Chapter

20

Gibberellins: Regulators of Plant Height

FOR NEARLY 30 YEARS after the discovery of auxin in 1927, and more than 20 years after its structural elucidation as indole-3-acetic acid, Western plant scientists tried to ascribe the regulation of all developmental phenomena in plants to auxin. However, as we will see in this and subsequent chapters, plant growth and development are regulated by several different types of hormones acting individually and in concert.

In the 1950s the second group of hormones, the gibberellins (GAs), was characterized. The gibberellins are a large group of related compounds (more than 125 are known) that, unlike the auxins, are defined by their chemical structure rather than by their biological activity. Gibberellins are most often associated with the promotion of stem growth, and the application of gibberellin to intact plants can induce large increases in plant height. As we will see, however, gibberellins play important roles in a variety of physiological phenomena.

The biosynthesis of gibberellins is under strict genetic, developmental, and environmental control, and numerous gibberellin-deficient mutants have been isolated. Mendel's tall/dwarf alleles in peas are a famous example. Such mutants have been useful in elucidating the complex pathways of gibberellin biosynthesis.

We begin this chapter by describing the discovery, chemical structure, and role of gibberellins in regulating various physiological processes, including seed germination, mobilization of endosperm storage reserves, shoot growth, flowering, floral development, and fruit set. We then examine biosynthesis of the gibberellins, as well as identification of the active form of the hormone.

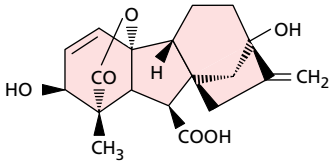
In recent years, the application of molecular genetic approaches has led to considerable progress in our understanding of the mechanism of gibberellin action at the molecular level. These advances will be discussed at the end of the chapter.

THE DISCOVERY OF THE GIBBERELLINS

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.

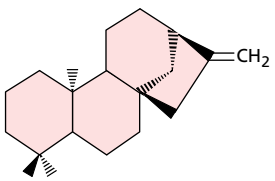
In the 1930s Japanese scientists succeeded in obtaining impure crystals of two fungal growth-active compounds, which they termed *gibberellin A* and *B*, but because of communication barriers and World War II, the information did not reach the West. Not until the mid-1950s did two groups—one at the Imperial Chemical Industries (ICI) research station at Welyn in Britain, the other at the U.S. Department of Agriculture (USDA) in Peoria, Illinois—succeed in elucidating the structure of the material that they had purified from fungal culture filtrates, which they named *gibberellic acid*:



Gibberellic acid (GA_3)

At about the same time scientists at Tokyo University isolated three gibberellins from the original gibberellin A and named them gibberellin A_1, gibberellin A_2, and gibberellin A_3. Gibberellin A_3 and gibberellic acid proved to be identical.

It became evident that an entire family of gibberellins exists and that in each fungal culture different gibberellins predominate, though gibberellic acid is always a principal component. As we will see, the structural feature that all gibberellins have in common, and that defines them as a family of molecules, is that they are derived from the *ent*-kaurene ring structure:



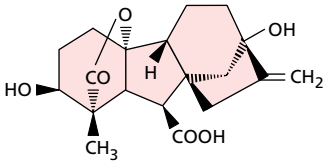
***ent*-Kaurene**

As gibberellic acid became available, physiologists began testing it on a wide variety of plants. Spectacular responses were obtained in the elongation growth of dwarf and rosette plants, particularly in genetically dwarf peas (*Pisum sativum*), dwarf maize (*Zea mays*), and many rosette plants.

In contrast, plants that were genetically very tall showed no further response to applied gibberellins. More recently, experiments with dwarf peas and dwarf corn have confirmed that the natural elongation growth of plants is regulated by gibberellins, as we will describe later.

Because applications of gibberellins could increase the height of dwarf plants, it was natural to ask whether plants contain their own gibberellins. Shortly after the discovery of the growth effects of gibberellic acid, gibberellin-like substances were isolated from several species of plants.[1] *Gibberellin-like substance* refers to a compound or an extract that has gibberellin-like biological activity, but whose chemical structure has not yet been defined. Such a response indicates, but does not prove, that the tested substance is a gibberellin.

In 1958 a gibberellin (gibberellin A_1) was conclusively identified from a higher plant (runner bean seeds, *Phaseolus coccineus*):

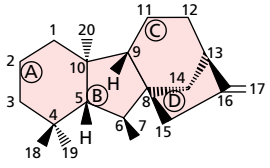


Gibberellin A_1 (GA_1)

Because the concentration of gibberellins in immature seeds far exceeds that in vegetative tissue, immature seeds were the tissue of choice for gibberellin extraction. However, because the concentration of gibberellins in plants is very low (usually 1–10 parts per billion for the active gibberellin in vegetative tissue and up to 1 part per million of total gibberellins in seeds), chemists had to use truckloads of seeds.

As more and more gibberellins from fungal and plant sources were characterized, they were numbered as gibberellin A_X (or GA_X), where *X* is a number, in the order of their discovery. This scheme was adopted for all gibberellins in 1968. However, the number of a gibberellin is simply a cataloging convenience, designed to prevent chaos in the naming of the gibberellins. The system implies no close chemical similarity or metabolic relationship between gibberellins with adjacent numbers.

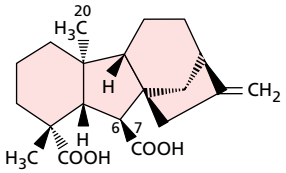
All gibberellins are based on the *ent*-gibberellane skeleton:



***ent*-Gibberellane structure**

[1] Phinney (1983) provides a wonderful personal account of the history of gibberellin discoveries.

Some gibberellins have the full complement of 20 carbons (C_{20}-GAs):



GA_{12} (a C_{20}-gibberellin)

Others have only 19 (C_{19}-GAs), having lost one carbon to metabolism.

There are other variations in the basic structure, especially the oxidation state of carbon 20 (in C_{20}-GAs) and the number and position of hydroxyl groups on the molecule (see **Web Topic 20.1**). Despite the plethora of gibberellins present in plants, genetic analyses have demonstrated that only a few are biologically active as hormones. All the others serve as precursors or represent inactivated forms.

EFFECTS OF GIBBERELLIN ON GROWTH AND DEVELOPMENT

Though they were originally discovered as the cause of a disease of rice that stimulated internode elongation, endogenous gibberellins influence a wide variety of developmental processes. In addition to stem elongation, gibberellins control various aspects of seed germination, including the loss of dormancy and the mobilization of endosperm reserves. In reproductive development, gibberellin can affect the transition from the juvenile to the mature stage, as well as floral initiation, sex determination, and fruit set. In this section we will review some of these gibberellin-regulated phenomena.

Gibberellins Stimulate Stem Growth in Dwarf and Rosette Plants

Applied gibberellin promotes internodal elongation in a wide range of species. However, the most dramatic stimulations are seen in dwarf and rosette species, as well as members of the grass family. Exogenous GA_3 causes such extreme stem elongation in dwarf plants that they resemble the tallest varieties of the same species (Figure 20.1). Accompanying this effect are a decrease in stem thickness, a decrease in leaf size, and a pale green color of the leaves.

Some plants assume a rosette form in short days and undergo shoot elongation and flowering only in long days (see Chapter 24). Gibberellin application results in *bolting* (stem growth) in plants kept in short days (Figure 20.2), and normal bolting is regulated by endogenous gibberellin.

In addition, as noted earlier, many long-day rosette plants have a cold requirement for stem elongation and flowering, and this requirement is overcome by applied gibberellin.

GA also promotes internodal elongation in members of the grass family. The target of gibberellin action is the **intercalary meristem**—a meristem near the base of the internode that produces derivatives above and below. Deepwater rice is a particularly striking example. We will examine the effects of gibberellin on the growth of deepwater rice in the section on the mechanism of gibberellin-induced stem elongation later in the chapter.

Although stem growth may be dramatically enhanced by GAs, gibberellins have little direct effect on root growth. However, the root growth of extreme dwarfs is less than that of wild-type plants, and gibberellin application to the shoot enhances both shoot and root growth. Whether the effect of gibberellin on root growth is direct or indirect is currently unresolved.

Gibberellins Regulate the Transition from Juvenile to Adult Phases

Many woody perennials do not flower until they reach a certain stage of maturity; up to that stage they are said to

FIGURE 20.1 The effect of exogenous GA_1 on normal and dwarf (*d1*) corn. Gibberellin stimulates dramatic stem elongation in the dwarf mutant but has little or no effect on the tall wild-type plant. (Courtesy of B. Phinney.)

FIGURE 20.2 Cabbage, a long-day plant, remains as a rosette in short days, but it can be induced to bolt and flower by applications of gibberellin. In the case illustrated, giant flowering stalks were produced. (© Sylvan Wittwer/Visuals Unlimited.)

be juvenile (see Chapter 24). The juvenile and mature stages often have different leaf forms, as in English ivy (*Hedera helix*) (see Figure 24.9). Applied gibberellins can regulate this juvenility in both directions, depending on the species. Thus, in English ivy GA_3 can cause a reversion from a mature to a juvenile state, and many juvenile conifers can be induced to enter the reproductive phase by applications of nonpolar gibberellins such as $GA_4 + GA_7$. (The latter example is one instance in which GA_3 is not effective.)

Gibberellins Influence Floral Initiation and Sex Determination

As already noted, gibberellin can substitute for the long-day or cold requirement for flowering in many plants, especially rosette species (see Chapter 24). Gibberellin is thus a component of the flowering stimulus in some plants, but apparently not in others.

In plants where flowers are unisexual rather than hermaphroditic, floral 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 gibberellin. In maize, for example, the staminate flowers (male) are restricted to the tassel, and the pistillate flowers (female) are contained in the ear. Exposure to short days and cool nights increases the endogenous gibberellin levels in the tassels 100-fold and simultaneously causes feminization of the tassel flowers. Application of exogenous gibberellic acid to the tassels can also induce pistillate flowers.

For studies on genetic regulation, a large collection of maize mutants that have altered patterns of sex determination have been isolated. Mutations in genes that affect either gibberellin biosynthesis or gibberellin signal transduction result in a failure to suppress stamen development in the flowers of the ear (Figure 20.3). Thus the primary role of gibberellin in sex determination in maize seems to be to suppress stamen development (Irish 1996).

In dicots such as cucumber, hemp, and spinach, gibberellin seems to have the opposite effect. In these species, application of gibberellin promotes the formation of staminate flowers, and inhibitors of gibberellin biosynthesis promote the formation of pistillate flowers.

Gibberellins Promote Fruit Set

Applications of gibberellins can cause *fruit set* (the initiation of fruit growth following pollination) and growth of some fruits, in cases where auxin may have no effect. For example, stimulation of fruit set by gibberellin has been observed in apple (*Malus sylvestris*).

Gibberellins Promote Seed Germination

Seed germination may require gibberellins for one of several possible steps: the activation of vegetative growth of

FIGURE 20.3 Anthers develop in the ears of a gibberellin-deficient dwarf mutant of corn (*Zea mays*). (Bottom) Unfertilized ear of the dwarf mutant *an1*, showing conspicuous anthers. (Top) Ear from a plant that has been treated with gibberellin. (Courtesy of M. G. Neuffer.)

the embryo, the weakening of a growth-constraining endosperm layer surrounding the embryo, and the mobilization of stored food reserves of the endosperm. Some seeds, particularly those of wild plants, require light or cold to induce germination. In such seeds this dormancy (see Chapter 23) can often be overcome by application of gibberellin. Since changes in gibberellin levels are often, but not always, seen in response to chilling of seeds, gibberellins may represent a natural regulator of one or more of the processes involved in germination.

Gibberellin application also stimulates the production of numerous hydrolases, notably α-amylase, by the aleurone layers of germinating cereal grains. This aspect of gibberellin action has led to its use in the brewing industry in the production of malt (discussed in the next section). Because this is the principal system in which gibberellin signal transduction pathways have been analyzed, it will be treated in detail later in the chapter.

FIGURE 20.4 Gibberellin induces growth in Thompson's seedless grapes. The bunch on the left is an untreated control. The bunch on the right was sprayed with gibberellin during fruit development. (© Sylvan Wittwer/Visuals Unlimited.)

Gibberellins Have Commercial Applications

The major uses of gibberellins (GA_3, unless noted otherwise), 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 (see **Web Topic 20.1**).

Fruit production. A major use of gibberellins is to increase the stalk length of seedless grapes. Because of the shortness of the individual fruit stalks, bunches of seedless grapes are too compact and the growth of the berries is restricted. Gibberellin stimulates the stalks to grow longer, thereby allowing the grapes to grow larger by alleviating compaction, and it promotes elongation of the fruit (Figure 20.4).

