synthesis. The control of ethylene in the commercial handling of fruit is discussed in Chapter 22.

In addition to these applications, today auxins are widely used as herbicides. The chemicals 2,4-D and dicamba (see Figure 19.4) are probably the most widely used synthetic auxins. Synthetic auxins are very effective because they are not metabolized by the plant as quickly as IAA is. Because maize and other monocotyledons can rapidly inactivate synthetic auxins by conjugation, these auxins are used by farmers for the control of dicot weeds, also called *broad-leaved weeds*, in commercial cereal fields, and by home gardeners for the control of weeds such as dandelions and daisies in lawns.

AUXIN SIGNAL TRANSDUCTION PATHWAYS

The ultimate goal of research on the molecular mechanism of hormone action is to reconstruct each step in the signal transduction pathway, from receptor binding to the physiological response. In this last section of the chapter, we will examine candidates for the auxin receptor and then discuss the various signaling pathways that have been implicated in auxin action. Finally we will turn our attention to auxin-regulated gene expression.

ABP1 Functions as an Auxin Receptor

In addition to its possible direct role in plasma membrane H^+-ATPase activation (discussed earlier), the auxin-binding protein ABP1 appears to function as an auxin receptor in other signal transduction pathways. ABP1 homologs have been identified in a variety of monocot and dicot species (Venis and Napier 1997). Knockouts of the *ABP1* gene in *Arabidopsis* are lethal, and less severe mutations result in altered development (Chen et al. 2001). Recent studies indicate that, despite being localized primarily on the endoplasmic reticulum (ER), a small amount of ABP1 is secreted to the plasma membrane outer surface where it interacts with auxin to cause protoplast swelling and H^+-pumping (Venis et al. 1996; Steffens et al. 2001).

However, it is unlikely that ABP1 mediates all auxin response pathways because expression of a number of auxin-responsive genes is not affected when protoplasts are incubated with anti-ABP1 antibodies. It is also unclear what role the ABP1 in the ER plays in auxin-responsive signal transduction. Finally, it remains to be determined whether ABP_{57}, the soluble and unrelated ABP from rice that activates the H^+-ATPase (see Figure 19.24), is involved in a signal transduction pathway.

Calcium and Intracellular pH Are Possible Signaling Intermediates

Calcium plays an important role in signal transduction in animals and is thought to be involved in the action of certain plant hormones as well. The role of calcium in auxin action seems very complex and, at this point in time, very uncertain. Nevertheless, some experimental evidence shows that auxin increases the level of free calcium in the cell.

Changes in cytoplasmic pH can also serve as a second messenger in animals and plants. In plants, auxin induces a decrease in cytosolic pH of about 0.2 units within 4 minutes of application. The cause of this pH drop is not known. Since the cytosolic pH is normally around 7.4, and the pH optimum of the plasma membrane H^+-ATPase is 6.5, a decrease in the cytosolic pH of 0.2 units could cause a marked increase in the activity of the plasma membrane H^+-ATPase. The decrease in cytosolic pH might also account for the auxin-induced increase in free intracellular calcium, by promoting the dissociation of bound forms.

MAP kinases (see Chapter 14 on the web site) that play a role in signal transduction by phosphorylating proteins in a cascade that ultimately activates transcription factors have also been implicated in auxin responses. When tobacco cells are deprived of auxin, they arrest at the end of either the G_1 or the G_2 phase and cease dividing; if auxin is added back into the culture medium, the cell cycle resumes (Koens et al. 1995). (For a description of the cell cycle, see Chapter 1.) Auxin appears to exert its effect on the cell cycle primarily by stimulating the synthesis of the major cyclin-dependent protein kinase (CDK): Cdc2 (*c*ell *d*ivision *c*ycle 2) (see Chapter 14 on the web site).

Auxin-Induced Genes Fall into Two Classes: Early and Late

One of the important functions of the signal transduction pathway(s) initiated when auxin binds to its receptor is the activation of a select group of transcription factors. The activated transcription factors enter the nucleus and promote the expression of specific genes. Genes whose expression is stimulated by the activation of preexisting transcription factors are called **primary response genes** or **early genes**.

