*The hormone concept in plants*

The concept of hormones, the chemical messengers that enable cells to communicate with one another, arose in the study of mammalian physiology. The latter half of the nineteenth century witnessed exciting advances in physiology and medicine. The German botanist Julius Sachs (ca. 1860) postulated specific organ-forming substances in plants. He postulated that root-forming substances, for example, produced in the leaves and migrating down the stem, would account for the initiation of roots above the wound. The real beginning of plant hormone research, however, is found in a series of simple but elegant experiments conducted by Charles Darwin. It was Darwin's observations and experiments that ultimately led F. W. Went, almost half a century later, to describe a hormonal-like substance as the causative agent when plants grew toward the light. At about the same time, H. Fitting introduced the term hormone into the plant physiology literature.

Hormones are naturally occurring, organic molecules that, at low concentration, exert a profound influence on physiological processes. In addition, hormones, as defined by animal physiologists, are (1) *synthesized in a discrete organ or tissue*, and (2) *transported in the bloodstream to a specific target tissue* where they (3) *control a physiological response in a concentration-dependent manner*. While there are many parallels between animal and plant hormones, there are also some significant differences. Like animal hormones, plant hormones are naturally occurring organic substances that profoundly influence physiological processes at low concentration. The site of synthesis and mode of transport for plant hormones, however, is not always so clearly localized. Although some tissues or parts of tissues may be characterized by higher hormone levels than others, synthesis of plant hormones appears to be much more diffuse and cannot always be localized to discrete organs.

**3.3. Auxins**

*The discovery of auxin: the first plant growth hormone*

Plant hormones have been the subject of intensive investigation since auxin was first discovered almost a century ago. Darwin developed an interest in certain aspects of plant physiology. Some of these studies were summarized in the book “The Power of Movement in Plants”, co-authored by his son, Francis. One of several “movements” studied by the Darwins was the tendency of canary grass (*Phalaris canariensis*) seedlings to bend toward the light coming from a window, a phenomenon we now know as *phototropism*.

Following the publication of Darwin's book, a number of scientists confirmed and extended their observations. In 1910, Boysen-Jensen demonstrated that the stimulus would pass through an agar block and was therefore chemical in nature. In 1918, Paál showed that if the apex were removed and replaced asymmetrically, curvature would occur even in darkness (**Figure 3.10**). The active substance was first successfully isolated in 1928 by F. W. Went, then a graduate student working in his father's laboratory in Holland. Following up on the earlier work of Boysen-Jensen and Paál, Went removed the apex of oat (*Avena sativa*) coleoptiles and stood the apical pieces on small blocks of agar. Allowing a period of time for the substance to diffuse from the tissue into the agar block, he then placed each agar block asymmetrically on a freshly decapitated coleoptile. The substance then diffused from the block into the coleoptile, preferentially stimulating elongation of the cells on the side of the coleoptile below the agar block. Curvature of the coleoptile was due to differential cell elongation on the two sides. Moreover, the curvature proved to be proportional to the amount of active substance in the agar. Went's work was particularly significant in two respects: first, he confirmed the existence of regulatory substances in the coleoptile apex, and second, he developed a means for isolation and quantitative analysis of the active substance. Because Went used coleoptiles from *Avena* seedlings, his quantitative test became known as the *Avena curvature test.* Substances active in this test were called auxin, from the Greek auxein (to increase). Physiology of plant growth and development

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**Figure 3.10** The growth promoting stimulus has chemical in nature (*source: Taiz L., Zeiger E., 2010*)

*Chemical structure and biosynthesis of auxin*

*The pricipal natural auxin is indole-3-acetic acid*

Although a large number of compounds have been discovered with auxin activity, **indole-3-acetic acid (IAA)** is the most widely distributed natural auxin. Several other auxins in higher plants were discovered later, but IAA is by far the most abundant and physiologically important. Because the structure of IAA is relatively simple (**Figure 3.11**), academic and industrial laboratories were quickly able to synthesize a wide array of molecules with auxin activity. Some of these compounds are now used widely as herbicides in horticulture and agriculture. 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.

F**igure 3.11** Sructures of naturally occuring auxins (*source: Taiz L., Zeiger E., 2010*)

*IAA is synthesized in meristems, young leaves, and developing fruits and seeds*

IAA biosynthesis is associated with rapidly dividing and growing tissues, especially in shoots. Although virtually all plant tissues appear to be capable of producing low levels of IAA, shoot apical meristems and young leaves are the primary sites of auxin synthesis. Root apical meristems are also important sites of auxin synthesis, especially as the roots elongate and mature, although the root remains dependent on the shoot for much of its auxin. Young fruits and seeds contain high levels of auxin, but it is unclear whether this auxin is newly synthesized or transported from maternal tissues during development.

*Multiple pathways exist for the biosynthesis of IAA*

IAA is structurally related to the amino acid tryptophan, and to the tryptophan precursor indole-3-glycerol phosphate, both of which can serve as precursors for IAA biosynthesis. Molecular genetic and radioisotope labeling studies have been used to identify the enzymes and intermediate molecules involved in tryptophan-dependent IAA biosynthesis, and the order in which they function. Multiple biosynthetic pathways using tryptophan as a precursor have been shown to produce IAA in plants, and a bacterial pathway of tryptophan-dependent IAA biosynthesis has also been identified. Auxin can be covalently bound to both high and low molecular weight compounds, particularly in seeds and storage organs such as cotyledons. IAA can be conjugated to many different low molecular weight compounds like amino acids or sugars, or to high molecular weight molecules like peptides, complex glycans (multiple sugar units), or glycoproteins. IAA is rapidly released from many, but not all, conjugates by enzymatic processes. Those conjugates that can release free auxin serve as reversible storage forms of the hormone. Physiology of plant growth and development

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*IAA is degraded by multiple pathways*

To be effective developmental signals, hormones must be short-lived and should not accumulate over time. Auxin catabolism ensures the degradation of active hormone when the concentration exceeds the optimal level or when the response to the hormone is complete. Like IAA biosynthesis, the enzymatic breakdown (oxidation) of IAA involves more than one pathway. On the basis of isotopic labeling and metabolite identification, two oxidative pathways are probably involved in the controlled degradation of IAA. In one pathway, the indole moiety of IAA is oxidized to form oxindole-3-acetic acid (OxIAA) and subsequently, OxIAA-glucose (OxIAA-Gluc). In another pathway, IAA-aspartate conjugates are oxidized to OxIAA.

*Auxin transport*

The main axes of shoots and roots, along with their branches, exhibit apex-base structural polarity, and this structural polarity is dependent on 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 that has been clearly shown to be transported polarly, and polar transport of this hormone is found in almost all plants.

Because the shoot apex serves as the primary source of auxin in the 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 major sites of polar auxin transport in the stems, leaves, and roots of most plants are the vascular parenchyma tissues, most likely those associated with the xylem. In grass coleoptiles, basipetal polar transport may also occur in nonvascular parenchyma tissues. Embryonic polar auxin transport is initially described as entirely basipetal, as the embryo has no root. The downward direction of auxin transport in the embryonic vascular parenchyma is maintained in the root vascular cylinder throughout the life of the plant.

A chemiosmotic model for polar auxin transport proposes that auxin uptake is driven by the proton motive force across the plasma membrane, while auxin efflux is driven by the membrane potential (**Figure 3.12**). The first step in polar transport is auxin influx. Auxin enters plant cells nondirectionally via passive diffusion of the protonated form (IAAH) across the phospholipid bilayer or via secondary active transport of the dissociated form (IAA-) through a 2H+-IAA-symporter. Once IAA enters the cytosol, which has a pH of approximately 7.2, nearly all of it dissociates to the anionic form. Because the membrane is impermeable to the anion, auxin accumulates inside the cell or along membrane surfaces unless it is exported by transport proteins on the plasma membrane. According to the chemiosmotic model, transport of IAA- out of the cell is driven by the negative membrane potential inside the cell.