A mixture of benzyladenine (a cytokinin; see Chapter 21) and $GA_4 + GA_7$ can cause apple fruit to elongate and is used to improve the shape of Delicious-type apples under certain conditions. Although this treatment does not affect yield or taste, it is considered commercially desirable.

In citrus fruits, gibberellins delay senescence, allowing the fruits to be left on the tree longer to extend the market period.

Malting of barley. Malting is the first step in the brewing process. During malting, barley seeds (*Hordeum vulgare*) are allowed to germinate at temperatures that maximize the production of hydrolytic enzymes by the aleurone layer. Gibberellin is sometimes used to speed up the malting process. The germinated seeds are then dried and pulverized to produce "malt," consisting mainly of a mixture of amylolytic (starch-degrading) enzymes and partly digested starch.

During the subsequent "mashing" step, water is added and the amylases in the malt convert the residual starch, as well as added starch, to the disaccharide maltose, which is converted to glucose by the enzyme maltase. The resulting "wort" is then boiled to stop the reaction. In the final step, yeast converts the glucose in the wort to ethanol by fermentation.

Increasing sugarcane yields. Sugarcane (*Saccharum officinarum*) is one of relatively few plants that store their carbohydrate as sugar (sucrose) instead of starch (the other important sugar-storing crop is sugar beet). Originally from New Guinea, sugarcane is a giant perennial grass that can grow from 4 to 6 m tall. The sucrose is stored in the central vacuoles of the internode parenchyma cells. Spraying the crop with gibberellin can increase the yield of raw cane by up to 20 tons per acre, and the sugar yield by 2 tons per acre. This increase is a result of the stimulation of internode elongation during the winter season.

Uses in plant breeding. The long juvenility period in conifers can be detrimental to a breeding program by preventing the reproduction of desirable trees for many years. Spraying with $GA_4 + GA_7$ can considerably reduce the time to seed production by inducing cones to form on very young trees. In addition, the promotion of male flowers in cucurbits, and the stimulation of bolting in biennial rosette crops such as beet (*Beta vulgaris*) and cabbage (*Brassica oleracea*), are beneficial effects of gibberellins that are occasionally used commercially in seed production.

Gibberellin biosynthesis inhibitors. Bigger is not always better. Thus, gibberellin biosynthesis inhibitors are used commercially to prevent elongation growth in some plants. In floral crops, short, stocky plants such as lilies, chrysanthemums, and poinsettias are desirable, and restrictions on elongation growth can be achieved by applications of gibberellin synthesis inhibitors such as ancymidol (known commercially as A-Rest) or paclobutrazol (known as Bonzi).

Tallness is also a disadvantage for cereal crops grown in cool, damp climates, as occur in Europe, where lodging can be a problem. *Lodging*—the bending of stems to the ground caused by the weight of water collecting on the ripened heads—makes it difficult to harvest the grain with a combine harvester. Shorter internodes reduce the tendency of the plants to lodge, increasing the yield of the crop. Even genetically dwarf wheats grown in Europe are sprayed with gibberellin biosynthesis inhibitors to further reduce stem length and lodging.

Yet another application of gibberellin biosynthesis inhibitors is the restriction of growth in roadside shrub plantings.

BIOSYNTHESIS AND METABOLISM OF GIBBERELLIN

Gibberellins constitute a large family of diterpene acids and are synthesized by a branch of the **terpenoid pathway**, which was described in Chapter 13. The elucidation of the gibberellin biosynthetic pathway would not have been possible without the development of sensitive methods of detection. As noted earlier, plants contain a bewildering array of gibberellins, many of which are *biologically inactive*. In this section we will discuss the biosynthesis of GAs, as well as other factors that regulate the steady-state levels of the biologically active form of the hormone in different plant tissues.

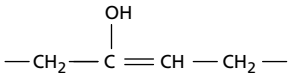
Gibberellins Are Measured via Highly Sensitive Physical Techniques

Systems of measurement using a biological response, called *bioassays*, were originally important for detecting gibberellin-like activity in partly purified extracts and for assessing the biological activity of known gibberellins (Figure 20.5). The use of bioassays, however, has declined with the development of highly sensitive physical techniques that allow precise identification and quantification of specific gibberellins from small amounts of tissue.

High-performance liquid chromatography (HPLC) of plant extracts, followed by the highly sensitive and selective analytical method of gas chromatography combined with mass spectrometry (GC-MS), has now become the method of choice. With the availability of published mass spectra, researchers can now identify gibberellins without possessing pure standards. The availability of heavy-isotope-labeled standards of common gibberellins, which can themselves be separately detected on a mass spectrometer, allows the accurate measurement of levels in plant tissues by mass spectrometry with these heavy-isotope-labeled gibberellins as internal standards for quantification (see **Web Topic 20.2**).

Gibberellins Are Synthesized via the Terpenoid Pathway in Three Stages

Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. Terpenoids are compounds made up of five-carbon (isoprene) building blocks:



joined head to tail. Researchers have determined the entire gibberellin biosynthetic pathway in seed and vegetative tissues of several species by feeding various radioactive precursors and intermediates and examining the production of the other compounds of the pathway (Kobayashi et al. 1996).

The gibberellin biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment (Figure 20.6) (Hedden and Phillips 2000).

FIGURE 20.5 Gibberellin causes elongation of the leaf sheath of rice seedlings, and this response is used in the dwarf rice leaf sheath bioassay. Here 4-day-old seedlings were treated with different amounts of GA and allowed to grow for another 5 days. (Courtesy of P. Davies.)

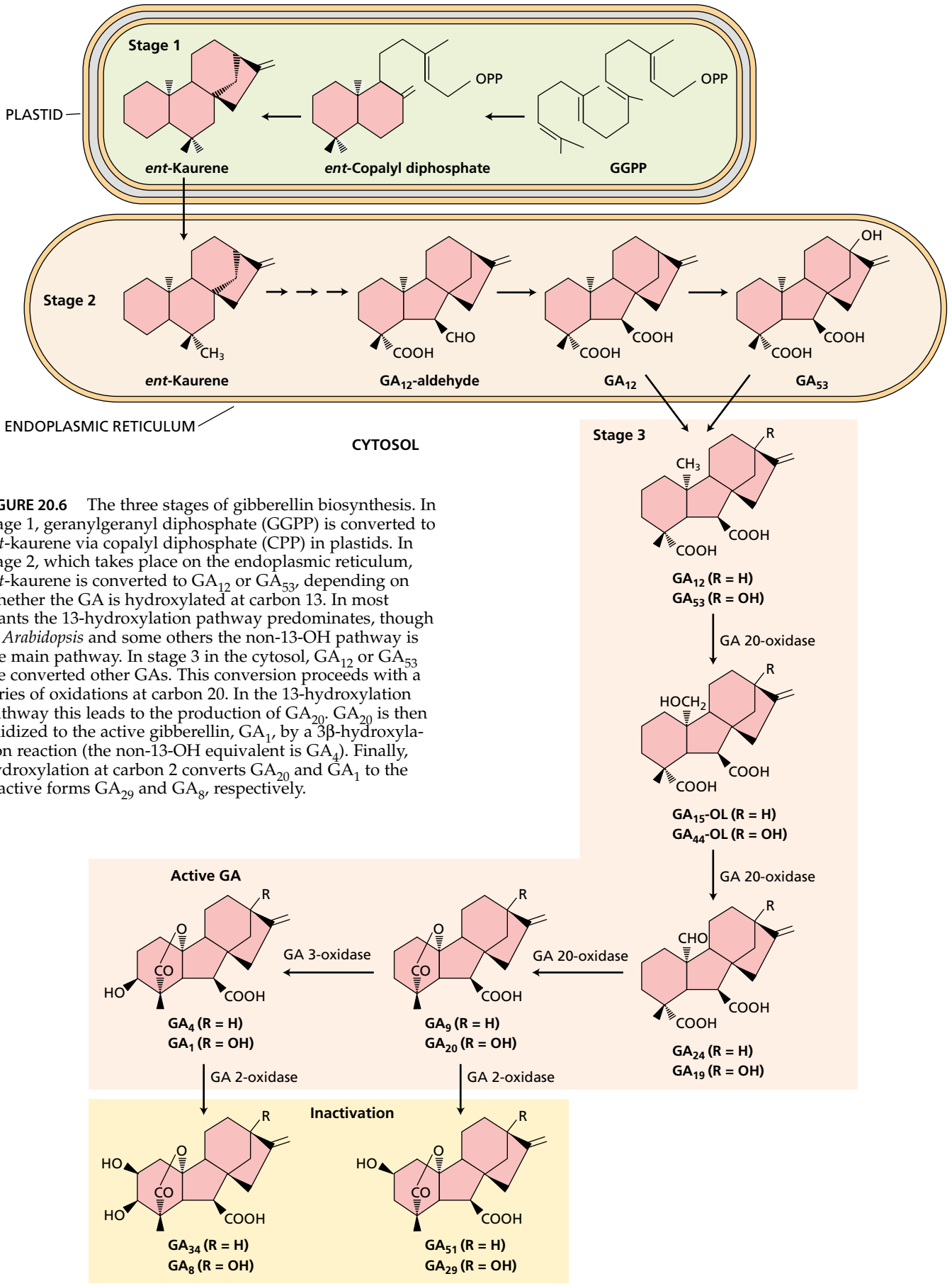


FIGURE 20.6 The three stages of gibberellin biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene via copalyl diphosphate (CPP) in plastids. In stage 2, which takes place on the endoplasmic reticulum, *ent*-kaurene is converted to GA_{12} or GA_{53}, depending on whether the GA is hydroxylated at carbon 13. In most plants the 13-hydroxylation pathway predominates, though in *Arabidopsis* and some others the non-13-OH pathway is the main pathway. In stage 3 in the cytosol, GA_{12} or GA_{53} are converted other GAs. This conversion proceeds with a series of oxidations at carbon 20. In the 13-hydroxylation pathway this leads to the production of GA_{20}. GA_{20} is then oxidized to the active gibberellin, GA_1, by a 3β-hydroxylation reaction (the non-13-OH equivalent is GA_4). Finally, hydroxylation at carbon 2 converts GA_{20} and GA_1 to the inactive forms GA_{29} and GA_8, respectively.

Stage 1: Production of terpenoid precursors and ent-kaurene in plastids. The basic biological isoprene unit is isopentenyl diphosphate (IPP).[2] IPP used in gibberellin biosynthesis in green tissues is synthesized in plastids from glyceraldehyde-3-phosphate and pyruvate (Lichtenthaler et al. 1997). However, in the endosperm of pumpkin seeds, which are very rich in gibberellin, IPP is formed in the cytosol from mevalonic acid, which is itself derived from acetyl-CoA. Thus the IPP used to make gibberellins may arise from different cellular compartments in different tissues.

Once synthesized, the IPP isoprene units are added successively to produce intermediates of 10 carbons (geranyl diphosphate), 15 carbons (farnesyl diphosphate), and 20 carbons (geranylgeranyl diphosphate, GGPP). GGPP is a precursor of many terpenoid compounds, including carotenoids and many essential oils, and it is only after GGPP that the pathway becomes specific for gibberellins.

The cyclization reactions that convert GGPP to *ent*-kaurene represent the first step that is specific for the gibberellins (Figure 20.7). The two enzymes that catalyze the reactions are localized in the proplastids of meristematic shoot tissues, and they are not present in mature chloroplasts (Aach et al. 1997). Thus, leaves lose their ability to synthesize gibberellins from IPP once their chloroplasts mature.

Compounds such as AMO-1618, Cycocel, and Phosphon D are specific inhibitors of the first stage of gibberellin biosynthesis, and they are used as growth height reducers.

Stage 2: Oxidation reactions on the ER form GA_{12} and GA_{53}. In the second stage of gibberellin biosynthesis, a methyl group on *ent*-kaurene is oxidized to a carboxylic acid, followed by contraction of the B ring from a six- to a five-carbon ring to give GA_{12}-aldehyde. GA_{12}-aldehyde is then oxidized to **GA_{12}**, the first gibberellin in the pathway in all plants and thus the precursor of all the other gibberellins (see Figure 20.6).

Many gibberellins in plants are also hydroxylated on carbon 13. The hydroxylation of carbon 13 occurs next, forming GA_{53} from GA_{12}. All the enzymes involved are monooxygenases that utilize cytochrome P450 in their reactions. These P450 monooxygenases are localized on the endoplasmic reticulum. Kaurene is transported from the plastid to the endoplasmic reticulum, and is oxidized *en route* to kaurenoic acid by kaurene oxidase, which is associated with the plastid envelope (Helliwell et al. 2001).