This definition implies that all of the proteins required for auxin-induced expression of the early genes are present in the cell at the time of exposure to the hormone; thus, early-gene expression cannot be blocked by inhibitors of protein synthesis such as cycloheximide. As a consequence, the time required for the expression of the early genes can be quite short, ranging from a few minutes to several hours (Abel and Theologis 1996).

In general, primary response genes have three main functions: (1) Some of the early genes encode proteins that regulate the transcription of **secondary response genes**, or **late genes**, that are required for the long-term responses to the hormone. Because late genes require de novo protein synthesis, their expression can be blocked by protein synthesis inhibitors. (2) Other early genes are involved in intercellular communication, or cell-to-cell signaling. (3) Another group of early genes is involved in adaptation to stress.

Five major classes of early auxin-responsive genes have been identified:

- Genes involved in auxin-regulated growth and development:
    1. The *AUX/IAA* gene family
    2. The *SAUR* gene family
    3. The *GH3* gene family
- Stress response genes:
    1. Genes encoding glutathione S-transferases
    2. Genes encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, the key enzyme in the ethylene biosynthetic pathway (see Chapter 22)

Early genes for growth and development. Members of the *AUX/IAA* gene family encode short-lived transcription factors that function as repressors or activators of the expression of late auxin-inducible genes. The expression of most of the *AUX/IAA* family of genes is stimulated by auxin within 5 to 60 minutes of hormone addition All the genes encode small hydrophilic polypeptides that have putative DNA-binding motifs similar to those of bacterial repressors. They also have short half-lives (about 7 minutes), indicating that they are turning over rapidly.

The *SAUR* gene family was mentioned earlier in the chapter in relation to tropisms. Auxin stimulates the expression of *SAUR* genes within 2 to 5 minutes of treatment, and the response is insensitive to cycloheximide. The five *SAUR* genes of soybean are clustered together, contain no introns, and encode highly similar polypeptides of unknown function. Because of the rapidity of the response, expression of *SAUR* genes has proven to be a convenient probe for the lateral transport of auxin during photo- and gravitropism.

GH3 early-gene family members, identified in both soybean and *Arabidopsis*, are stimulated by auxin within 5 minutes. Mutations in *Arabidopsis GH3*-like genes result in dwarfism (Nakazawa et al. 2001) and appear to function in light-regulated auxin responses (Hsieh et al. 2000). Because *GH3* expression is a good reflection of the presence of endogenous auxin, a synthetic *GH3*-based **reporter** gene known as *DR5* is widely used in auxin bioassays (see Figure 19.5 and **Web Topic 19.12**) (Ulmasov et al. 1997).

Early genes for stress adaptations. As mentioned earlier in the chapter, auxin is involved in stress responses, such as wounding. Several genes encoding glutathione-S-transferases (GSTs), a class of proteins stimulated by various stress conditions, are induced by elevated auxin concentrations. Likewise, ACC synthase, which is also induced by stress and is the rate-limiting step in ethylene biosynthesis (see Chapter 22), is induced by high levels of auxin.

To be induced, the promoters of the early auxin genes must contain response elements that bind to the transcription factors that become activated in the presence of auxin. A limited number of these response elements appear to be arranged combinatorily within the promoters of a variety of auxin-induced genes.

Auxin-Responsive Domains Are Composite Structures

A conserved **auxin response element** (**AuxRE**) within the promoters of the early auxin genes, like *GH3*, is usually combined with other response elements to form **auxin response domains** (**AuxRDs**). For example, the *GH3* gene promoter of soybean is composed of three independently acting AuxRDs (each containing multiple AuxREs) that contribute incrementally to the strong auxin inducibility of the promoter.

Early Auxin Genes Are Regulated by Auxin Response Factors

As noted previously, early auxin genes are by definition insensitive to protein synthesis inhibitors such as cycloheximide. Instead of being inhibited, the expression of many of the early auxin genes has been found to be stimulated by cycloheximide.

Cycloheximide stimulation of gene expression is accomplished both by transcriptional activation and by mRNA stabilization. Transcriptional activation of a gene by inhibitors of protein synthesis usually indicates that the gene is being repressed by a short-lived repressor protein or by a regulatory pathway that involves a protein with a high turnover rate.