Several compounds have been synthesized that can act as auxin transport inhibitors, including NPA (l-N-naphthylphthalamic acid), TIBA (2,3,5-triiodobenzoic acid), CPD (2-carboxyphenyl-3-phenylpropane-l,3-dione), NOA (l-napthoxyacetic acid), 2-[4-(diethylamino) -Z-hydroxybenzoyl] benzoic acid, and gravacin. NPA, TIBA, CPD, and gravacin are auxin efflux inhibitors (AEIs), while NOA is an auxin influx inhibitor. Some AEIs, such as TIBA, have weak auxin activity and inhibit polar transport in part by competing with auxin at the efflux carrier site. Other AEIs, such as CPD, NPA, and gravacin interfere with auxin transport by binding to a regulatory site. Some natural compounds, primarily flavonoids, also function as auxin efflux inhibitors. Physiology of plant growth and development

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**Figure 3.12** The chemiosmotic model for polar auxin transport (*source: Taiz L., Zeiger E., 2010*)

*Auxin signal transduction pathway*

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 the case of auxin, this would seem to be a particularly daunting task because auxin affects so many physiological and developmental processes. However, the initial steps in auxin signaling are surprisingly simple, and involve binding to a small group of receptor-enzyme complexes that regulate protein degradation via the ubiquitin-proteasome pathway. Upon activation by auxin, the receptor-enzyme complex targets specific transcriptional repressors for proteolysis, resulting in the activation and derepression of auxin-responsive genes. While this mechanism appears to account for most auxin responses, a different type of auxin receptor protein may function in nontranscriptional activation and mobilization of plasma membrane H+-ATPases to cause rapid cell wall acidification and cell elongation.

*Effects of auxin on growth and development*

Although originally discovered in relation to growth, auxin influences nearly every stage of a plant's life cycle from germination to senescence. The morphology of a plant depends on the directed movement of auxin via the polar transport system, which maintains both basic shoot-root polarity and polarized outgrowth throughout development.

*Auxins promote growth in stems and coleoptiles, while inhibiting growth in roots*

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. Such spraying may even be inhibitory in the case of dark-grown seedlings, which are more sensitive to supraoptimal auxin concentrations than light-grown plants.

In long-term experiments, auxin treatment of excised sections of coleoptiles or dicot stems stimulates the rate of elongation of the section for up to 20 hours. The optimal auxin concentration for elongation growth in pea stems and oat coleoptiles is typically 10-6 to 10-5 M. The inhibition observed when auxin concentrations exceed optimal levels is attributed mainly to auxin-induced ethylene biosynthesis.

Auxin control of root elongation has been more difficult to demonstrate, perhaps because auxin induces the production of ethylene, which also inhibits root growth. Recent evidence shows that these two hormones interact differentially in root tissue to control growth. 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, while roots may require a minimum concentration of auxin to Physiology of plant growth and development

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grow, root growth is strongly inhibited by auxin concentrations that promote elongation in stems and coleoptiles.

*The minimum lag time for auxin-induced elongation 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. Beyond the optimum concentration, auxin becomes inhibitory. Both oat (*Avena sativa*) coleoptiles and soybean (*Glycine max*) hypocotyls (dicot stems) reach a maximum growth rate after 30 to 60 minutes of auxin treatment. This maximum represents a five to tenfold increase over the basal rate. The stimulation of growth by auxin requires energy, and metabolic inhibitors inhibit the response within minutes

*Auxin induced proton extrusion increases cell extension*

According to the widely accepted **acid growth hypothesis**, hydrogen ions act as the intermediate between auxin and cell wall loosening (**Figure 3.13**). The source of the hydrogen ions is the plasma membrane H+-ATPase, whose activity is thought to increase in response to auxin. Auxin should increase the rate of proton extrusion (wall acidification), and the kinetics of proton extrusion should closely match those of auxin-induced growth. Cell walls should contain a “wall-loosening factor” with an acidic pH optimum.

**Figure 3.13** Kinetics of auxin-induced elongation and cell wall acidification in maize coleoptiles (*source: Taiz L., Zeiger E., 2010*)

*Phototropism is mediated by the lateral redistribution of auxin*

*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. 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 independently in the 1920s by two plant physiologists: Nicolai Cholodny in Russia and Frits Went in the Netherlands. According to the **Cholodny-Went model of phototropism**, the tips of grass coleoptiles are sites of high auxin concentration and have two other specialized functions: (1) the perception of a unilateral light stimulus, and (2) decrease in basipetal IAA transport and diversion to lateral transport 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.

*Gravitropism involves lateral redistribution of auxin*

*Gravitropism*, growth in response to gravity, enables roots to grow downward into the soil and shoots to grow upward away from the soil, responses that are especially critical during the early stages of germination. 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 could perceive gravity and redistribute auxin to the lower side. Physiology of plant growth and development

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For example, if coleoptile tips are oriented horizontally, a greater amount of auxin diffuses into an agar block from the lower half than from the upper half.

*Auxin regulates apical dominance*

In most higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds – a phenomenon called **apical dominance**. Removal of the shoot apex (decapitation) results in the outgrowth 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. The classic experiment performed on kidney bean (*Phaseolus vulgaris*) plants by Kenneth Thimann and Folke Skoog in the 1920s (**Figure 3.14**).

**Figure 3.14** Auxin supresses the growth of axillary buds in bean plants (*source: Taiz L., Zeiger E., 2002*)

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 terminal bud. This hypothesis was supported by experiments that showed that a ring of the auxin transport inhibitor TIBA in lanolin paste (as a carrier) placed below the shoot apex releases the axillary buds from inhibition. Measurements of auxin levels in axillary buds have shown that following decapitation, the auxin content of the buds actually increases. In addition, application of auxin directly to the terminal bud raises the auxin concentration in the shoot but fails to inhibit normal axillary bud outgrowth. Finally, experiments with radiolabeled auxin have shown that the auxin applied at the terminal bud does not enter apical buds.

*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. 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.

Increased auxin levels or application of auxin can promote the formation of adventitious roots (roots originating from nonroot tissue). Adventitious roots arise from differentiated cells that begin to divide and develop into a root apical meristem in a manner somewhat analogous to the formation of a lateral root primordium. 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.

*Auxin delays the onset of leaf abscision*

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, in the case of leaves, is located near the base of the petiole. 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. Physiology of plant growth and development

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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. However ethylene also plays a crucial role in the regulation of abscission.

*Auxin promotes fruit development*

Much evidence suggests that auxin is involved in the regulation of fruit development. Auxin is produced or mobilized from storage in pollen, 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 from 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.

**3.4. Gibberellins**

Gibberellins (**GAs**) are best known for their promotion of stem elongation, and GA-deficient mutants that have dwarf phenotypes have been isolated. Mendel's tall/dwarf alleles in peas are a famous example of a single gene locus that can control the level of bioactive GA and hence stem length. Such mutants have been useful in elucidating the complex pathways of GA biosynthesis, and in determining which of the GAs in a plant has intrinsic biological activity.

Although gibberellins did not become known to American and British scientists until the 1950s, they had been discovered much earlier by Japanese scientists. Rice farmers in Asia **3.4. Gibberellins**

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Although gibberellins did not become known to American and British scientists until the 1950s, they had been discovered much earlier by Japanese scientists. Rice farmers in Asia had long known of a disease that makes the rice plants grow tall but eliminates seed production. In Japan this disease was called the “foolish seedling” or *bakanae* disease. Plant pathologists investigating the disease found that the tallness of these plants was induced by a chemical secreted by a fungus that had infected the tall plants. This chemical was isolated from filtrates of the cultured fungus and called gibberellin after *Gibberella fujikuroi*, the name of the fungus.

*Their structure is made up from isoprenoid units, and they are synthesized via the terpenoid pathway*

Terpenes are a functionally and chemically diversegroup of molecules. With nearly 15,000 structures known, terpenes are probably the largest and most diverse class of organic compounds found in plants. The terpene family includes, in addition to the GAs, the hormones abscisic acid and brassinosteroids, the carotenoid pigments (carotene and xanthophyll), sterols (e.g., ergosterol, sitosterol, cholesterol) and sterol derivatives (e.g., cardiatic glycosides), latex (the basis for natural rubber), and many essential oils that give plants their distinctive odors and flavors.

The GAs are diterpenoids that are formed from four isoprenoid units each consisting of five carbons. They possess a tetra cyclic (four-ringed) ent-gibberellane skeleton (containing 20 carbon atoms) (**Figure 3.15**). Terpenoids are compounds made up of five-carbon isoprenoid building blocks. The GAs are diterpenoids that are formed from four such isoprenoid units. The GA biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment: plastid, ER, or cytosol. As more GAs from *Gibberella* and different plant sources were characterized, a scheme was adopted to number them (GA1-GAn) in chronological order of their discovery.