Further conversions to GA_{12} take place on the endoplasmic reticulum. Paclobutrazol and other inhibitors of P450 monooxygenases specifically inhibit this stage of gibberellin biosynthesis before GA_{12}-aldehyde, and they are also growth retardants.

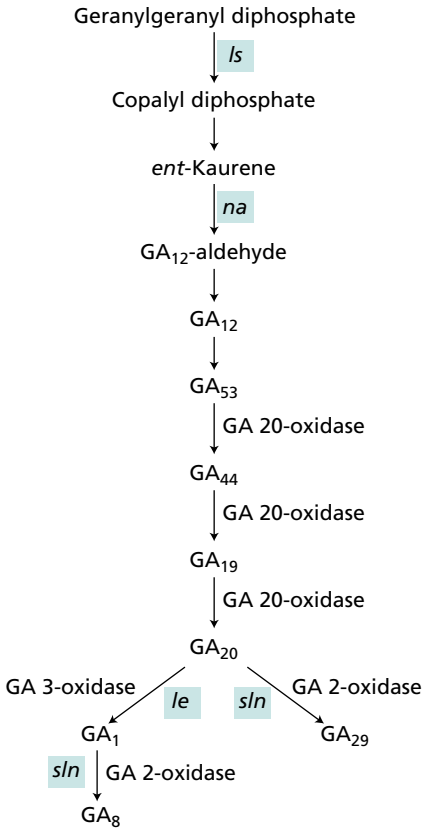


FIGURE 20.7 A portion of the gibberellin biosynthetic pathway showing the abbreviations and location of the mutant genes that block the pathway in pea and the enzymes involved in the metabolic steps after GA_{53}.

Stage 3: Formation in the cytosol of all other gibberellins from GA_{12} or GA_{53}. All subsequent steps in the pathway (see Figure 20.6) are carried out by a group of soluble dioxygenases in the cytosol. These enzymes require 2-oxoglutarate and molecular oxygen as cosubstrates, and they use Fe^{2+} and ascorbate as cofactors.

The specific steps in the modification of GA_{12} vary from species to species, and between organs of the same species. Two basic chemical changes occur in most plants:

1. Hydroxylation at carbon 13 (on the endoplasmic reticulum) or carbon 3, or both.
2. A successive oxidation at carbon 20 ($CH_2 \rightarrow CH_2OH \rightarrow CHO$). The final step of this oxidation is the loss of carbon 20 as CO_2 (see Figure 20.6).

When these reactions involve gibberellins initially hydroxylated at C-13, the resulting gibberellin is GA_{20}. GA_{20} is then converted to the biologically active form,

[2] As noted in Chapter 13, IPP is the abbreviation for isopentenyl *pyro*phosphate, an earlier name for this compound. Similarly, the other pyrophosphorylated intermediates in the pathway are now referred to as *di*phosphates, but they continue to be abbreviated as if they were called *pyro*phosphates.

GA_1, by hydroxylation of carbon 3. (Because this is in the beta configuration [drawn as if the bond to the hydroxyl group were toward the viewer], it is referred to as 3β-hydroxylation.)

Finally, GA_1 is inactivated by its conversion to GA_8 by a hydroxylation on carbon 2. This hydroxylation can also remove GA_{20} from the biosynthetic pathway by converting it to GA_{29}.

Inhibitors of the third stage of the gibberellin biosynthetic pathway interfere with enzymes that utilize 2-oxoglutarate as cosubstrates. Among these, the compound prohexadione (BX-112), is especially useful because it specifically inhibits GA 3-oxidase, the enzyme that converts inactive GA_{20} to growth-active GA_1.

The Enzymes and Genes of the Gibberellin Biosynthetic Pathway Have Been Characterized

The enzymes of the gibberellin biosynthetic pathway are now known, and the genes for many of these enzymes have been isolated and characterized (see Figure 20.7). Most notable from a regulatory standpoint are two biosynthetic enzymes—GA 20-oxidase (GA20ox)[3] and GA 3-oxidase (GA3ox)—and an enzyme involved in gibberellin metabolism, GA 2-oxidase (GA2ox):

- **GA 20-oxidase** catalyzes all the reactions involving the successive oxidation steps of carbon 20 between GA_{53} and GA_{20}, including the removal of C-20 as CO_2.
- **GA 3-oxidase** functions as a 3β-hydroxylase, adding a hydroxyl group to C-3 to form the active gibberellin, GA_1. (The evidence demonstrating that GA_1 is the active gibberellin will be discussed shortly.)
- **GA 2-oxidase** *inactivates* GA_1 by catalyzing the addition of a hydroxyl group to C-2.

The transcription of the genes for the two gibberellin biosynthetic enzymes, as well as for GA 2-oxidase, is highly regulated. All three of these genes have sequences in common with each other and with other enzymes utilizing 2-oxoglutarate and Fe^{2+} as cofactors. The common sequences represent the binding sites for 2-oxoglutarate and Fe^{2+}.

Gibberellins May Be Covalently Linked to Sugars

Although active gibberellins are free, a variety of gibberellin glycosides are formed by a covalent linkage between gibberellin and a sugar. These gibberellin conjugates are particularly prevalent in some seeds. The conjugating sugar is usually glucose, and it may be attached to the gibberellin via a carboxyl group forming a gibberellin glycoside, or via a hydroxyl group forming a gibberellin glycosyl ether.

When gibberellins are applied to a plant, a certain proportion usually becomes glycosylated. Glycosylation therefore represents another form of inactivation. In some cases, applied glucosides are metabolized back to free GAs, so glucosides may also be a storage form of gibberellins (Schneider and Schmidt 1990).

GA_1 Is the Biologically Active Gibberellin Controlling Stem Growth

Knowledge of biosynthetic pathways for gibberellins reveals where and how dwarf mutations act. Although it had long been assumed that gibberellins were natural growth regulators because gibberellin application caused dwarf plants to grow tall, direct evidence was initially lacking. In the early 1980s it was demonstrated that tall stems do contain more bioactive gibberellin than dwarf stems have, and that the level of the endogenous bioactive gibberellin mediates the genetic control of tallness (Reid and Howell 1995).

The gibberellins of tall pea plants containing the homozygous *Le* allele (wild type) were compared with dwarf plants having the same genetic makeup, except containing the *le* allele (mutant). *Le* and *le* are the two alleles of the gene that regulates tallness in peas, the genetic trait first investigated by Gregor Mendel in his pioneering study in 1866. We now know that tall peas contain much more bioactive GA_1 than dwarf peas have (Ingram et al. 1983).

As we have seen, the precursor of GA_1 in higher plants is GA_{20} (GA_1 is 3β-OH GA_{20}). If GA_{20} is applied to homozygous dwarf (*le*) pea plants, they fail to respond, although they do respond to applied GA_1. The implication is that the *Le* gene enables the plants to convert GA_{20} to GA_1. Metabolic studies using both stable and radioactive isotopes demonstrated conclusively that the *Le* gene encodes an enzyme that 3β-hydroxylates GA_{20} to produce GA_1 (Figure 20.8).

Mendel's *Le* gene was isolated, and the recessive *le* allele was shown to have a single base change leading to a defective enzyme only one-twentieth as active as the wild-type

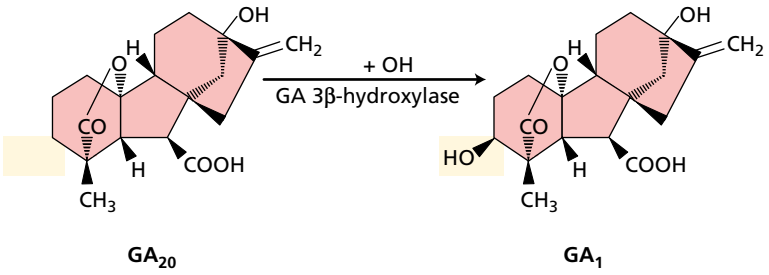


FIGURE 20.8 Conversion of GA_{20} to GA_1 by GA 3β-hydroxylase, which adds a hydroxyl group (OH) to carbon 3 of GA_{20}.

[3] *GA 20-oxidase* means an enzyme that oxidizes at carbon 20; it is not the same as GA_{20}, which is gibberellin 20 in the GA numbering scheme.

enzyme, so much less GA_1 is produced and the plants are dwarf (Lester et al. 1997).

Endogenous GA_1 Levels Are Correlated with Tallness

Although the shoots of gibberellin-deficient *le* dwarf peas are much shorter than those of normal plants (internodes of 3 cm in mature dwarf plants versus 15 cm in mature normal plants), the mutation is "leaky" (i.e., the mutated gene produces a partially active enzyme) and some endogenous GA_1 remains to cause growth. Different *le* alleles give rise to peas differing in their height, and the height of the plant has been correlated with the amount of endogenous GA_1 (Figure 20.9).

There is also an extreme dwarf mutant of pea that has even fewer gibberellins. This dwarf has the allele *na* (the wild-type allele is *Na*), which completely blocks gibberellin biosynthesis between *ent*-kaurene and GA_{12}-aldehyde (Reid and Howell 1995). As a result, homozygous (*nana*) mutants, which are almost completely free of gibberellins, achieve a stature of only about 1 cm at maturity (Figure 20.10).

However, *nana* plants may still possess an active GA 3β-hydroxylase encoded by *Le*, and thus can convert GA_{20} to GA_1. If a *nana naLe* shoot is grafted onto a dwarf *le* plant, the resulting plant is tall because the *nana* shoot tip can convert the GA_{20} from the dwarf into GA_1.

Such observations have led to the conclusion that GA_1 is the biologically active gibberellin that regulates tallness in peas (Ingram et al. 1986; Davies 1995). The same result has been obtained for maize, a monocot, in parallel studies using genotypes that have blocks in the gibberellin biosynthetic pathway. Thus the control of stem elongation by GA_1 appears to be universal.

Although GA_1 appears to be the primary active gibberellin in stem growth for most species, a few other gib-

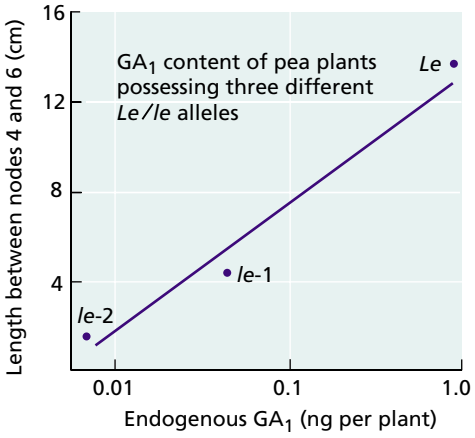


FIGURE 20.9 Stem elongation corresponds closely to the level of GA_1. Here the GA_1 content in peas with three different alleles at the *Le* locus is plotted against the internode elongation in plants with those alleles. The allele *le*-2 is a more intense dwarfing allele of *Le* than is the regular *le*-1 allele. There is a close correlation between the GA level and internode elongation. (After Ross et al. 1989.)