A family of **auxin response factors** (**ARFs**) function as transcriptional activators by binding to the auxin response element TGTCTC, which is present in the promoters of *GH3* and other early auxin response genes. Mutations in ARF genes result in severe developmental defects. To bind the AuxRE stably, ARFs must form dimers. It has been proposed that ARF dimers promote transcription by binding to two AuxREs arranged in a palindrome (Ulmasov et al. 1997).

Recent studies also indicate that proteins encoded by the *AUX/IAA* gene family (itself one of the early auxin response gene families) can inhibit the transcription of early auxin response genes by forming inactive heterodimers with ARFs. These inactive heterodimers may act to inhibit ARF–AuxRE binding, thereby blocking either gene activation or repression. AUX/IAA proteins may thus function as ARF inhibitors.

It is now believed that auxin induces the transcription of the early response genes by promoting the proteolytic degradation of the inhibitory AUX/IAA proteins so that active ARF dimers can form. The precise mechanism by

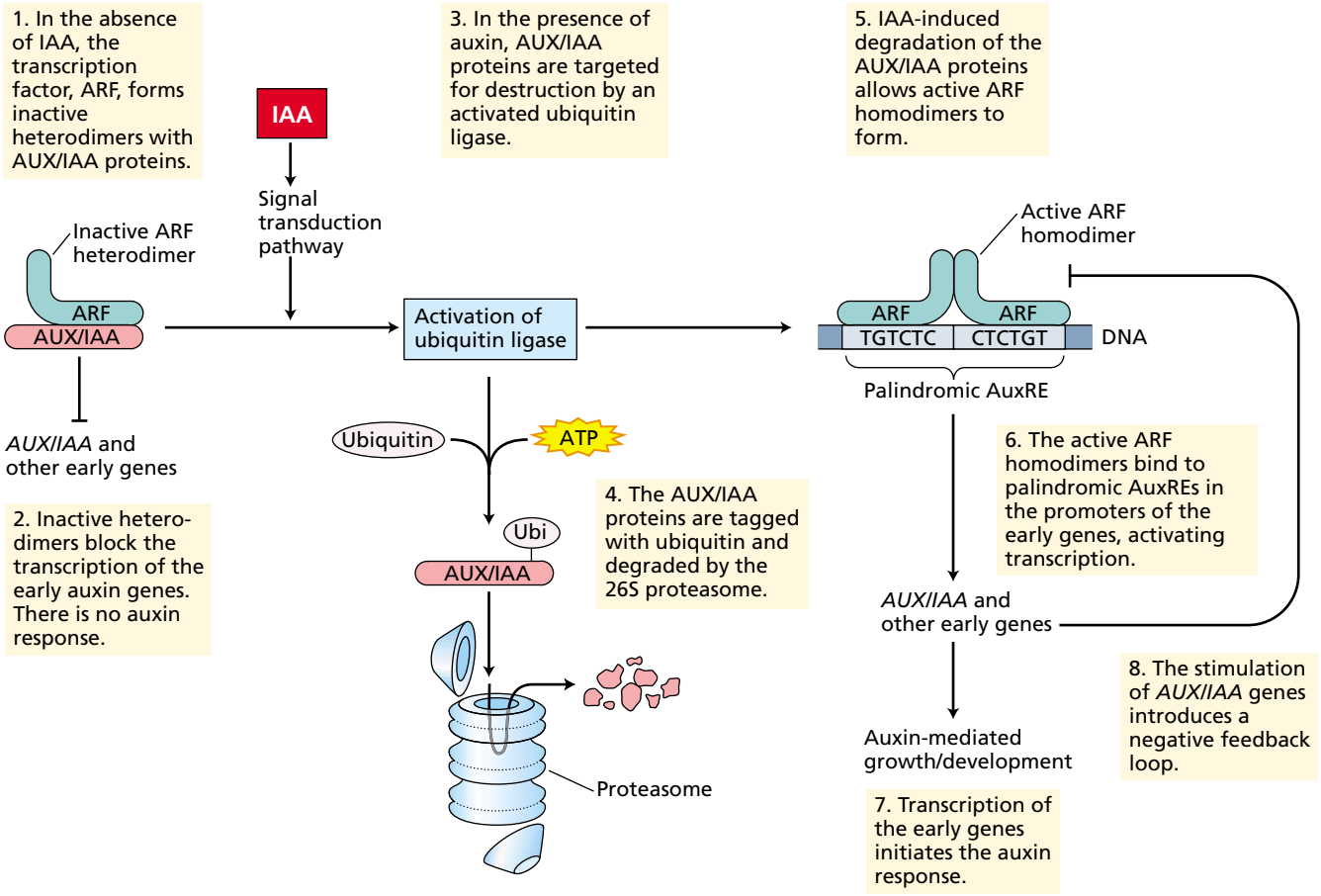


FIGURE 19.41 A model for auxin regulation of transcriptional activation of early response genes by auxin. (After Gray et al. 2001.)