**Figure 3.15** The structures of GA4, GA1, GA7, and GA3 (*source: Taiz L., Zeiger E., 2010*) Physiology of plant growth and development

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*GA biosynthesis occurs in multiple plant organs, like germinating embryos, young seedlings, shoot apices, developing seeds, and even in some fungi*

It is generally accepted that there are three principal sites of gibberellin biosynthesis: (1) developing seeds and fruits, (2) the young leaves of developing aical buds and elongating shoots, and (3) the apical regions of roots. Immature seeds and fruits are prominent sites of gibberellin biosynthesis. This is based on the observation that young fruits, seeds, and seed parts contain large amount of gibberellins, particularly during stages of rapid increase in size. In addition, cell-free preparations from seeds are able to actively synthesize gibberellins. The site of gibberellin biosynthesis may be developing endosperm, the young cotyledons of legumes, or the scutellum of cereal grains. As the seed matures, metabolism appears to shift in favor of gibberellin-sugar conjugates. It is not as easy to obtain clear evidence that gibberellin biosynthesis occurs in shoots and roots. This is partly because gibberellin levels are much lower in vegetative tissues. Vegetative tissues also yield cell-free preparations that are less active, suggesting that enzyme levels for gibberellin metabolism are also lower than for reproductive tissues.

*The gibberellins are mobile and may act either locally or distant from their sites of synthesis*

Gibberellin transport studies have been conducted largely by application of radioactively labeled GAs to either stem or coleoptile sections. Gibberellins have been detected in both phloem and xylem saps. Transport of gibberellins does not appear to be polar, as it is with auxin, but moves along with other phloem-translocated organic materials according to a source-sink relationship. Whether gibberellins are actually transported in the xylem is not clear; they could end up there simply by lateral translocation from the phloem. On the other hand, it is likely that any gibberellins synthesized in the root tip are distributed to the aerial portions of the plant through the xylem stream. It is not known whether gibberellins are transported as free hormones or in conjugated form.

*Effects of gibberellins on growth and development*

Though they were originally identified as the cause of disease symptoms of rice that resulted in internode elongation, endogenous GAs can influence a large number of developmental processes in addition to stem elongation. Many of these properties of GAs have been exploited in agriculture for decades, and manipulation of the GA content of crop plants affects shoot size, fruit set, and fruit growth.

*Gibberellins promote seed germination via interrupting of dormancy*

Many seeds, particularly those of wild plant species, do not germinate immediately after dispersal from the mother plant, and may experience a period of dormancy. Dormant seeds will not germinate even if provided with water. Abscisic acid (ABA) and bioactive GA act in an antagonistic manner, and the relative amounts of the two hormones within the seed can, in many species, determine the degree of dormancy. Light or cold treatments of dormant seeds have been shown to lower the amount of ABA and increase the concentration of bioactive GA, ending dormancy and promoting germination. Treatment of seeds with bioactive GA can often substitute for the light or cold treatment needed to break dormancy.

During germination, GAs induce the synthesis of hydrolytic enzymes, such as amylases and proteases in cereal grains (**Figure 3.16**). These enzymes degrade the stored food reserves accumulated in the endosperm or embryo as the seed matured. This degradation of carbohydrates and storage proteins provides nourishment and energy to support seedling growth. Physiology of plant growth and development

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**Figure 3.16** Gibberellin effect on enzyme synthesis (*source: Taiz L., Zeiger E., 2002*)

*GAs can stimulate stem and root growth*

GAs may not have dramatic effects on stem elongation in plants that are already “tall”, since bioactive GA may not to be limiting in some tall plants. However, applied GAs can promote internode elongation very dramatically in genetically dwarf mutants, in “rosette” species, and in various members of the *Poaceae* (grass family). Exogenous GA causes such extreme stem elongation in dwarf maize plants that they resemble the tallest varieties of the same species.

Gibberellins are also important for root growth. Extreme dwarf mutants of pea and *Arabidopsis*, in which GA biosynthesis is blocked, have shorter roots than wild-type plants, and GA application to the shoot enhances both shoot and root elongation

*They regulate the transition from juvenile to adult phase*

Many woody perennials do not flower or produce cones until they reach a certain stage of maturity; up to that stage they are said to be juvenile. Applied GAs can regulate phase change, though whether GA hastens or retards the juvenile-to-adult transition will depend on the species. In many conifers, the juvenile phase, which may last up to 20 years, can be shortened by treatment with GA3 or with a mixture of GA4 and GA7, and much younger plants can be induced to enter the reproductive, cone-producing phase precociously.

*They have influence on floral initiation and sex determination*

GAs can substitute for the long-day requirement for flowering in many plants, especially rosette species. The interaction of photoperiod and GAs in flowering is complex. In plants with imperfect (unisexual) rather than perfect (hermaphroditic) flowers, sex determination is genetically regulated. However, it is also influenced by environmental factors such as photoperiod and nutritional status, and these environmental effects may be mediated by GAs. Just as in the case of the juvenile-to-adult transition, the nature of the effect of GA on sex determination can vary with species. In dicots such as cucumber (*Cucumis sativus*), hemp (*Cannabis sativa*), and spinach, GAs promote the formation of staminate (male) flowers, and inhibitors of GA biosynthesis promote the formation of pistillate (female) flowers. In some other plants, such as maize, GAs suppress stamen formation and promote pistil formation.

*GAs promote pollen development and tube growth*

Gibberellin-deficient dwarf mutants (e.g., in *Arabidopsis* and rice) have impaired anther development and pollen formation, and both these defects, which lead to male sterility, can be reversed by treatment with bioactive GA. In other mutants in which GA response (rather than GA biosynthesis) is blocked, the defects in anther and pollen development cannot be reversed by GA treatment, so these mutants are male-sterile. In addition, reducing the level of bioactive GA in *Arabidopsis* by overexpressing a GA deactivating enzyme severely inhibits pollen tube growth. Thus GAs seem to be required for both the development of the pollen grain and the formation of the pollen tube. Physiology of plant growth and development

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*Gibberellins promote fruit set and parthenocarpy*

Gibberellin application can cause fruit set (the initiation of fruit growth following pollination) and growth of some fruits. For example, stimulation of fruit set by GA has been observed in pear (*Pyrus communis*). GA-induced fruit set may occur in the absence of pollination, resulting in parthenocarpic fruit (fruit without seeds). In grape (*Vitis vinifera*), the “Thompson Seedless” variety normally produces small fruits because of early seed abortion. Fruits can be stimulated to enlarge by treatment with GA3. Both these effects of GAs on grapes are exploited commercially to produce large, seedless fruits.

*They promote early seed development and germination*

Some GA-deficient mutants, or transgenic plants with enhanced GA inactivation, have increased seed abortion. The failure of seeds to develop normally can be attributed to reduced levels of bioactive GAs in very young seeds. Treatment with GA will not restore normal seed development, because exogenous GA cannot enter the new seeds. However, the effect of GA deficiency on seed abortion can be negated by simultaneous expression of mutations that give a constitutive GA response. Taken together, these results provide evidence for a role for GA in the early stages of seed development.

**3.5. Cytokinins**

The cytokinins were discovered in the search for factors that stimulate plant cells to divide (i.e., undergo cytokinesis). A great many substances were tested in an effort to initiate and sustain the proliferation of normal stem tissues in culture. Materials ranging from yeast extract to tomato juice were found to have a positive effect, at least with some tissues. However, *in vitro* tissue culture growth was stimulated most dramatically when the liquid endosperm of coconut (also known as coconut water) was added to the culture medium.

In the 1940s and 1950s, Folke Skoog and co-workers at the University of Wisconsin tested many substances for their ability to initiate and sustain the proliferation of cultured tobacco pith tissue. They had observed that the nucleic acid base adenine had a slight promotive effect, so they tested the possibility that nucleic acids would stimulate division in this tissue. Surprisingly, aged or autoclaved herring sperm DNA had a powerful cell division-promoting effect. After much work, a small molecule was identified from the autoclaved DNA and named **kinetin**. It was shown to be an adenine (6-aminopurine) derivative, **6-furfurylaminopurine**. Kinetin is not a naturally occurring plant growth regulator, and it does not occur as a base in the DNA of any species. It is a by-product of the heat-induced degradation of DNA. Of greater importance, the discovery of kinetin suggested that naturally occurring molecules with structures similar to that of kinetin regulate cell division activity within the plant.

*Cytokinins N6-substituted adenine derivatives*

Naturally occurring cytokinins are all adenine derivatives with either an isoprene-related side chain or an aromatic (cyclic) side chain. The former are called *isoprenoid cytokinins* and the latter are called *aromatic cytokinins*. Although there is some variation depending on species and developmental stage, the most common isoprenoid cytokinins are **N6-(2-isopentenyl)-adenine (iP), *trans*-zeatin (tZ), and dihydrozeatin (DZ)** (**Figure 3.17**). The aromatic cytokinins, such as benzyladenine (BA) are less common and are found in only a few species. Physiology of plant growth and development

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**Figure 3.17** Structures of some naturally occurring cytokinins (*source: Taiz L., Zeiger E., 2010*)

**Zeatin** is the most abundant naturally occurring free cytokinin. Its molecular structure is similar to that of kinetin. Although they have different side chains, in both cases the side chain is linked to the nitrogen attached to C6 *(=N6)* of adenine. Because the side chain of zeatin has a double bond, it can exist in either the cis or the *trans* configuration. Since its discovery in immature maize endosperm, zeatin has been found in many plants and in some bacteria.