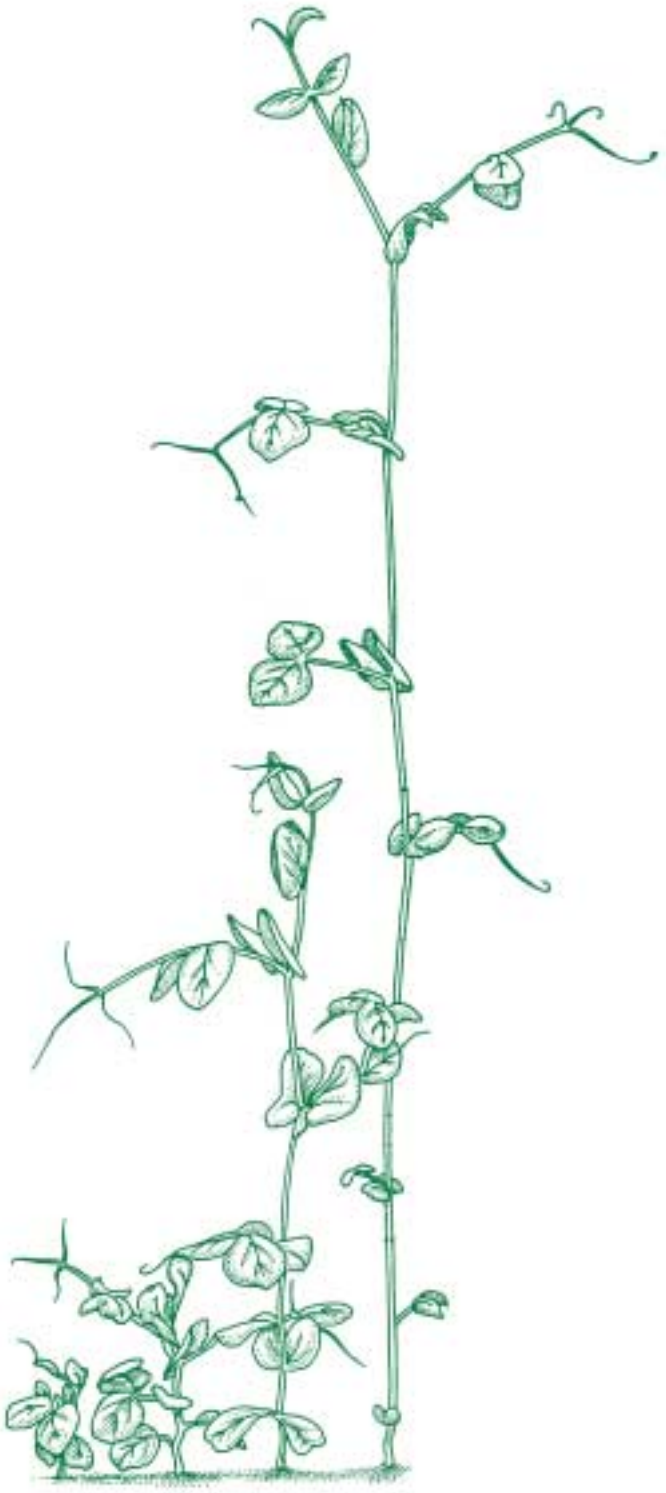
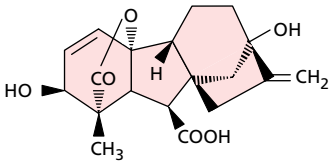


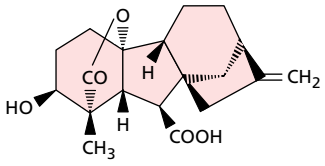
FIGURE 20.10 Phenotypes and genotypes of peas that differ in the gibberellin content of their vegetative tissue. (All alleles are homozygous.) (After Davies 1995.)

berellins have biological activity in other species or tissues. For example, GA_3, which differs from GA_1 only in having one double bond, is relatively rare in higher plants but is able to substitute for GA_1 in most bioassays:



Gibberellic acid (GA_3)

GA_4, which lacks an OH group at C-13, is present in both *Arabidopsis* and members of the squash family (Cucurbitaceae). It is as active as GA_1, or even more active, in some bioassays, indicating that GA_4 is a bioactive gibberellin in the species where it occurs (Xu et al. 1997). The structure of GA_4 looks like this:



Gibberellin A_4 (GA_4)

Gibberellins Are Biosynthesized in Apical Tissues

The highest levels of gibberellins are found in immature seeds and developing fruits. However, because the gibberellin level normally decreases to zero in mature seeds, there is no evidence that seedlings obtain any active gibberellins from their seeds.

Work with pea seedlings indicates that the gibberellin biosynthetic enzymes and GA3ox are specifically localized in young, actively growing buds, leaves, and upper internodes (Elliott et al. 2001). In *Arabidopsis*, GA20ox is expressed primarily in the apical bud and young leaves, which thus appear to be the principal sites of gibberellin synthesis (Figure 20.11).

The gibberellins that are synthesized in the shoot can be transported to the rest of the plant via the phloem. Intermediates of gibberellin biosynthesis may also be translocated in the phloem. Indeed, the initial steps of gibberellin biosynthesis may occur in one tissue, and metabolism to active gibberellins in another.

Gibberellins also have been identified in root exudates and root extracts, suggesting that roots can also synthesize gibberellins and transport them to the shoot via the xylem.

Gibberellin Regulates Its Own Metabolism

Endogenous gibberellin regulates its own metabolism by either switching on or inhibiting the transcription of the genes that encode enzymes of gibberellin biosynthesis and degradation (feedback and feed-forward regulation, respectively). In this way the level of active gibberellins is kept within a narrow range, provided that precursors are available and the enzymes of gibberellin biosynthesis and degradation are functional.

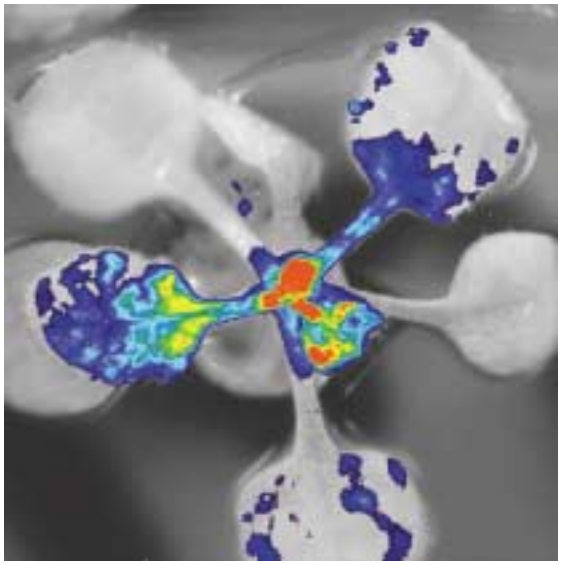


FIGURE 20.11 Gibberellin is synthesized mainly in the shoot apex and in young developing leaves. This false color image shows light emitted by transgenic *Arabidopsis* plants expressing the firefly luciferase coding sequence coupled to the GA20ox gene promoter. The emitted light was recorded by a CCD camera after the rosette was sprayed with the substrate luciferin. The image was then color-coded for intensity and superimposed on a photograph of the same plant. The red and yellow regions correspond to the highest light intensity. (Courtesy of Jeremy P. Coles, Andrew L. Phillips, and Peter Hedden, IACR-Long Ashton Research Station.)

For example, the application of gibberellin causes a down-regulation of the biosynthetic genes—GA20ox and GA3ox—and an elevation in transcription of the degradative gene—GA2ox (Hedden and Phillips 2000; Elliott et al. 2001). A mutation in the GA 2-oxidase gene, which prevents GA_1 from being degraded, is functionally equivalent to applying exogenous gibberellin to the plant, and produces the same effect on the biosynthetic gene transcription.

Conversely, a mutation that lowers the level of active gibberellin, such as GA_1, in the plant stimulates the transcription of the biosynthetic genes—GA20ox and GA3ox—and down-regulates the degradative enzyme—GA2ox. In peas this is particularly evident in very dwarf plants, such as those with a mutation in the *LS* gene (CPP synthase) or even more severely dwarf *na* plants (defective GA_{12}-aldehyde synthase) (Figure 20.12).

Environmental Conditions Can Alter the Transcription of Gibberellin Biosynthesis Genes

Gibberellins play an important role in mediating the effects of environmental stimuli on plant development. Environmental factors such as photoperiod and temperature can alter the levels of active gibberellins by affecting gene transcription for specific steps in the biosynthetic pathway (Yamaguchi and Kamiya 2000).

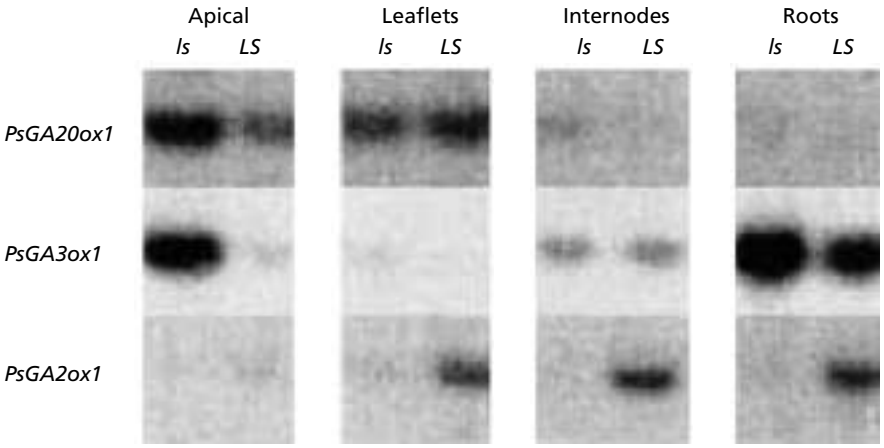


FIGURE 20.12 Northern blots of the mRNA for the enzymes of gibberellin biosynthesis in different tissues of peas. The more intense the band, the more mRNA was present. The plants designated *LS* are tall wild-type plants. Those designated *ls* are very dwarf mutants due to a defective copalyl diphosphate synthase that creates a block in the GA biosynthesis pathway. The differences in the spot intensity show that a low level of GA_1 in the mutant *ls* plants causes the upregulation of GA_1 biosynthesis by GA20ox and GA3ox, and a repression of GA_1 breakdown by GA2ox. (From Elliott et al. 2001.)

Light regulation of GA_1 biosynthesis. The presence of light has many profound effects. Some seeds germinate only in the light, and in such cases gibberellin application can stimulate germination in darkness. The promotion of germination by light has been shown to be due to increases in GA_1 levels resulting from a light-induced increase in the transcription of the gene for GA3ox, which converts GA_{20} to GA_1 (Toyomasu et al. 1998). This effect shows red/far-red photoreversibility and is mediated by phytochrome (see Chapter 17).

When a seedling becomes exposed to light as it emerges from the soil, it changes its form (see Chapter 17)—a process referred to as de-etiolation. One of the most striking changes is a decrease in the rate of stem elongation such that the stem in the light is shorter than the one in the dark. Initially it was assumed that the light-grown plants would contain less GA_1 than dark-grown plants. However, light-grown plants turned out to contain *more* GA_1 than dark-grown plants—indicating that de-etiolation is a complex process involving changes in the level of GA_1, as well as changes in the responsiveness of the plant to GA_1.

In peas, for example, the level of GA_1 initially falls within 4 hours of exposure to light because of an increase in transcription of the gene for GA2ox, leading to an increase in GA_1 breakdown (Figure 20.13A). The level of GA_1 remains low for a day but then increases, so that by

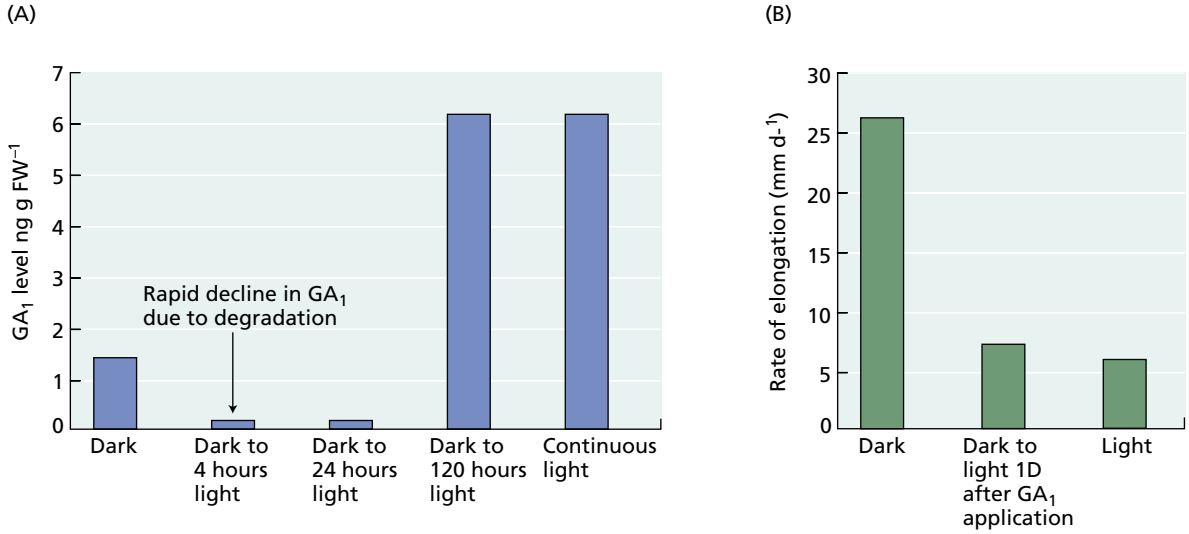


FIGURE 20.13 When a plant grows in the light, the rate of extension slows down through regulation by changes in hormone levels and sensitivity. (A) When dark-grown pea seedlings are transferred to light, GA_1 level drops rapidly because of metabolism of GA_1, but then increases to a higher level, similar to that of light-grown plants, over the next 4 days. (B) To investigate the GA_1 response in various light regimes, 10 mg of GA_1 was applied to the internode of GA-deficient *na* plants in darkness, 1 day after the start of the light, or 6 days of continuous light, and growth in the next 24 hours was measured. The results show that the gibberellin sensitivity of pea seedlings falls rapidly upon transfer from darkness to light, so the elongation rate of plants in the light is lower than in the dark, even though their total GA_1 content is higher. (After O'Neill et al. 2000.)