which auxin causes AUX/IAA turnover is unknown, although it is known to involve ubiquitination by a ubiquitin ligase and proteolysis by the massive 26S proteasome complex (see Chapter 14 on the web site) (Gray et al. 2001; Zenser et al. 2001). Note that a negative feedback loop is introduced into the pathway by virtue of the fact that one of the gene families turned on by auxin is *AUX/IAA*, which inhibits the response.

A model for auxin regulation of the early response genes based on the findings described here is shown in Figure 19.41.

SUMMARY

Auxin was the first hormone to be discovered in plants and is one of an expanding list of chemical signaling agents that regulate plant development. The most common naturally occurring form of auxin is indole-3-acetic acid (IAA). One of the most important roles of auxin in higher plants is the regulation of elongation growth in young stems and coleoptiles. Low levels of auxin are also required for root elongation, although at higher concentrations auxin acts as a root growth inhibitor.

Accurate measurement of the amount of auxin in plant tissues is critical for understanding the role of this hormone in plant physiology. Early coleoptile-based bioassays have been replaced by more accurate techniques, including physicochemical methods and immunoassay.

Regulation of growth in plants may depend in part on the amount of free auxin present in plant cells, tissues, and organs. There are two main pools of auxin in the cell: the cytosol and the chloroplasts. Levels of free auxin can be modulated by several factors, including the synthesis and breakdown of conjugated IAA, IAA metabolism, compartmentation, and polar auxin transport. Several pathways have been implicated in IAA biosynthesis, including tryptophan-dependent and tryptophan-independent pathways. Several degradative pathways for IAA have also been identified.

IAA is synthesized primarily in the apical bud and is transported polarly to the root. Polar transport is thought to occur mainly in the parenchyma cells associated with the vascular tissue. Polar auxin transport can be divided into two main processes: IAA influx and IAA efflux. In accord with the chemiosmotic model for polar transport, there are two modes of IAA influx: by a pH-dependent passive transport of the undissociated form, or by an active H^+

cotransport mechanism driven by the plasma membrane H^+-ATPase.

Auxin efflux is thought to occur preferentially at the basal ends of the transporting cells via anion efflux carriers and to be driven by the membrane potential generated by the plasma membrane H^+-ATPase. Auxin transport inhibitors (ATIs) can interrupt auxin transport directly by competing with auxin for the efflux channel pore or by binding to regulatory or structural proteins associated with the efflux channel. Auxin can be transported nonpolarly in the phloem.

Auxin-induced cell elongation begins after a lag time of about 10 minutes. Auxin promotes elongation growth primarily by increasing cell wall extensibility. Auxin-induced wall loosening requires continuous metabolic input and is mimicked in part by treatment with acidic buffers.

According to the acid growth hypothesis, one of the important actions of auxin is to induce cells to transport protons into the cell wall by stimulating the plasma membrane H^+-ATPase. Two mechanisms have been proposed for auxin-induced proton extrusion: direct activation of the proton pump and enhanced synthesis of the plasma membrane H^+-ATPase. The ability of protons to cause cell wall loosening is mediated by a class of proteins called expansins. Expansins loosen the cell wall by breaking hydrogen bonds between the polysaccharide components of the wall. In addition to proton extrusion, long-term auxin-induced growth involves the uptake of solutes and the synthesis and deposition of polysaccharides and proteins needed to maintain the acid-induced wall-loosening capacity.

Promotion of growth in stems and coleoptiles and inhibition of growth in roots are the best-studied physiological effects of auxins. Auxin-promoted differential growth in these organs is responsible for the responses to directional stimuli (i.e., light, gravity) called tropisms. According to the Cholodny–Went model, auxin is transported laterally to the shaded side during phototropism and to the lower side during gravitropism. Statoliths (starch-filled amyloplasts) in the statocytes are involved in the normal percepton of gravity, but they are not absolutely required.