Cytokinin biosynthesis begins with the condensation of an isopentenyl group with the amino group of adenosine monophosphate. The reaction is catalyzed by the enzyme **adenosine phosphate-isopentenyl transferase (IPT)**. The IPT-catalyzed reaction is also the rate limiting reaction in cytokinin biosynthesis, a factor that has enabled many investigators to manipulate the cytokinin content of issues by transforming plants with genes that cause an overexpression of IPT.

Zeatin and iP are thought to be the most biologically active cytokinins in most plants. Reduction of the double bond in the side chain of zeatin would give the dihydrozeatin derivative, which is particularly active in some species of legumes.

*Cytokinins are synthesized in roots, developing embryos, young leaves, fruits*

A major site of cytokinin biosynthesis in higher plants is the root. High cytokinin levels have been found in roots, especially the mitotically active root tip, and in the xylem sap of roots from a variety of sources. It is generally concluded that roots are a principal source of cytokinins in most, if not all, plants and that they are transported to the aerial portion of the plant through the xylem. The delayed senescence when roots are allowed to form is apparently due to the presence of cytokinins, which are synthesized in the root and transported to the leaf through the vascular tissue.

Immature seeds and developing fruits also contain high levels of cytokinins; the first naturally occurring cytokinins were isolated from milky endosperm of maize and developing plum fruits. While there is some evidence that seeds and fruits are capable of synthesizing cytokinins, there is also evidence to the contrary. Thus it remains equally possible that developing seeds, because of their high metabolic activity and rapid growth, may simply function as a sink for cytokinins transported from the roots. On the other hand, there is now evidence that cytokinins are not always a long-distance messenger. Meristematic cells in the shoot apical meristem and floral meristems in particular are under the control of locally produced cytokinins.

Certain insects secrete cytokinins, which play a role in the formation of the galls these insects use as feeding sites. Root-knot nematodes also produce cytokinins, which may be involved in manipulating host development to produce the giant cells from which the nematode feeds. Physiology of plant growth and development

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*Cytokinin receptor and signaling*

In spite of the fundamental role played by cytokinins in cell division, the multiple other effects that cytokinins have on plant development have made it difficult to identify cytokinin receptors and signal chains. It has only been within the last decade, more than fifty years after Skoog and Miller purified the first cytokinin, that the first genes involved in cytokinin signaling have been identified.

The cytokinin receptor was finally discovered by T. Kakimoto and his colleagues who developed an *Arabidopsis* hypocotyl test to screen for mutants. Hypocotyl sections, or explants, respond to added cytokinins by typical cytokinin responses; rapid cell proliferation, greening, and shoot formation. The *cytokinin response 1 (cre1)* mutant shows none of these responses, even rith a tenfold increase in cytokinin concentration. This could be expected if the cytokinin receptor were either missing or nonfunctional in the mutant. Subsequent experiments confirmed that the wildtype protein CRE1 as in fact a cytokinin receptor.

*CRE1* is the first component of a **two-component regulatory system** – a type of regulatory system previously known to operate in bacteria and other prokaryotes. The name comes from the bacterial configuration where **receptor** (or sensor) – the first component – activate a **response regulator (RR)** – the second component. Response regulators in turn either regulate the transcription of target genes or modulate other metabolic reactions. In addition to serving as hormone receptors, two-component regulatory systems also function in osmosensing, light sensing, and other forms of sensory perception.

*Developmental and physiological effects of cytokinins*

Although discovered as a cell division factor, cytokinins can stimulate or inhibit a variety of physiological, metabolic, biochemical, and developmental processes when they are applied to higher plants, and it is increasingly clear that endogenous cytokinins play an important role in the regulation of these events in the intact plant.

*Cytokinins promote shoot growth by increasing cell proliferation in the shoot apical meristem*

Several lines of evidence suggest that cytokinins also play key roles in the regulation of cell division *in vivo*. Much of the cell division in an adult plant occurs in the meristems. Cytokinin plays a positive role in the proliferation of cells in the shoot apical meristem. Recall that elevated levels of cytokinins may result in fasciation of shoots, a condition resulting from over-proliferation of the shoot apical meristem. Reduction of cytokinin function by reducing endogenous cytokinin levels via overexpression of cytokinin oxidase or by mutation of the IPT genes results in the opposite effect, a substantial retardation of shoot development. Disruption of cytokinin perception (e.g., in a triple-receptor mutant) also results in a reduced shoot apical meristem, leading to a stunted shoot and little or no flower production.

*They inhibit root growth by promoting the exit of cells from the root apical meristem*

Cytokinin plays a very different role in the root apical meristem than it does in the shoot apical meristem. In contrast to its effect on the shoot, overexpression of cytokinin oxidase in tobacco increases root growth, primarily by increasing the size of the root apical meristem. Similarly, mutations that partially disrupt cytokinin perception also cause enhanced root growth. The mechanism by which cytokinins negatively regulate root apical meristems has recently been explored. The size of a meristem is determined by the rate at which cells divide minus the rate at which cells exit the meristem by growth and differentiation. Cytokinins accelerate the process of vascular differentiation in the root tip.

*Both cytokinin and auxin regulate the plant cell cycle and are needed for cell division*

Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle. Zeatin levels peak in synchronized culture tobacco cells at the end of S phase, the G2/M phase transition, and in late G1. Inhibition of cytokinin biosynthesis blocks cell division, and application of exogenous cytokinin allows cell division to proceed. Cytokinins were discovered in relation to their ability to stimulate cell division in tissues supplied with an optimal level of auxin. Evidence suggests that both auxin and cytokinins participate in regulating the cell cycle and that they do so by controlling the activity of cyclin-dependent kinases. *Cyclin-dependent protein kinases* (CDKs), in concert with their regulatory subunits, the cyclins, are enzymes that regulate the eukaryotic cell cycle.

*The auxin:cytokinin ratio regulates morphogenesis in cultured tissues* Physiology of plant growth and development

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Shortly after the discovery of kinetin, it was observed that the differentiation of cultured callus tissue derived from tobacco pith segments into either roots or shoots depends on the ratio of auxin to cytokinin in the culture medium. Whereas high auxin:cytokinin ratios stimulated the formation of roots, low auxin:cytokinin ratios led to the formation of shoots. At intermediate levels, the tissue grew as an undifferentiated tissue, called *callus* (**Figure 3.18**).

**Figure 3.18** Plate 4 from Skoog and Miller (1957) showing the effect of the auxin to cytokinin ratio on the pattern of development (*source: http://www.plantphysiol.org/cgi/doi/10.1104/pp.104.900160*)

*Cytokinins modify apical dominance and promote lateral bud growth*

One of the primary determinants of plant form is the degree of apical dominance. Plants with strong apical dominance, such as maize, have a single growing axis with few lateral branches. In contrast, many lateral buds initiate growth in shrubby plants. Branching patterns are normally determined by light, nutrients, and genotype. Physiologically, branching is regulated by a complex interplay of hormones, including auxin, cytokinin, and a recently identified root-derived signal. Auxin transported polarly from the apical bud suppresses the growth of axillary buds. In contrast, cytokinin stimulates cell division activity and outgrowth when applied directly to the axillary buds of many species, and cytokinin-overproducing mutants tend to be bushy. In the nodal region of pea stems, auxin was found to inhibit the expression of a subset of IPT genes, which encode the enzyme catalyzing the rate-limiting step in cytokinin biosynthesis, and to elevate the expression of cytokinin oxidase, which degrades cytokinins. The combined effect of the regulation of these genes by auxin is to keep cytokinin levels low in the apical buds. Removal of the shoot apex results in a decreased auxin flow, which allows IPT levels to rise and cytokinin oxidase levels to fall. The net effect of terminal bud removal is thus an increased concentration of cytokinin in the nodal area of the stem.