FIGURE 20.14 Spinach plants undergo stem and petiole elongation only in long days, remaining in a rosette form in short days. Treatment with the GA biosynthesis inhibitor AMO-1618 prevents stem and petiole elongation and maintains the rosette growth habit even under long days. Gibberellic acid can reverse the inhibitory effect of AMO-1618 on stem and petiole elongation. As shown in Figure 20.16, long days cause changes in the gibberellin content of the plant. (Courtesy of J. A. D. Zeevaart.)

5 days there is a fivefold increase in the GA_1 content of the stems, even though the stem elongation rate is lower (Figure 20.13B) (O'Neill et al. 2000). The reason that growth slows down despite the increase in GA_1 level is that the plants are now severalfold *less sensitive* to the GA_1 present.

As will be discussed later in the chapter, sensitivity to active gibberellin is governed by components of the gibberellin signal transduction pathway.

Photoperiod regulation of GA_1 biosynthesis. When plants that require long days to flower (see Chapter 24) are shifted from short days to long days, gibberellin metabolism is altered. In spinach (*Spinacia oleracea*), in short days, when the plants maintain a rosette form (Figure 20.14), the level of gibberellins hydroxylated at carbon 13 is relatively low. In response to increasing day length, the shoots of spinach plants begin to elongate after about 14 long days.

The levels of all the gibberellins of the carbon 13–hydroxylated gibberellin pathway ($GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1 \rightarrow GA_8$) start to increase after about 4 days (Figure 20.15). Although the level of GA_{20} increases 16-fold during the first 12 days, it is the fivefold increase in GA_1 that induces stem growth (Zeevaart et al. 1993).

The dependence of stem growth on GA_1 has been shown through the use of different inhibitors of gibberellin synthesis and metabolism. The inhibitors AMO-1618 and BX-112 both prevent internode elongation (bolting). The effect of AMO-1618, which blocks gibberellin biosynthesis prior to GA_{12}-aldehyde, can be overcome by applications of GA_{20} (Figure 20.16A). However, the effect of another inhibitor, BX-112, which blocks the production of GA_1 from GA_{20}, can be overcome only by GA_1 (Figure 20.16B). This result demonstrates that the rise in GA_1 is the crucial factor in regulating spinach stem growth.

The level of GA 20-oxidase mRNA in spinach tissues, which occurs in the highest amount in shoot tips and elongating stems (see Figure 20.11), is increased under long-day conditions (Wu et al. 1996). The fact that GA 20-oxidase is the enzyme that converts GA_{53} to GA_{20} (see Figure 20.7) explains why the concentration of GA_{20} was found to be higher in spinach under long-day conditions (Zeevaart et al. 1993).

Photoperiod control of tuber formation. Potato tuberization is another process regulated by photoperiod (Figure 20.17). Tubers form on wild potatoes only in short days (although the requirement for short days has been bred out of many cultivated varieties), and this tuberization can be blocked by applications of gibberellin. The transcription of GA20ox was found to fluctuate during the light–dark cycle, leading to lower levels of GA_1 in short days. Potato plants overexpressing the GA20ox gene showed delayed tuberization, whereas trans-

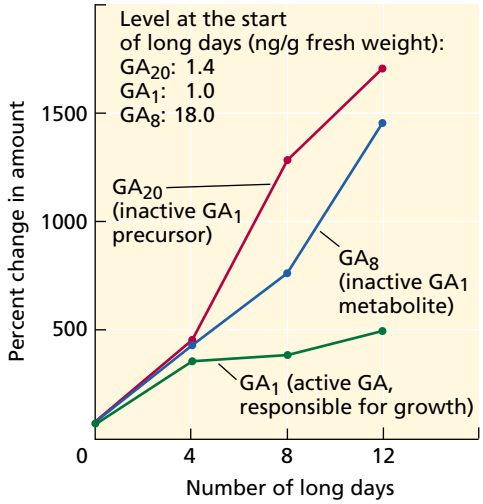


FIGURE 20.15 The fivefold increase in GA_1 is what causes growth in spinach exposed to an increasing number of long days but before stem elongation starts at about 14 days. (After Davies 1995; redrawn from data in Zeevaart et al. 1993.)

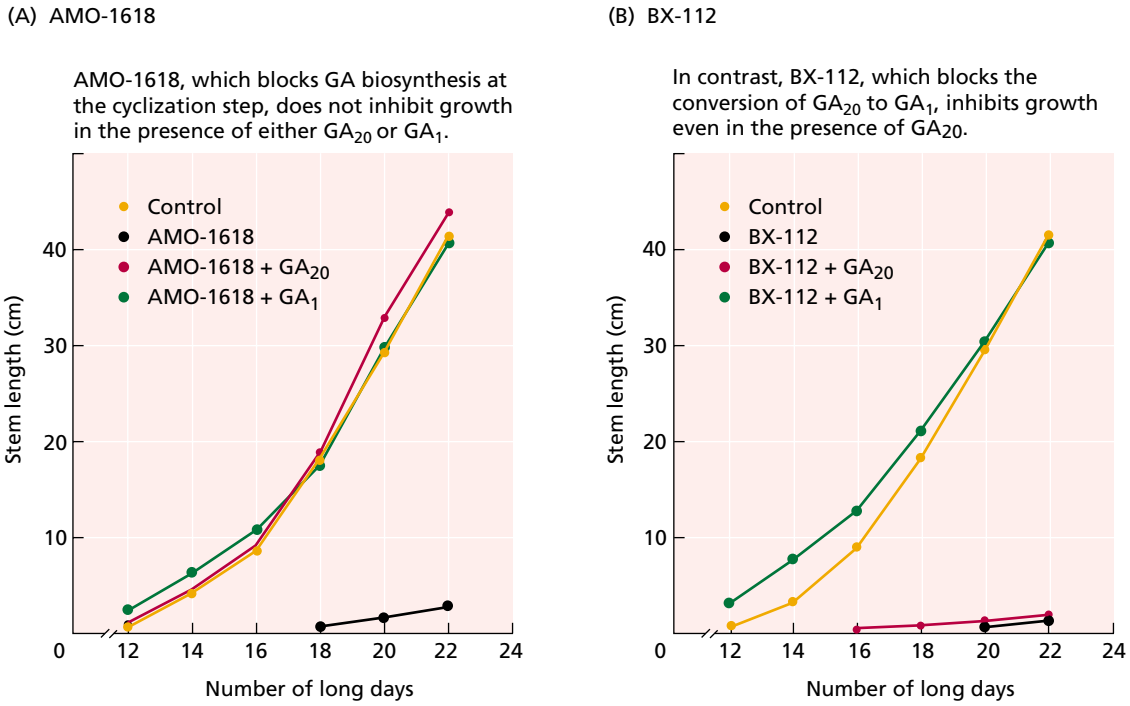


FIGURE 20.16 The use of specific growth retardants (GA biosynthesis inhibitors) and the reversal of the effects of the growth retardants by different GAs can show which steps in GA biosynthesis are regulated by environmental change, in this case the effect of long days on stem growth in spinach. The control lacks inhibitors or added GA. (After Zeevaart et al. 1993.)

formation with the antisense gene for GA20ox promoted tuberization, demonstrating the importance of the transcription of this gene in the regulation of potato tuberization (Carrera et al. 2000).

In general, de-etiolation, light-dependent seed germination, and the photoperiodic control of stem growth in rosette plants and tuberization in potato are all mediated by phytochromes (see Chapter 17). There is mounting evidence that many phytochrome effects are in part due to modulation of the levels of gibberellins through changes in the transcription of the genes for gibberellin biosynthesis and degradation.

Temperature effects. Cold temperatures are required for the germination of certain seeds (stratification) and for flowering in certain species (vernalization) (see Chapter

FIGURE 20.17 Tuberization of potatoes is promoted by short days. Potato (*Solanum tuberosum* spp. *Andigena*) plants were grown under either long days or short days. The formation of tubers in short days is associated with a decline in GA_1 levels (see Chapter 24). (Courtesy of S. Jackson.)

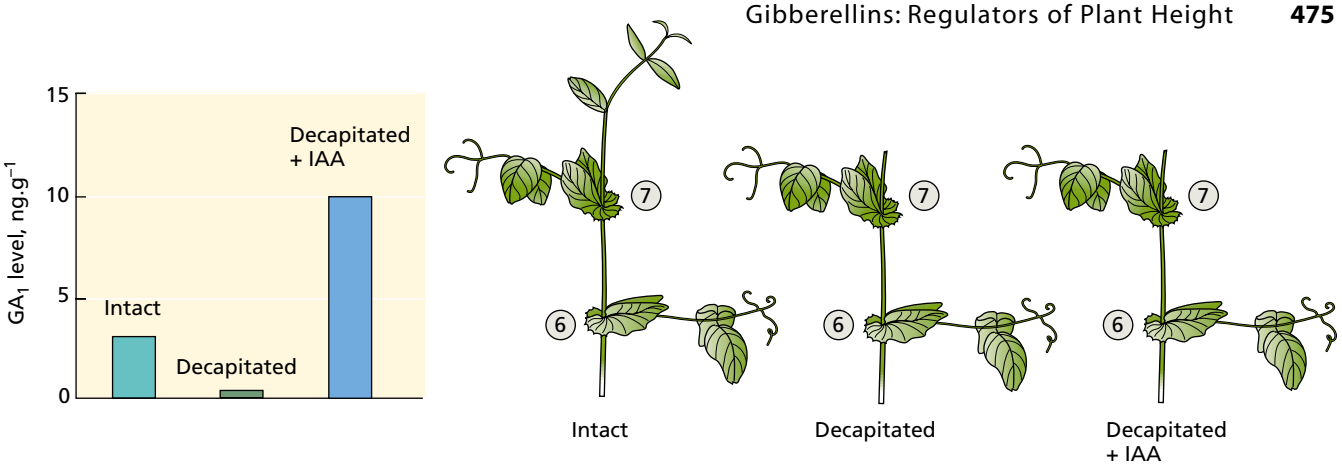


FIGURE 20.18 Decapitation reduces, and IAA (auxin) restores, endogenous GA_1 content in pea plants. Numbers refer to the leaf node. (From Ross et al. 2000.)

24). For example, a prolonged cold treatment is required for both the stem elongation and the flowering of *Thlaspi arvense* (field pennycress), and gibberellins can substitute for the cold treatment.

In the absence of the cold treatment, *ent*-kaurenoic acid accumulates to high levels in the shoot tip, which is also the site of perception of the cold stimulus. After cold treatment and a return to high temperatures, the *ent*-kaurenoic acid is converted to GA_9, the most active gibberellin for stimulating the flowering response. These results are consistent with a cold-induced increase in the activity of *ent*-kaurenoic acid hydroxylase in the shoot tip (Hazebroek and Metzger 1990).

Auxin Promotes Gibberellin Biosynthesis

Although we often discuss the action of hormones as if they act singly, the net growth and development of the plant are the results of many combined signals. In addition, hormones can influence each other's biosynthesis so that the effects produced by one hormone may in fact be mediated by others.

For example, it has long been known that auxin induces ethylene biosynthesis. It is now evident that gibberellin can induce auxin biosynthesis and that auxin can induce gibberellin biosynthesis. If pea plants are decapitated, leading to a cessation in stem elongation, not only is the level of auxin lowered because its source has been removed, but the level of GA_1 in the upper stem drops sharply. This change can be shown to be an auxin effect because replacing the bud with a supply of auxin restores the GA_1 level (Figure 20.18).

The presence of auxin has been shown to promote the transcription of *GA3ox* and to repress the transcription of *GA2ox* (Figure 20.19). In the absence of auxin the reverse occurs. Thus the apical bud promotes growth not only through the direct biosynthesis of auxin, but also through the auxin-induced biosynthesis of GA_1 (Figure 20.20) (Ross et al. 2000; Ross and O'Neill 2001).

Figure 20.21 summarizes some of the factors that modulate the active gibberellin level through regulation of the transcription of the genes for gibberellin biosynthesis or metabolism.