In addition to its roles in growth and tropisms, auxin plays central regulatory roles in apical dominance, lateral-root initiation, leaf abscission, vascular differentiation, floral bud formation, and fruit development. Commercial applications of auxins include rooting compounds and herbicides.

The auxin-binding soluble protein ABP1 is a strong candidate for the auxin receptor. ABP1 is located primarily in the ER lumen. Studies of the signal transduction pathways involved in auxin action have implicated other signaling intermediates such as Ca^{2+}, intracellular pH, and kinases in auxin-induced cell division.

Auxin-induced genes fall into two categories: early and late. Induction of early genes by auxin does not require protein synthesis and is insensitive to protein synthesis inhibitors. The early genes fall into three functional classes: expression of the late genes (secondary response genes), stress adaptation, and intercellular signaling. The auxin response domains of the promoters of the auxin early genes have a composite structure in which an auxin-inducible response element is combined with a constitutive response element. Auxin-induced genes may be negatively regulated by repressor proteins that are degraded via a ubiquitin activation pathway.

Web Material

Web Topics

19.1 Additional Synthetic Auxins

Biologically active synthetic auxins have suprisingly diverse structures.

19.2 The Structural Requirements for Auxin Activity

Comparisons of a wide variety of compounds that possess auxin activity have revealed common features at the molecular level that are essential for biological activity.

19.3 Auxin Measurement by Radioimmunoassy

Radioimmunoassay (RIA) allows the measurement of physiological levels (10^{-9} g = 1 ng) of IAA in plant tissues.

19.4 Evidence for the Tryptophan-Independent Biosynthesis of IAA

Additional experimental evidence for the tryptophan-independent biosynthesis of IAA is provided.

19.5 The Multiple Factors That Regulate Steady-State IAA Levels

The steady-state level of free IAA in the cytosol is determined by several interconnected processes, including synthesis, degradation, conjugation, compartmentation and transport.

19.6 The Mechanism of Fusicoccin Activation of the Plasma Membrane H^+-ATPase

Fusicoccin, a phytotoxin produced by the fungus *Fusicoccum amygdale*, causes membrane hyperpolarization and proton extrusion in nearly all plant tissues, and acts as a "super-auxin" in elongation assays.

19.7 The Fluence Response of Phototropism

The effect of light dose on phototropism is described and a model explaining the phenomenon is presented.

19.8 Differential *SAUR* Gene Expression during Gravitropism

SAUR gene expression is used to detect the lateral auxin gradient during gravitropism.

19.9 Gravity Perception without Statoliths in *Chara*

The giant-celled freshwater alga, *Chara*, bends in response to gravity without any apparent statoliths.

19.10 The Role of Cytokinins in Apical Dominance

In Douglas fir *Psuedotsuga menziesii*, there is a correlation between cytokinin levels and axillary bud growth.

19.11 The Role of ABA in Apical Dominance

In Quackgrass (*Elytrigia repens*) axillary bud growth is correlated with a reduction in ABA.

19.12 The Facilitation of IAA Measurements by *GH3*-Based Reporter Constructs

Because *GH3* expression is a good reflection of the presence of endogenous auxin, a *GH3*-based **reporter** gene, known as *DR5*, is widely used in auxin bioassays.

19.13 The Effect of Auxin on Ubiquitin-Mediated Degradation of AUX/IAA Proteins

A model for auxin-regulated degradation of AUX/IAA proteins is discussed.

Web Essays

19.1 Brassinosteroids: A New Class of Plant Steroid Hormones

Brassinosteroids have been implicated in a wide range of developmental phenomena in plants, including stem elongation, inhibition of root growth, and ethylene biosynthesis.

19.2 Exploring the Cellular Basis of Polar Auxin Transport.

Experimental evidence indicates that the polar transport of the plant hormone auxin is regulated at the cellular level. This implies that proteins involved in auxin transport must be asymmetrically distributed on the plasma membrane. How those transport proteins get to their destination is the focus of ongoing research.

19.3 Phototropism: From Photoperception to Auxin-Dependent Changes in Gene Expression

How photoperception by phototropins is coupled to auxin signaling is the subject of this essay.

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