*Cytokinins delay leaf senescence, promote nutrient mobilization, help regulate the synthesis of photosynthetic pigments and proteins*

Leaves detached from the plant slowly lose chlorophyll, RNA, lipids, and protein, even if they are kept moist and provided with minerals. This programmed aging process leading to death is termed senescence. Leaf senescence is more rapid in the dark than in the light. Treating isolated leaves of many species with cytokinins delays their senescence. Although applied cytokinins do not prevent senescence completely, their effects can be dramatic, particularly when the cytokinin is sprayed directly on the intact plant. If only one leaf is treated, it remains green after other leaves of similar developmental age have yellowed and dropped off the plant. If a small spot on a leaf is treated with cytokinin, that spot will remain green after the surrounding tissues on the same leaf begin to senesce. The cytokinins involved in delaying senescence are primarily zeatin riboside and dihydrozeatin riboside, which may be transported into the leaves from the roots through the xylem, along with the transpiration stream.

Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as cytokinin-induced nutrient mobilization. Thus, the nutrient status of the plant regulates cytokinin levels, and in turn the ratio of cytokinin to auxin determines the relative growth rates of roots and shoots: High cytokinin concentrations promote shoot growth, and, conversely, high auxin levels promote root growth. In the presence Physiology of plant growth and development

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of low nutrient levels, cytokinin levels are also low, resulting in an increase in root growth and allowing the plant to more effectively acquire the nutrients present in the soil. In contrast, optimal levels of soil nutrients promote increased cytokinin levels, which favor shoot growth, thus maximizing photosynthetic capacity.

If etiolated leaves are treated with cytokinin before being illuminated, they form chloroplasts with more extensive grana, and chlorophyll and photosynthetic enzy.mes are synthesized at a greater rate upon illumination. These results suggest that cytokinins – along with other factors, such as light, nutrition, and development – regulate the synthesis of photosynthetic pigments and proteins.

*Cytokinin-overproducing plants have delayed senescence and yield more grain*

Some of the consequences of altering cytokinin function could be highly beneficial for agriculture if synthesis of the hormone can be controlled. Because leaf senescence is delayed in the cytokinin-overproducing plants, it should be possible to extend their photosynthetic productivity. Indeed, when an ipt gene is expressed in lettuce from a senescence-inducible promoter, leaf senescence is strongly retarded, similar to the results observed in tobacco (**Figure 3.19**).

**Figure 3.19** Leaf senescence is retarded in a transgenic tobacco plant containing a cytokinin biosynthesis gene, ipt (*source: Taiz L., Zeiger E., 2010*)

**3.6. Ethylene**

**Ethylene** is another class of hormones with a single representative. It is a simple gaseous hydrocarbon with the chemical structure H2C=CH2. Ethylene is apparently not required for normal vegetative growth, although it can have a significant impact on the development of roots and shoots. Ethylene appears to be synthesized primarily in response to stress and may be produced in large amounts by tissues undergoing senescence or ripening. It is commonly used to enhance ripening in bananas and other fruits that are picked green for shipment as well.

*Ethylene can be produced by almost all parts of higher plants*

Ethylene occurs in all plant organs – roots, stems, leaves, bulbs, tubers, fruits, seeds, and so on – although the rate of production may vary depending on the stage of development. Ethylene production will also vary from tissue to tissue within the organ, but is frequently located in peripheral tissues. In peach and avocado seeds, for example, ethylene production appears to be localized primarily in the seed coats, while in tomato fruit and mung bean hypocotyls it originates from the epidermal regions.

Ethylene production increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as can physiological stresses such as flooding, disease, and temperature or drought stress. In additon, infection by various pathogens can also elevate ethylene biosynthesis. Physiology of plant growth and development

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*The amino acid methionine is the precursor of ethylene*

M. Lieberman and L. W. Mapson first demonstrated in 1964 that methionine was rapidly converted to ethylene in a cell-free, nonenzymatic model system. In subsequent studies, Lieberman and coworkers confirmed that plant tissues such as apple fruit converted [l4C]-methionine to [14C]-ethylene and that the ethylene was derived from the third and fourth carbons of methionine. Little progress was made until 1977 when D. Adams and F. Yang demonstrated that S-adenosylmethionine (SAM) was an intermediate in the conversion of methionine to ethylene by apple tissue. In 1979, Adams and Yang further demonstrated the accumulation of *1-aminocyclopropane-1-carboxylic acid* (ACC) in apple tissue fed [13C]-methionine under anaerobic conditions – conditions that inhibit the production of ethylene. However, upon reintroduction of oxygen, the labeled ACC was rapidly converted to ethylene. ACC is a nonprotein amino acid that had been isolated from ripe apples in 1957, but its relationship to ethylene was not obvious at that time. These results established that ACC is an intermediate in the biosynthesis of ethylene.

The ethylene biosynthesis is a three-step pathway in higher plants is shown. In the first step, an adenosine group (i.e., adenine plus ribose) is donated to methionine by a molecule of ATP, thus forming SAM. An ATP requirement is consistent with earlier evidence that ethylene production is blocked by inhibitors of oxidative phosphorylation, such as 2,4-dinitrophenol. Conversion of methionine to SAM is catalyzed by the enzyme methionine adenosyltransferase or SAM synthetase.

The cleavage of SAM to yield 5'-methylthio-adenosine (MTA) and ACC, mediated by the enzyme *ACC synthase*, is the rate-limiting step. ACC synthase was the first enzyme in the pathway to be studied in detail. The enzyme has been partially purified from tomato and apple fruit but, because of its instability and low abundance, progress toward its purification and characterization has been slow. Ethylene biosynthesis is stimulated by several factors, including developmental state, environmental conditions, other plant hormones, and physical and chemical injury. Ethylene biosynthesis also varies in a circadian manner, peaking during the day and reaching a minimum at night.

*The primary steps in ethylene action are likely similar: binding to a receptor, followed by activation of signal transduction pathways*

Unbound ethylene receptors are negative regulators of the response pathway. In *Arabidopsis*, tomato, and probably most other plant species, the ethylene receptors are encoded by multi gene families. Targeted disruption (complete inactivation) of the five *Arabidopsis* ethylene receptors (ETRl, ETR2, ERSl, ERS2, and EIN4) has revealed that they are functionally redundant. That is, disruption of any single gene encoding one of these proteins has no effect, but a plant with disruptions in multiple receptor genes exhibits a constitutive ethylene response phenotype.

The observation that ethylene responses, such as the triple response, become constitutive when the receptors are disrupted indicates that the receptors are normally “on” (i.e., in the active state) in the absence of ethylene, and that the function of the receptor minus its ligand (ethylene), is to shut off the signaling pathway that leads to the response. Binding of ethylene “turns off” (inactivates *Effects of ethylene on plant growth and development*

*Ethylene affects the transcription of numerous genes via specific transcription factors*

One of the primary effects of ethylene signaling is an alteration in the expression of various target genes. Ethylene affects the mRNA transcript levels of numerous genes, including those that encode cellulase and genes related to ripening and ethylene biosynthesis. Regulatory sequences called *ethylene response elements*, or EREs, have been identified among the ethylene-regulated genes.

*The hormone promotes the ripening of some fruits*

In everyday usage, the term fruit ripening refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins.

Because of their importance in agriculture, the vast majority of studies on fruit ripening have focused on edible fruits. Ethylene has long been recognized as the hormone that accelerates the ripening of edible fruits. Exposure of such fruits to ethylene hastens the processes associated with ripening, and a dramatic increase in ethylene Physiology of plant growth and development

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production accompanies the initiation of ripening. However, surveys of a wide range of fruits have shown that not all of them respond to ethylene.

All fruits that ripen in response to ethylene exhibit a characteristic respiratory rise called a *climacteric* before the ripening phase. Such fruits also show a spike of ethylene production immediately before the respiratory rise (**Figure 3.20**). Apples, bananas, avocados, and tomatoes are examples of climacteric fruits. In contrast, fruits such as citrus fruits and grapes do not exhibit the respiration and ethylene production rise and are called *nonclimacteric* fruits. In climacteric fruits, treatment with ethylene induces the fruit to produce additional ethylene, a response that can be described as autocatalytic.

*Ethylene inhibits hypocotyl elongation*

At concentrations above 0.1 μL L-1, ethylene changes the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the hypocotyl or the epicotyl. In dicots, this swelling is part of the *triple response*, which, in *Arabidopsis*, consists of inhibition of hypocotyl elongation combined with hypocotyl swelling, inhibition of root elongation, and exaggeration of the curvature of the apical hook.

**Figure 3.20** Ethylene production and respiration during banana ripening (*source: Taiz L., Zeiger E., 2010*)

*It regulates flowering, sex determination, and defence responses in some species*

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially for synchronization of pineapple fruit set. Flowering of other species, such as mango, is also initiated by ethylene. On plants that have separate male and female flowers (monoecious species), ethylene may change the sex of developing flowers. The promotion of female flower formation in cucumber is one example of this effect. Recently, a gene responsible for andromonoecy (plants carrying both male and bisexual flowers) in melons was identified as encoding an ACC synthase. A mutation that reduces the activity of this ACC synthase gene results in the formation of the bisexual flowers in these andromonoecious lines.