Dwarfness Can Now Be Genetically Engineered

The characterization of the gibberellin biosynthesis and metabolism genes—*GA20ox*, *GA3ox*, and *GA2ox*—has

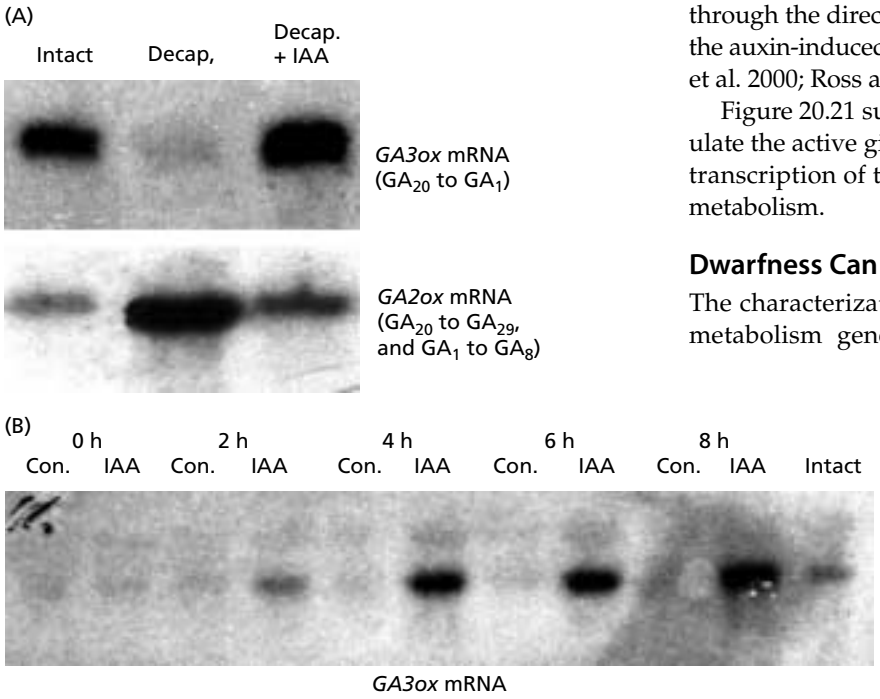


FIGURE 20.19 (A) IAA up-regulates the transcription of GA 3β-hydroxylase (forming GA_1), and down-regulates that of GA 2-oxidase, which destroys GA_1. (B) The increase in GA 3β-hydroxylase in response to IAA can be seen by 2 hours. Con., control. (From Ross et al. 2000.)

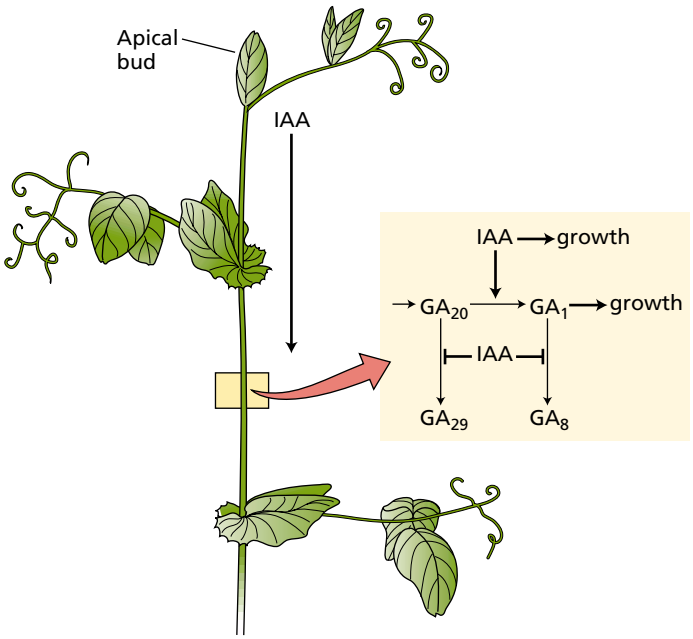


FIGURE 20.20 IAA (from the apical bud) promotes and is required for GA_1 biosynthesis in subtending internodes. IAA also inhibits GA_1 breakdown. (From Ross and O'Neill 2001.)

FIGURE 20.22 Genetically engineered dwarf wheat plants. The untransformed wheat is shown on the extreme left. The three plants on the right were transformed with a gibberellin 2-oxidase cDNA from bean under the control of a constitutive promoter, so that the endogenous active GA_1 was degraded. The varying degrees of dwarfing reflects varying degrees of overexpression of the foreign gene. (Photo from Hedden and Phillips 2000, courtesy of Andy Phillips.)

enabled genetic engineers to modify the transcription of these genes to alter the gibberellin level in plants, and thus affect their height (Hedden and Phillips 2000). The desired effect is usually to increase dwarfness because plants grown in dense crop communities, such as cereals, often grow too tall and thus are prone to lodging. In addition, because gibberellin regulates bolting, one can prevent bolting by inhibiting the rise in gibberellin. An example of the latter is the inhibition of bolting in sugar beet.

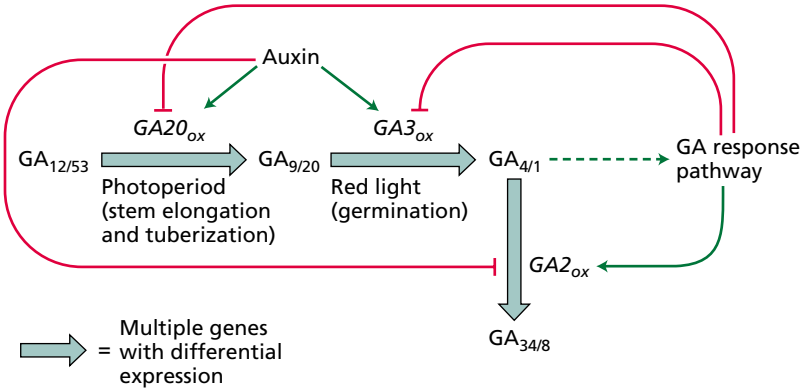


FIGURE 20.21 The pathway of gibberellin biosynthesis showing the identities of the genes for the metabolic enzymes and the way that their transcription is regulated by feedback, environment, and other endogenous hormones.

Sugar beet is a biennial, forming a swollen storage root in the first season and a flower and seed stalk in the second. To extend the growing season and obtain bigger beets, farmers sow the beets as early as possible in the spring, but sowing too early leads to bolting in the first year, with the result that no storage roots form. A reduction in the capacity to make gibberellin inhibits bolting, allowing earlier sowing of the seeds and thus the growth of larger beets.

Reductions in GA_1 levels have recently been achieved in such crops as sugar beet and wheat, either by the transformation of plants with antisense constructs of the *GA20ox* or *GA3ox* genes, which encode the enzymes leading to the synthesis of GA_1, or by overexpressing the gene responsible for GA_1 metabolism: *GA2ox*. Either approach results in dwarfing in wheat (Figure 20.22) or an inhibition of bolting in rosette plants such as beet.

The inhibition of seed production in such transgenic plants can be overcome by sprays of gibberellin solution, provided that the reduction in gibberellin has been achieved by blocking the genes for GA20ox or GA3ox, the gibberellin biosynthetic enzymes. A similar strategy has recently been applied to turf grass, keeping the grass short with no seedheads, so that mowing can be virtually eliminated—a boon for homeowners!

PHYSIOLOGICAL MECHANISMS OF GIBBERELLIN-INDUCED GROWTH

As we have seen, the growth-promoting effects of gibberellin are most evident in dwarf and rosette plants. When dwarf plants are treated with gibberellin, they resemble the tallest varieties of the same species (see Figure 20.1). Other examples of gibberellin action include the elongation of hypocotyls and of grass internodes.

A particularly striking example of internode elongation is found in deep-water rice (*Oryza sativa*). In general, rice plants are adapted to conditions of partial submergence. To enable the upper foliage of the plant to stay above water, the internodes elongate as the water level rises. Deep-water rice has the greatest potential for rapid internode elongation. Under field conditions, growth rates of up to 25 cm per day have been measured.

The initial signal is the reduced partial pressure of O_2 resulting from submergence, which induces ethylene biosynthesis (see Chapter 22). The ethylene trapped in the submerged tissues, in turn, reduces the level of abscisic acid (see Chapter 23), which acts as an antagonist of gibberellin. The end result is that the tissue becomes more responsive to its endogenous gibberellin (Kende et al. 1998). Because inhibitors of gibberellin biosynthesis block the stimulatory effect of both submergence and ethylene on growth, and exogenous gibberellin can stimulate growth in the absence of submergence, gibberellin appears to be the hormone directly responsible for growth stimulation.

GA-stimulated growth in deep-water rice can be studied in an excised stem system (Figure 20.23). The addition of gibberellin causes a marked increase in the growth rate after a lag period of about 40 minutes. Cell elongation accounts for about 90% of the length increase during the first 2 hours of gibberellin treatment.

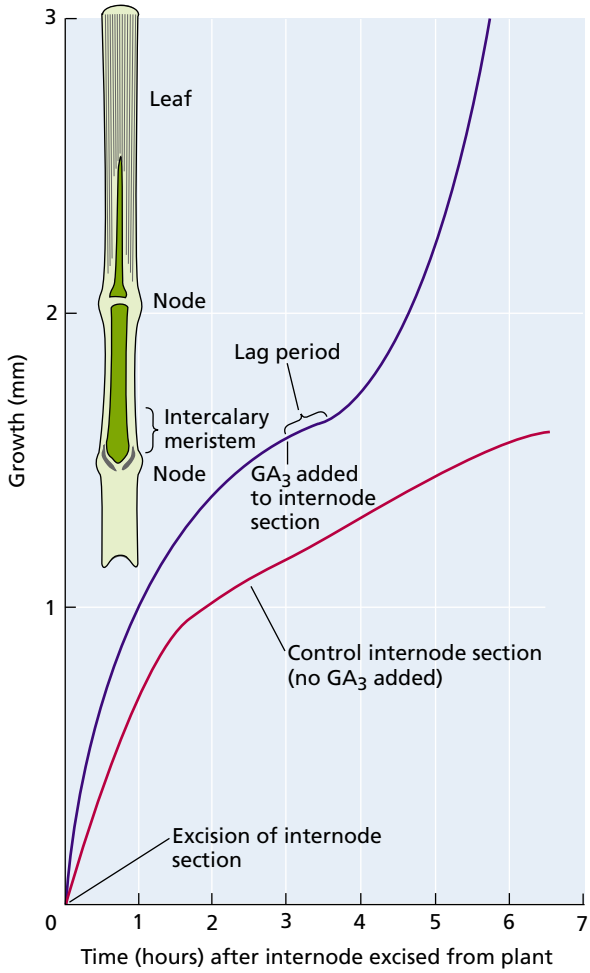


FIGURE 20.23 Continuous recording of the growth of the upper internode of deep-water rice in the presence or absence of exogenous GA_3. The control internode elongates at a constant rate after an initial growth burst during the first 2 hours after excision of the section. Addition of GA after 3 hours induced a sharp increase in the growth rate after a 40-minute lag period (upper curve). The difference in the initial growth rates of the two treatments is not significant here, but reflects slight variation in experimental materials. The inset shows the internode section of the rice stem used in the experiment. The intercalary meristem just above the node responds to GA. (After Sauter and Kende 1992.)

Gibberellins Stimulate Cell Elongation and Cell Division

The effect of gibberellins applied to intact dwarf plants is so dramatic that it would seem to be a simple task to determine how they act. Unfortunately, this is not the case because, as we have seen with auxin, so much about plant cell growth is not understood. However, we do know some characteristics of gibberellin-induced stem elongation.

Gibberellin increases both cell elongation and cell division, as evidenced by increases in cell length and cell number in response to applications of gibberellin. For example, internodes of tall peas have more cells than those of dwarf peas, and the cells are longer. Mitosis increases markedly in the subapical region of the meristem of rosette long-day plants after treatment with gibberellin (Figure 20.24). The dramatic stimulation of internode elongation in deep-water rice is due in part to increased cell division activity in the intercalary meristem. Moreover, only the cells of the intercalary meristem whose division is increased by gibberellin exhibit gibberellin-stimulated cell elongation.

Because gibberellin-induced cell elongation appears to precede gibberellin-induced cell division, we begin our discussion with the role of gibberellin in regulating cell elongation.

Gibberellins Enhance Cell Wall Extensibility without Acidification

As discussed in Chapter 15, the elongation rate can be influenced by both cell wall extensibility and the osmotically driven rate of water uptake. Gibberellin has no effect

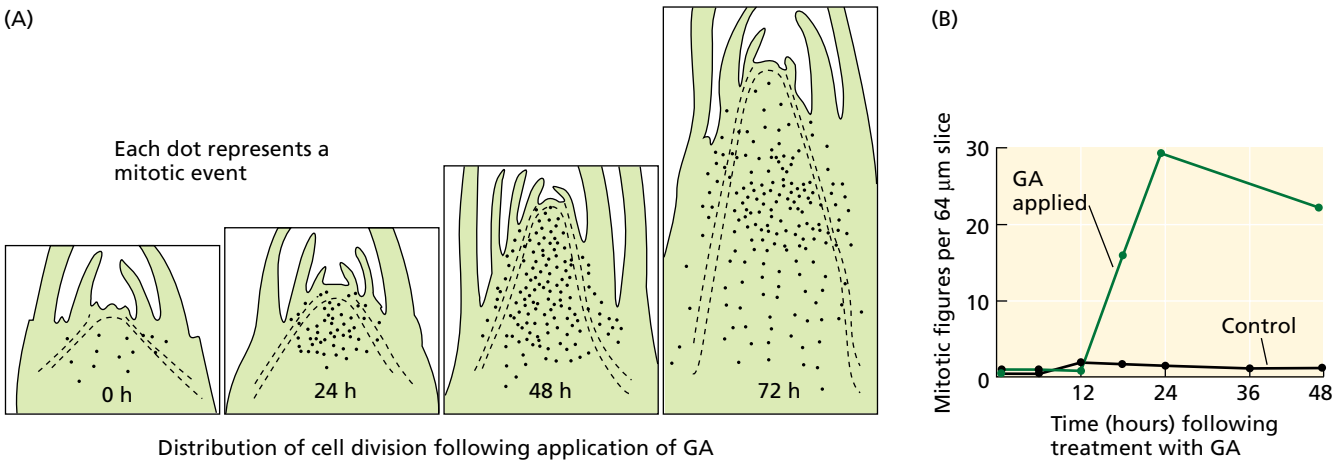


FIGURE 20.24 Gibberellin applications to rosette plants induce stem internode elongation in part by increasing cell division. (A) Longitudinal sections through the axis of *Samolus parviflorus* (brookweed) show an increase in cell division after application of GA. (Each dot represents one mitotic figure in a section 64 µm thick.) (B) The number of such mitotic figures with and without GA in stem apices of *Hyoscyamus niger* (black henbane). (After Sachs 1965.)

on the osmotic parameters but has consistently been observed to cause an increase in both the mechanical extensibility of cell walls and the stress relaxation of the walls of living cells. An analysis of pea genotypes differing in gibberellin content or sensitivity showed that gibberellin decreases the minimum force that will cause wall extension (the wall yield threshold) (Behringer et al. 1990). Thus, both gibberellin and auxin seem to exert their effects by modifying cell wall properties.

In the case of auxin, cell wall loosening appears to be mediated in part by cell wall acidification (see Chapter 19). However, this does not appear to be the mechanism of gibberellin action. In no case has a gibberellin-stimulated increase in proton extrusion been demonstrated. On the other hand, gibberellin is never present in tissues in the complete absence of auxin, and the effects of gibberellin on growth may depend on auxin-induced wall acidification.

The typical lag time before gibberellin-stimulated growth begins is longer than for auxin; as noted already, in deepwater rice it is about 40 minutes (see Figure 20.23), and in peas it is 2 to 3 hours (Yang et al. 1996). These longer lag times point to a growth-promoting mechanism distinct from that of auxin. Consistent with the existence of a separate gibberellin-specific wall-loosening mechanism, the growth responses to applied gibberellin and auxin are additive.

Various suggestions have been made regarding the mechanism of gibberellin-stimulated stem elongation, and all have some experimental support, but as yet none provide a clear-cut answer. For example, there is evidence that the enzyme xyloglucan endotransglycosylase (XET) is involved in gibberellin-promoted wall extension. The function of XET may be to facilitate the penetration of expansins into the cell wall. (Recall that expansins are cell wall proteins that cause wall loosening in acidic conditions by weakening hydrogen bonds between wall polysaccharides [see Chapter 15].) Both expansins and XET may be required for gibberellin-stimulated cell elongation (see **Web Topic 20.3**).

Gibberellins Regulate the Transcription of Cell Cycle Kinases in Intercalary Meristems

As noted earlier, the growth rate of the internodes of deepwater rice dramatically increases in response to submergence, and part of this response is due to increased cell divisions in the intercalary meristem. To study the effect of gibberellin on the cell cycle, researchers isolated nuclei from the intercalary meristem and quantified the amount of DNA per nucleus (Figure 20.25) (Sauter and Kende 1992).

In submergence-induced plants, gibberellin activates the cell division cycle first at the transition from G_1 to S phase, leading to an increase in mitotic activity. To do this, gibberellin induces the expression of the genes for several **cyclin-dependent protein kinases** (**CDKs**), which are involved in regulation of the cell cycle (see Chapter 1). The transcription of these genes—first those regulating the transition from G_1 to S phase, followed by those regulating the transition from G_2 to M phase—is induced in the intercalary meristem by gibberellin. The result is a gibberellin-induced increase in the progression from the G_1 to the S phase through to mitosis and cell division (see **Web Topic 20.4**) (Fabian et al. 2000).

Gibberellin Response Mutants Have Defects in Signal Transduction

Single-gene mutants impaired in their response to gibberellin provide valuable tools for identifying genes that encode possible gibberellin receptors or components of signal transduction pathways. In screenings for such mutants,

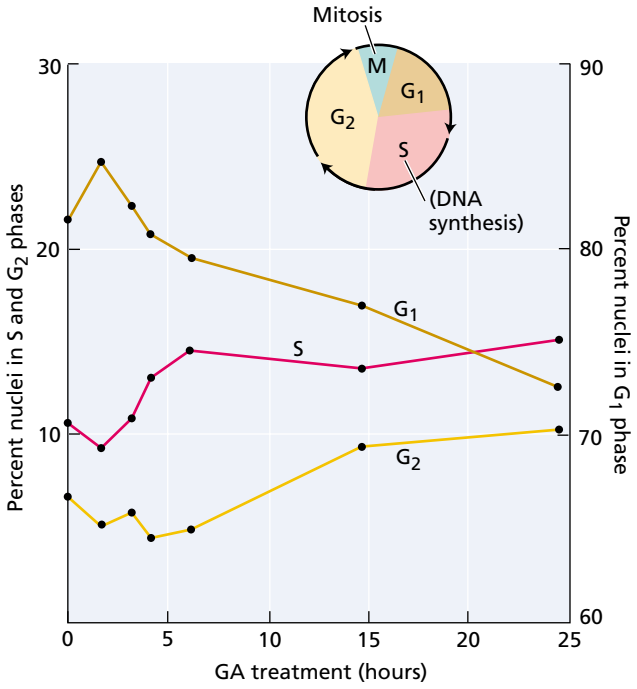


FIGURE 20.25 Changes in the cell cycle status of nuclei from the intercalary meristems of deep-water rice internodes treated with GA_3. Note that the scale for the G_1 nuclei is on the right side of the graph. (After Sauter and Kende 1992.)

three main classes of mutations affecting plant height have been selected:

1. Gibberellin-insensitive dwarfs
2. Gibberellin-deficient mutants in which the gibberellin deficiency has been overcome by a second "suppressor" mutation, so the plants look closer to normal
3. Mutants with a constitutive gibberellin response ("slender" mutants)

All three types of gibberellin response mutants have been generated in *Arabidopsis*, but equivalent mutations have also been found in several other species; in fact, some have been in agricultural use for many years.

The three types of mutant screens have sometimes identified genes encoding the same signal transduction components, even though the phenotypes being selected are completely different. This is possible because mutations at different sites in the same protein can produce vastly different phenotypes, depending on whether the mutation is in a regulatory domain or in an activity, or functional, domain. Some examples of the different phenotypes that can result from changes at different sites in the same protein are described in the sections that follow.

Functional domain (repression). The principal gibberellin signal transduction components that have been identified so far are *repressors of gibberellin signaling*; that is, they repress what we regard as gibberellin-induced tall growth and make the plant dwarf. The repressor proteins are negated or turned off by gibberellin so that the default-type growth—namely, tall—is allowed to proceed. The loss of function resulting from a mutation in the functional domain of such a *negative regulator* results in the mutant appearing as if it has been treated with gibberellin; that is, it has a tall phenotype. Thus a loss-of-function mutation of a negative regulator is like a double negative in English grammar: It translates into a positive.

Because the effects of these loss-of-function mutations are pleiotropic—that is, they also affect developmental processes other than stem elongation—the steps in the pathway involved in the growth response are probably common to all gibberellin responses.

Regulatory domain. If a mutation in the gene for the same negative regulator causes a change in the *regulatory domain* (i.e., that part of the protein that receives a signal from the gibberellin receptor indicating the presence of gibberellin), the protein is unable to receive the signal, and it retains its growth-repressing activity. The phenotype of such a mutant will be that of a gibberellin-insensitive dwarf. Thus, different mutations in the same gene can give opposite phenotypes (tall versus dwarf), depending on whether the mutation is located in the repression domain or the regulatory domain.

The regulatory domain mutations that confer loss of gibberellin sensitivity result in the synthesis of a constitutively active form of the repressor than cannot be turned off by gibberellin. The more of this type of mutant repressor that is present in the cell, the more dwarf the plant will be. Hence, such regulatory domain mutations are semidominant.

In contrast, mutations in the repression domain inactivate the negative regulator (i.e., they act as "knockout" alleles) so that it no longer represses growth; such mutations are recessive because in a heterozygote half the proteins will still be able to repress growth in the absence of gibberellin. *All* of the negative regulators have to be nonfunctional for the plant to grow tall without gibberellin.

With this as background, we now examine specific examples of mutations in the genes that encode proteins in the gibberellin signal transduction pathway.

Different Genetic Screens Have Identified the Related Repressors GAI and RGA

Several gibberellin-insensitive dwarf mutants have been isolated from various species. The first to be isolated in *Arabidopsis* was the *gai-1* mutant (Figure 20.26) (Sun 2000). The *gai-1* mutants resemble gibberellin-deficient mutants, except that they do not respond to exogenous gibberellin.

Another mutant was obtained by screening for a second mutation in a gibberellin-deficient *Arabidopsis* mutant that restores, or partially restores, wild-type growth. The origi-

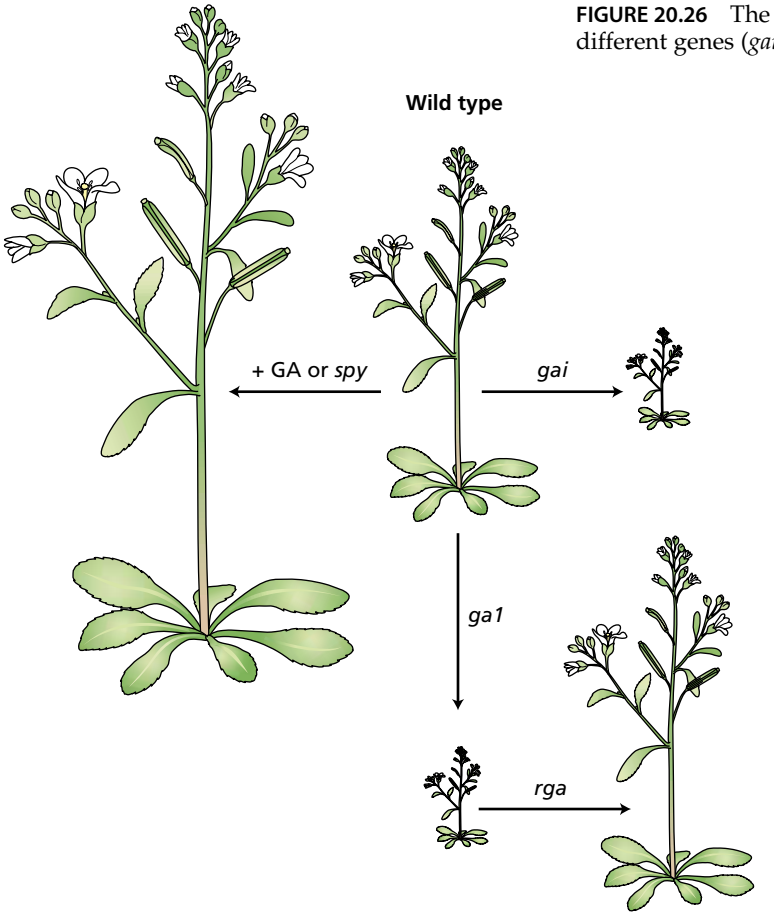


FIGURE 20.26 The effects of gibberellin treatment and mutations in three different genes (*gai*, *ga1*, and *rga*) on the phenotype of *Arabidopsis*.

The reason that *gai-1* is dwarf, while *rga* is tall, is that the mutations are in different parts of the protein. Whereas the *gai-1* mutation (which negates sensitivity of the repressor to gibberellin) is in the regulatory domain, the *rga* mutation (which prevents the action of the repressor in blocking growth) is located in the repression domain, as illustrated in Figure 20.28.

The mutant *gai-1* gene has been shown to encode a mutant protein with a deletion of 17 amino acids, which corresponds to the regulatory domain of the repressor (Dill et al. 2001). A similar mutation in the receptor domain of the *RGA* gene also produces a gibberellin-insensitive dwarf, demonstrating that the two related proteins have overlapping functions. Because of this deletion in the *gai-1* mutant, the action of the repressor cannot be alleviated by gibberellin, and growth is constitutively inhibited.