Pathogen infection and disease will occur only if the interactions between host and pathogen are genetically compatible. However, ethylene production generally increases in response to pathogen attack in both compatible (i.e., pathogenic) and noncompatible (nonpathogenic) interactions. The discovery of ethylene-insensitive mutants has facilitated the assessment of the role of ethylene in the response to various pathogens. The involvement of ethylene in pathogenesis is complex and depends on the particular host-pathogen interaction. For example, blocking ethylene responsiveness does not affect the resistance responses of *Arabidopsis* to *Pseudomonas* bacteria or of tobacco to tobacco mosaic virus. In compatible interactions of these pathogens and hosts, however, elimination of ethylene responsiveness prevents the development of disease symptoms, even though the growth of the pathogen appears to be unaffected.

*Ethylene is active in leaf and flower senescence and in leaf abscision* Physiology of plant growth and development

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Senescence is a genetically programmed developmental process that affects all tissues of the plant. Research has provided several lines of physiological evidence that support roles for ethylene and cytokinins in the control of leaf senescence:

• exogenous applications of ethylene or ACC (the precursor of ethylene) accelerate leaf senescence, and treatment with exogenous cytokinins delays leaf senescence;

• enhanced ethylene production is associated with chlorophyll loss and color fading, which are characteristic features of leaf and flower senescence; an inverse correlation has been found between cytokinin levels in leaves and the onset of senescence;

• inhibitors of ethylene synthesis (e.g., AVG or Co2+) and action (e.g., Ag+ -STS- or CO2) retard leaf and flower senescence (**Figure 3.21**).

Taken together, these physiological studies suggest that senescence is regulated by the balance of ethylene and cytokinin. In addition, abscisic acid has been implicated in the control of leaf senescence.

**Figure 3.21** Inhibition of flower senescence by inhibition of ethylene action (*source: Taiz L., Zeiger E., 2010*)

The shedding of leaves, fruits, flowers, and other plant organs is termed abscission. Abscission takes place in specific layers of cells called abscission layers, which become morphologically and biochemically differentiated during organ development. Weakening of the cell walls at the abscission layer depends on cell wall-degrading enzymes such as cellulase and polygalacturonase. Ethylene appears to be the primary regulator of the abscission process, with auxin acting as a suppressor of the ethylene effect. However, supraoptimal auxin concentrations stimulate ethylene production, which has led to the use of auxin analogs as defoliants. Its action is based on its ability to increase ethylene biosynthesis, thereby stimulating leaf abscission.

During the *early phase of leaf maintenance*, auxin from the leaf prevents abscission by maintaining the cells of the abscission zone in an ethylene-insensitive state. It has long been known that removal of the leaf blade (the site of auxin production) promotes petiole abscission. Application of exogenous auxin to petioles from which the leaf blade has been removed delays the abscission process. However, application of auxin to the proximal side of the abscission zone (i.e., the side closest to the stem) actually accelerates the abscission process.

In the *shedding induction phase*, the amount of auxin from the leaf decreases and the ethylene level rises. Ethylene appears to decrease the activity of auxin both by reducing its synthesis and transport and by increasing its destruction. The reduction in the concentration of free auxin increases the response of specific target cells to ethylene. The shedding phase is characterized by the induction of genes encoding specific hydrolytic enzymes of cell wall polysaccharides and proteins.

The target cells, located in the *abscission zone*, synthesize cellulase and other polysaccharide-degrading enzymes, and secrete them into the cell wall. The activities of these enzymes lead to cell wall loosening, cell separation, and abscission.

**3.7. Abscisic acid** Physiology of plant growth and development

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Unlike auxins, gibberellins, and cytokinins, the hormone **abscisic acid (ABA)** is represented by a single 15-carbon sesquiterpene. ABA also appears to have a more limited range of specific effects than auxins, gibberellins, and cytokinins. The name is based on the once held belief that it was involved in the abscission of leaves and other organs. It now appears to have nothing to do with abscision, but the name has stuck.

The primary functions of ABA are (1) prohibiting precocious germination and promoting dormancy in seeds and (2) inducing stomatal closure and the production of molecules that protect cells against desiccation in times of water stress.

*The chemical structure of ABA determines its physiological activity*

Once the structure of ABA had been determined, two possible pathways for the synthesis of ABA were proposed. In the “direct pathway”, ABA would be synthesized from a 15-carbon terpenoid precursor such as farnesyl diphosphate. By the late 1970s it had been clearly established that this pathway was operative in certain fungal plant pathogens that actively synthesized ABA, but not in plants themselves. According to the second, or “indirect pathway”, ABA was produced from the cleavage of a carotenoid such as β-carotene. Originally proposed in the late 1960s, the indirect pathway was based on structural similarities between carotenoid pigments and ABA and has since received support from a variety of biochemical studies, 18O2-labeling experiments, and, most recently, the characterization of ABA biosynthetic mutants. The cleavage of carotenoids, especially β -carotene, to produce useful biochemicals is not without precedent. The cyanobacterium Microcystis, for example, produces a C10 metabolite by cleavage of β-carotene. Mammals produce vitamin A by cleavage of β-carotene and cleavage of β-carotene to produce 2 molecules of the photoreceptor retinal (C20) has been reported.

*ABA signal transduction pathways*

ABA is involved in *short-term physiological effects* (e.g., stomatal closure), as well as *long-term developmental processes* (e.g., seed maturation):

• rapid physiological responses frequently involve alterations in the fluxes of ions across membranes and usually involve regulation of certain genes as well, as evidenced by the fact that a variety of ABA-stimulated transcription factors that are expressed in guard cells regulate stomatal aperture;

• in contrast, long-term processes inevitably involve major changes in the pattern of gene expression.

Comparisons of total transcript populations have shown that at least 10% of the genes in both *Arabidopsis* and rice are regulated by ABA. Signal transduction pathways, which amplify the primary signal generated when the hormone binds to its receptor, are required for both the short-term and the long-term effects of ABA.

*Developmental and physiological effects of ABA*

Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene, and brassinosteroids.

*In seed development, ABA promotes the synthesis of storage proteins and lipids, as well as special proteins*

The ABA content of seeds is very low early in embryogenesis, reaches a maximum at about the halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus there is a broad peak of ABA accumulation in the seed corresponding to mid-to late embryogenesis. The hormonal balance of seeds is complicated by the fact that not all the tissues have the same genotype. The seed coat is derived from maternal tissues, the zygote and endosperm are derived from both parents. Genetic studies with ABA -deficient mutants of Arabidopsis have shown that the zygotic genotype controls ABA synthesis in the embryo and endosperm and is essential to dormancy induction, whereas the maternal genotype controls the major, early peak of ABA accumulation and helps suppress vivipary in mid-embryogenesis. During mid-to late embryogenesis, when seed ABA levels are highest, seeds accumulate storage compounds that will support seedling growth at germination. Another important function of ABA in the developing seed is to promote the acquisition of desiccation tolerance. As maturing seeds begin to lose water, embryos accumulate sugars and so-called late embryogenesis-abundant (LEA) proteins. Physiological and genetic studies have shown that ABA affects the synthesis of LEAs and of storage proteins and lipids. Physiology of plant growth and development

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*Seed dormancy and germination are controlled by the ratio of ABA to gibberellic acid (GA)*

During seed maturation, the embryo desiccates and enters a quiescent phase. Seed germination can be defined as the resumption of growth of the embryo of the mature seed. Germination depends on the same environmental conditions as vegetative growth does: water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present.

In many cases a viable (living) seed will not germinate even if all the necessary environmental conditions for growth are satisfied. This phenomenon is termed *seed dormancy*. Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographic distances. It also maximizes seedling survival by preventing germination under unfavorable conditions. Seed dormancy may result from *coat-imposed dormancy, embryo dormancy*, or both. Dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extrafloral organs, is known as coat-imposed dormancy. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues have been either removed or damaged. Seed dormancy that is intrinsic to the embryo and is not due to any influence of the seed coat or other surrounding tissues is called embryo dormancy.

*Embryo dormancy* is thought to be due to the presence of inhibitors, especially ABA, as well as the absence of growth promoters, such as GA. Maintenance of dormancy in imbibed seeds requires *de novo* ABA biosynthesis (**Figure 3.22**), and the loss of embryo dormancy is often associated with a sharp decrease in the ratio of ABA to GA. The levels of ABA and GA are regulated by their synthesis and catabolism, which are catalyzed by specific isozymes whose expression is controlled by developmental and environmental factors.

**Figure 3.22** Germinating of ABA-deficient seeds in the fruit while still attached to the plant (*source: Taiz L., Zeiger E., 2010*)

*In germinating seeds, ABA inhibits the GA induced synthesis of hydrolitic enzymes*

In addition to the ABA-GA antagonism affecting seed dormancy, ABA inhibits the GA-induced synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in germinating seeds. For example, GA stimulates the aleurone layer of cereal grains to produce ex-amylase and other hydrolytic enzymes that break down stored resources in the endosperm during germination. ABA inhibits this GA-dependent enzyme synthesis by inhibiting the transcription of α-amylase mRNA. ABA exerts this inhibitory effect via at least two mechanisms, one direct and one indirect:

• a protein originally identified as an activator of ABA-induced gene expression, VPl, acts as a transcriptional repressor of some GA-regulated genes,

• ABA represses the GA-induced expression of GAMYB, a transcription factor that mediates the GA induction of α-amylase expression.

*ABA promotes root growth and inhibits shoot growth at low water potentials*

Despite the traditional view of ABA as a growth inhibitor, endogenous ABA restricts shoot growth only under water stress conditions. Moreover, under these conditions, when ABA levels are high, endogenous ABA exerts a strong positive effect on primary root growth by suppressing ethylene production. The overall effect is a Physiology of plant growth and development

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dramatic increase in the root:shoot ratio at low water potentials, which, along with the effect of ABA on stomatal closure, helps the plant cope with water stress. Furthermore, the temporary inhibition of lateral root outgrowth promotes exploration of new areas of soil, and permits replacement of dehydrated laterals following rehydration. It is not clear how different ABA levels lead to opposite effects on growth, but these effects may reflect signaling through receptors with different functional ranges of sensitivity or different downstream signaling elements in roots versus shoots.

*ABA greatly accelerates the senescence of leaves, thereby increasing ethylene formation and stimulating abscision*

Abscisic acid was originally isolated as an abscission causing factor. However, it has since become evident that ABA stimulates abscission of organs in only a few species and that the hormone primarily responsible for causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence, and through its promotion of senescence it might indirectly increase ethylene formation and stimulate abscission. Leaf senescence has been studied extensively. Leaf segments senesce faster in darkness than in light, and they turn yellow as a result of chlorophyll breakdown. In addition, the breakdown of proteins and nucleic acids is increased by the stimulation of several hydrolases. ABA greatly accelerates the senescence of both leaf segments and attached leaves.

*ABA accumulates in dormant buds, inhibiting their growth; it may interact with growth-promoting hormones*

ABA was originally suggested as the dormancy-inducing hormone because it accumulates in dormant buds and decreases after the tissue is exposed to low temperatures. However, later studies showed that the ABA content of buds does not always correlate with the degree of dormancy. As we saw in the case of seed dormancy, this apparent discrepancy might reflect interactions between ABA and other hormones; perhaps bud dormancy and growth are regulated by the balance between bud growth inhibitors, such as ABA, and growth-inducing substances, such as cytokinins and gibberellins. Much progress has been achieved in elucidating the role of ABA in seed dormancy by the use of ABA-deficient mutants. However, progress on the role of ABA in bud dormancy, a characteristic of woody perennials, has lagged because of the lack of a convenient genetic system. This discrepancy illustrates the tremendous contribution that genetics and molecular biology have made to plant physiology, and underscores the need for extending such approaches to woody species.

*Abscisic acid closes stomata in response to water stress*

ABA accumulates in water-stressed (that is, wilted) leaves and exogenous application of ABA is a powerful inhibitor of stomatal opening. The precise role of ABA in stomatal closure in water-stressed whole plants has, however, been difficult to decipher with certainty. This is because ABA is ubiquitous, often occurring in high concentrations in nonstressed tissue. Also, some early studies indicated that stomata would begin to close before increases in ABA content could be detected.

According to current thinking, the initial detection of water stress in leaves is related to its effects on photosynthesis. Inhibition of electron transport and photophosphorylation in the chloroplasts would disrupt proton accumulation in the thylakoid lumen and lower the stroma pH. At the same time, there is an increase in the pH of the apoplast surrounding the mesophyll cells. The resulting pH gradient stimulates a release of ABA from the mesophyll cells into the apoplast, where it can be carried in the transpiration stream to the guard cells.

Stomatal closure does not always rely on the perception of water deficits and signals arising within the leaves. In some cases it appears that the stomata close in response to soil desiccation well before there is any measurable reduction of turgor in the leaf mesophyll cells. Several studies have indicated a feed-forward control system that originates in the roots and transmits information to the stomata. In these experiments, plants are grown such that the roots are equally divided between two containers of soil. Water deficits can then be introduced by withholding water from one container while the other is watered regularly. Control plants receive regular watering of both containers. Stomatal opening along with factors such as ABA levels, water potential, and turgor are compared between half-watered plants and fully watered controls. Typically, stomatal conductance, a measure of stomatal opening, declines within a few days of withholding water from the roots (**Figure 3.23**), yet there is no measurable change in water potential or loss of turgor in the leaves. Furthermore, ABA is readily translocated from roots to the leaves in the transpiration stream, even when roots are exposed to dry air. These results suggest that ABA is involved in some kind of early warning system that communicates information about soil water potential to the leaves. Physiology of plant growth and development

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**Figure 3.23** Changes in water potential, stomatal resistance, and ABA content in corn in response to water stress (*source: Taiz L., Zeiger E., 2010*)

**3.8. Brassinosteroids**

**Brassinosteroids (BRs)** are steroid hormones with a chemical structure similar to the steroid hormones in animals. Brassinosteroids elicit an impressive array of developmental responses, including an increased rate of stem and pollen tube elongation, increased rates of cell division (in the presence of auxin and cytokinin), seed germination, leaf morphogenesis, apical dominance, inhibition of root elongation, vascular differentiation, accelerated senescence, and cell death. Brassinosteroids are also implicated in mediating responses to both abiotic and biotic stress, including salt, drought, temperature extremes, and pathogens.

*BRs cause dramatic changes in growth and differentiation at very low concentration*

The study of brassinosteroids as plant hormones dates back to the early 1970s, when a group of agricultural researchers began screening pollen, already known as a rich source of growth-promoting substances. The result was a complex mixture of lipids that stimulated elongation of bean second internodes (**Figure 3.24**). Because the most active preparations were isolated from pollen of the rape plant (*Brassica napus*), the active substances were referred to collectively as **brassins**. Many of the effects of the brassins were similar to those of GA, leading many to believe the extracts were simply crude extracts of gibberellins, rather than a new class of hormones as originally proposed. However, in 1979, M. D. Grove and his coworkers identified the active component as **brassinolide (BL)**.

**Figure 3.24** Bean second-internode bioassay for brassinosteroids (*source: Taiz L., Zeiger E., 2010*)

*Brassinosteroids are synthesized from campesterol* Physiology of plant growth and development

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Brassinosteroids are polyhydroxylated plant sterols – lipoidal substances related biosynthetically to the gibberellins and abscisic acid. Plants synthesize a large number and variety of sterols, including sitosterol, stigmasterol, cholesterol, and *campesterol*. Sterols are triterpenoids, C30 molecules that are derived from acetate through the mevalonic acid pathway. In the synthesis of terpenes, sequential additions of the 5-carbon isopentenyl pyrophosphate (IPP) produce terpenes with 10-, 15-, or 20-carbon atoms. Triterpenes are formed when two C15 (farnesyl) units join head to head to form the C30 molecule squalene. The subsequent biosynthesis of plant sterols is not yet fully understood, but the first step is a cyclization reaction to form cycloartenol. Using cycloartenol as a common precursor, there are probably multiple pathways leading to the several sterols found in plants. Decarboxylation and oxidation reactions are involved, as most common sterols have from 26 to 29 carbons and a single hydroxyl (-OH) group. It is thought that most sterols, with the exception of stigmasterol, may serve as precursors for various brassinosteroids. However, the pathway for the biosynthesis of brassinolide is best understood. The precursor to brassinolide is campesterol, a C28 sterol.

*BRs act near their sites of synthesis and do not undergo long-distance transport*

An important determinant of hormone responses in general is the extent and rate of hormone transport from the site of synthesis to the site of action. Exogenously applied 24-epibrassinolide (24-epiBL) undergoes long-distance transport from the root to the shoot. For example, when roots of cucumber, tomato, or wheat plants were treated with 14C-24-epiBL, the radioactivity was readily translocated to the shoot. Moreover, the dwarf phenotype of the BR-deficient *Arabidopsis* mutants could be restored (rescued) back to wild-type size, when grown on agar media supplemented with BL. In contrast, when 14C-24-epiBL was applied to the upper surface of a young cucumber leaf, it was readily taken up, but was only slowly transported out of the leaf. These results suggest that exogenous BRs are readily translocated from the root to the shoot, but are poorly translocated out of leaves.

*BRs promote both cell proliferation and cell elongation*

The growth-promoting effects of BRs are reflected in acceleration of both cell elongation and cell division. These were first characterized using the bean second-internode bioassay. The rice leaf lamina inclination bioassay is dependent on BR-induced cell expansion. Lamina inclination resembles the epinasty caused by ethylene. In response to BR, the cells on the adaxial (upper) surface of the leaf near the joint region expand more than the cells on the abaxial (lower) surface, causing the vertically oriented leaf to bend outward. An increase in cell wall loosening is required for BL-induced cell expansion on the adaxial side of the leaf. The stimulatory effect of BRs on growth is most pronounced in young, growing shoot tissues. The kinetics of cell expansion in response to nanomolar concentrations of BL differ from those of auxin-induced cell expansion. In soybean epicotyl sections, for example, BL begins to enhance the elongation rate after a 45-minute lag period, and reaches a maximum rate only after several hours of treatment. In contrast, auxin stimulates elongation after a 15-minute lag time and reaches a maximum rate within 45 minutes. These results suggest that the growth response to BRs may involve a slower pathway involving gene transcription, whereas the rapid response to auxin may not require gene transcription. In addition to cell elongation, BR also stimulates cell proliferation.

*BRs promote root growth at low concentrations and inhibit root growth at high concentration*

When applied exogenously, BRs promote root growth at low concentrations and inhibit root growth at high concentrations. The threshold concentration for inhibition depends on the activity of the BR analog used. The effects of BR on root growth are independent of both auxin and gibberellin action. An inhibitor of polar auxin transport, 2,3,5-triiodobenzoic acid (TIBA), does not prevent BR-induced growth. When BR and auxin are applied simultaneously, both the promotive and inhibitory effects on root growth are additive. Moreover, the reduced root growth phenotype of BR-deficient mutants is not reversed by gibberellin application. Taken together, these observations indicate that BR inhibition of root growth does not involve interactions with either auxin or GA. On the other hand, high concentrations of BR, like auxin, stimulate ethylene production, so it is possible that at least some of BR's inhibitory effects on root growth are due to ethylene. At low concentrations, BRs can also induce the formation of lateral roots. In these conditions, however, BRs and auxin act synergistically. The current model suggests that BRs promote lateral root development partially by influencing polar auxin transport.

*BRs promote differentiation of the xylem and supress that of the phloem*

BRs play an important role in vascular development, by promoting differentiation of the xylem and suppressing that of the phloem. This is evident in the impaired vasculature systems of BR mutants, which have a higher phloem-to-xylem ratio than the wild type. BR-deficient mutants also have a reduced number of vascular bundles Physiology of plant growth and development

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with irregular spacing between the bundles. In contrast, mutants overexpressing the BR receptor protein produce more xylem than the wild type.

*BRs promote seed germination by interacting with ther hormones, such as GA and ABA*

Seeds, like pollen grains, contain very high levels of BRs, and BRs promote seed germination as well. BRs promote seed germination by interacting with other plant hormones, although the molecular basis for these interactions is not known. It is well established that GA and abscisic acid (ABA) play positive and negative roles, respectively, in stimulating seed germination. BRs can enhance germination of tobacco seeds, independent of GA signaling. Moreover, BRs can rescue the delayed germination phenotype of both GA-deficient and GA-perception mutants, and BR mutants are more sensitive to the inhibition by ABA than the wild type. Thus, BRs can stimulate germination and are needed to overcome the inhibitory effect of ABA. As BRs are known to stimulate cell expansion and division, it is likely that BRs facilitate germination by stimulating the growth of the embryo.

**4. Synthetic and microbial plant hormones in plant production**

Hormones and other regulatory chemicals are now used in a variety of applications where it is desirable for commercial reasons to control some aspect of plant development.

*Commercial application of auxins*

Auxins have been used commercially in agriculture and horticulture for more than 50 years. The synthetic auxins are used in commercial applications largely because they are resistant to oxidation by enzymes that degrade IAA. In addition to their greater stability, the synthetic auxins are often more effective than IAA in specific applications. One of the most widespread uses of auxin encountered by the consumer is the use of 2,4-D in weed control. 2,4-D and other synthetic compounds, such as 2,4,5-T and dicamba, express auxin activity at low concentrations, but at higher concentrations are effective herbicides.

Indolebutyric acid and naphthaleneacetic acid are both widely used in vegetative propagation – the propagation of plants from stem and leaf cuttings. This application can be traced to the propensity for auxin to stimulate adventitious root formation. Generally marketed as “rooting hormone” preparations, the auxins, usually a synthetic auxin such as NAA or IBA, are mixed with an inert ingredient such as talcum powder. Stem cuttings are dipped in the powder prior to planting in a moist sand bed in order to encourage root formation.

4-CPA may be sprayed on tomatoes to increase flowering and fruit set while NAA is commonly used to induce flowering in pineapples. This latter effect is actually due to auxin-induced ethylene production. NAA is also used both to thin fruit set and prevent preharvest fruit drop in apples and pears. These seemingly opposite effects are dependent on timing the auxin application with the appropriate stage of flower and fruit development (**Figure 3.25**). Spraying in early fruit set, shortly after the flowers bloom, enhances abscission of the young fruits (again, due to auxin-induced ethylene production). Thinning is necessary in order to reduce the number of fruits and prevent too many small fruits from developing. Spraying as the fruit matures has the opposite effect, preventing premature fruit drop and keeping the fruit on the tree until it is fully mature and ready for harvest.

The use of synthetic auxins, especially the chlorinated forms, as herbicides has come under close scrutiny by environmental groups because of potential health hazards. 2,4,5-T, for example, has been banned in many jurisdictions because commercial preparations contain significant levels of dioxin, a highly carcinogenic chemical. Physiology of plant growth and development

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**Figure 3.25** Auxin promotes fruit development that produced by achenes (*source: Taiz L., Zeiger E., 2010*)

*Commercial use of gibberellins*

The major uses of gibberellins (GA3), applied as a spray or dip, are to manage fruit crops, to malt barley, and to increase sugar yield in sugarcane. In some crops a reduction in height is desirable, and this can be accomplished by the use of gibberellin synthesis inhibitors.

Many of the table grapes grown in the United States are a genetically seedless variety that would naturally produce small fruit on very compact clusters. Almost all seedless grapes on the market are treated with GA3. It substitutes for the presence of seeds, which would normally be the source of native GAs for fruit growth. Repeated spraying with GA3 increases both rachis length (producing looser clusters) and fruit size (**Figure 3.26**). The increased rachis length prevents the cluster from being too compact, and this reduces the chance of fungal growth inside the cluster. Two to three additional applications of GA3 during fruit development are thought to increase berry size by enhancing the import of carbohydrates into the developing fruit.

**Figure 3.26** Gibberellin induces growth in Thompson’s seedless grapes (left – control, right – sprayed with GA3) (*source: Taiz L., Zeiger E., 2010*)

Gibberellic acid is also used to boost cherry production. Sweet, bing cherries are sprayed 4 to 6 weeks before harvest to increase fruit size. Application of GA3 to tart cherries increases yield through enhanced bearing. Gibberellin A4 (GA4) is used to promote the fruit set of apple and pear trees. For example, in some apple cultivars the amount of fruit produced is often limited by biennial bearing, a phenomenon whereby the production of a heavy crop of fruit one year inhibits the subsequent production of flower buds, and hence, the yield of fruit the following year. The alternate bearing of some cultivars can be overcome by applying GA4 in the “off” year to promote the formation of flower buds, and subsequent fruit set. In regions of Europe where fruit set of apple and pear trees is often reduced by inclement weather at the time of pollination, the application of a hormone mixture can promote the production and subsequent growth of parthenocarpic (seedless) fruit. GA4/7 is also used on Golden Delicious apples to prevent abnormal cell divisions in the epidermal layer that Physiology of plant growth and development

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produce “russetting”. Gibberellic acid is also applied to citrus crops, though the actual use depends on the particular crop. For example GA3 is sprayed onto oranges and tangerines to delay or prevent rind-aging, so that fruit can be harvested later without adverse effects on rind quality and appearance. For lemons and limes, GA3 synchronizes ripening and enhances fruit size.

Gibberellins from the embryo of germinating grains are necessary for the synthesis of