Gibberellins Cause the Degradation of RGA Transcriptional Repressors

The *Arabidopsis* wild-type *GAI* and *RGA* genes are members of a large gene family encoding transcriptional gibberellin-deficient mutant was *ga1-3*, and the second mutation that partially "rescued" the phenotype (i.e., restored normal growth) was called *rga* (for *r*epressor of *ga1-3*).[4] The *rga* mutation is a recessive mutation that, when present in double copy, gives a plant of intermediate height (see Figure 20.26).

Despite the contrasting phenotypes of the mutants, the wild-type *GAI* and *RGA* genes turned out to be closely related, with a very high (82%) sequence identity. The *gai-1* mutation is semidominant, as are similar gibberellin-insensitive dwarf mutations in other species.

Genetic analyses have indicated that both the GAI and RGA proteins normally act as repressors of gibberellin responses. Gibberellin acts indirectly through an unidentified signaling intermediate, which is thought to bind to the regulatory domains of the GAI and RGA proteins (Figure 20.27). The repressor is no longer able to inhibit growth, and the resulting plant is tall.

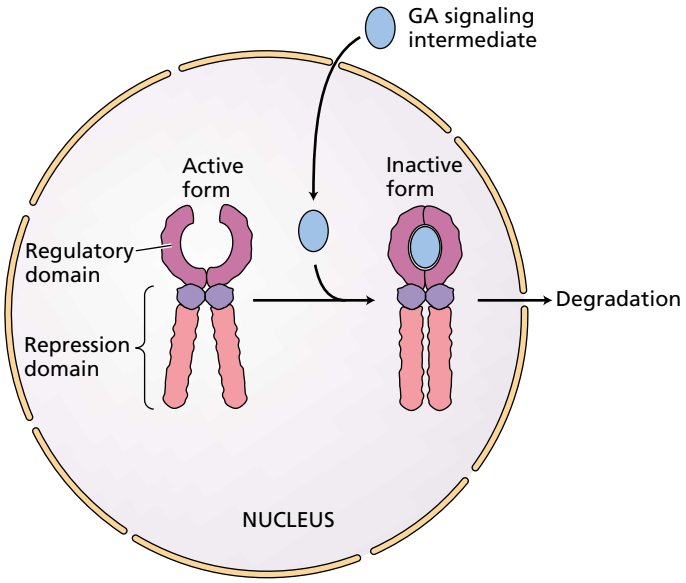


FIGURE 20.27 Two main functional domains of GAI and RGA: the regulatory domain and the repression domain. The repression domain is active in the absence of gibberellin. A gibberellin-induced signaling intermediate binds to the regulatory domain, targeting it for destruction. Note that the protein forms homodimers.

[4] Be careful not to confuse *gai* (gibberellin insensitive) and *ga1* (gibberellin-deficient #1), which can look alike in print.

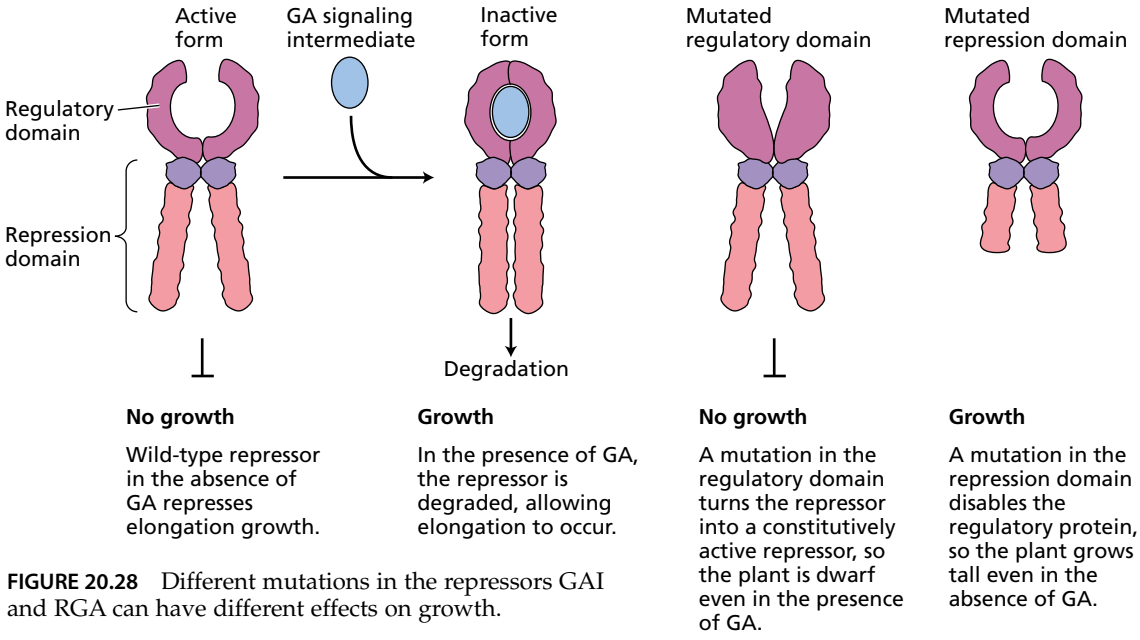


FIGURE 20.28 Different mutations in the repressors GAI and RGA can have different effects on growth.

scriptional repressors that have highly conserved regions with nuclear localization signals. To demonstrate the nuclear localization and repressor nature of the RGA product, the *RGA* promoter was fused to the gene for a green fluorescent protein whose product can be visualized under the microscope. The green color could be seen in cell nuclei.

When the plants were treated with gibberellin, there was no green color, showing that the RGA protein was not present following gibberellin treatment. However, when the gibberellin content was severely lowered by treatment with the gibberellin biosynthesis inhibitor paclobutrazol, the nuclei acquired a very intense green fluorescence, demonstrating both the presence and nuclear localization of the RGA protein only when gibberellin was absent or low (Figure 20.29) (Silverstone et al. 2001).

Both GAI and RGA also have a conserved region at the amino terminus of the protein referred to as DELLA, after

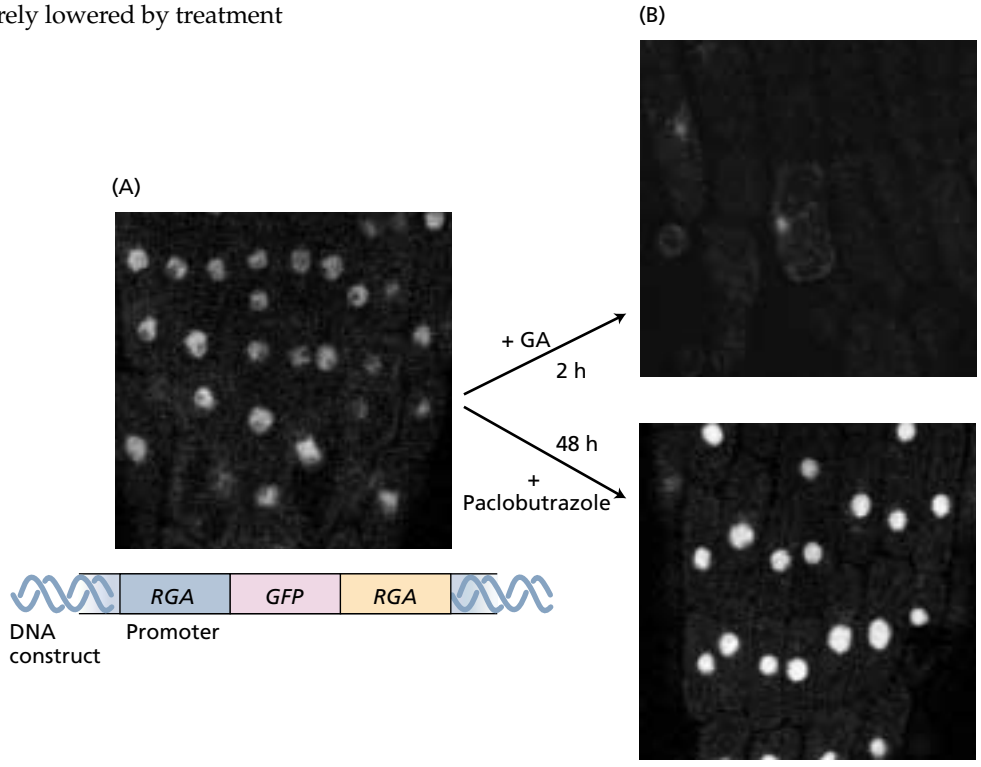


FIGURE 20.29 The RGA protein is found in the cell nucleus, consistent with its identity as a transcription factor, and its level is affected by the level of GA. (A) Plant cells were transformed with the gene for RGA fused to the gene for green fluorescent protein (GFP), allowing detection of RGA in the nucleus by fluorescence microscopy. (B) Effect of GA on RGA. A 2-hour pretreatment with gibberellin causes the loss of RGA from the cell (top). When the gibberellin biosynthesis is inhibited in the presence of paclobutrazole, the RGA content in the nucleus increases (bottom). (From Silverstone et al. 2001.)

the code letters for the amino acids in that sequence. This region is involved in the gibberellin response because it is the location of the mutation in *gai-1* that renders it nonresponsive to gibberellin. It turns out that the RGA protein is synthesized all the time; in the presence of gibberellin this protein is targeted for destruction, and the DELLA region is required for this response (Dill et al. 2001).

It is likely that gibberellin also brings about the turnover of GAI. *RGA* and *GAI* have partially redundant functions in maintaining the repressed state of the gibberellin signaling pathway. However, *RGA* appears to play a more dominant role than *GAI* because in a gibberellin-deficient mutant, a second mutation in the repression domain of *gai* (*gai-t6*) does not restore growth, whereas a comparable mutation in *rga* does. On the other hand, the existence of repression domain mutations in both of these genes allows for complete expression of many characteristics induced by GA, including plant height, in the absence of gibberellin (see Figure 20.26) (Dill and Sun 2001; King et al. 2001).

DELLA Repressors Have Been Identified in Crop Plants

Functional DELLA repressors have been found in several crop plants that have dwarfing mutations, analogous to *gai-1*, in the genes encoding these proteins. Most notable are the *rht* (*r*educed *h*eigh*t*) mutations of wheat that have been in use in agriculture for 30 years. These alleles encode gibberellin response modulators that lack gibberellin responsiveness, leading to dwarfness (Peng et al. 1999; Silverstone and Sun 2000).

Cereal dwarfs such as these are very important as the foundations of the green revolution that enabled large increases in yield to be obtained. Normal cereals grow too tall when close together in a field, especially with high levels of fertilizer. The result is that plants fall down (lodge), and the yield decreases concomitantly. The use of these stiff-strawed dwarf varieties that resist lodging enables high yields.

The Negative Regulator SPINDLY Is an Enzyme That Alters Protein Activity

"Slender mutants" resemble wild-type plants that have been treated with gibberellin repeatedly. They exhibit elongated internodes, parthenocarpic (seed-free) fruit growth (in dicots), and poor pollen production. Slender mutants are rare compared to dwarf mutants.

One possible explanation of the slender phenotype could be simply that the mutants have higher-than-normal levels of endogenous gibberellins. For example, in the *sln* mutation of peas, a gibberellin deactivation step is blocked in the seed. As a result, the mature seed, which in the wild type contains little or no GA, has abnormally high levels of GA_{20}. The GA_{20} from the seed is then taken up by the germinating seedling and converted to the bioactive GA_1, giving rise to the slender phenotype. However, once the seedling runs out of GA_{20} from the seed, its phenotype returns to normal (Reid and Howell 1995).

If, on the other hand, the slender phenotype is *not* due to an overproduction of endogenous gibberellin, the mutant is considered to be a **constitutive response mutant** (Sun 2000). The best characterized of such mutants are the ultratall mutants: *la cry*^{s} in pea, (representing mutations at two loci: *La* and *Cry*^{s}) (see Figure 20.10); *procera* (*pro*) in tomato; *slender* (*sln*) in barley; and *spindly* (*spy*) in *Arabidopsis* (Figure 20.30). All of these mutations are recessive and appear to be loss-of-function mutations in negative regulators of the gibberellin response pathway, as in the case of the DELLA regulators.

SPINDLY (*SPY*) in *Arabidopsis* and related genes in other species are similar in sequence to genes that encode glucosamine transferases in animals (Thornton et al. 1999). These enzymes modify target proteins by the glycosylation of serine or threonine residues. Glycosylation can modify protein activity either directly or indirectly by interfering with or blocking sites of phosphorylation by protein kinases. The target protein for spindly proteins has not yet been identified.

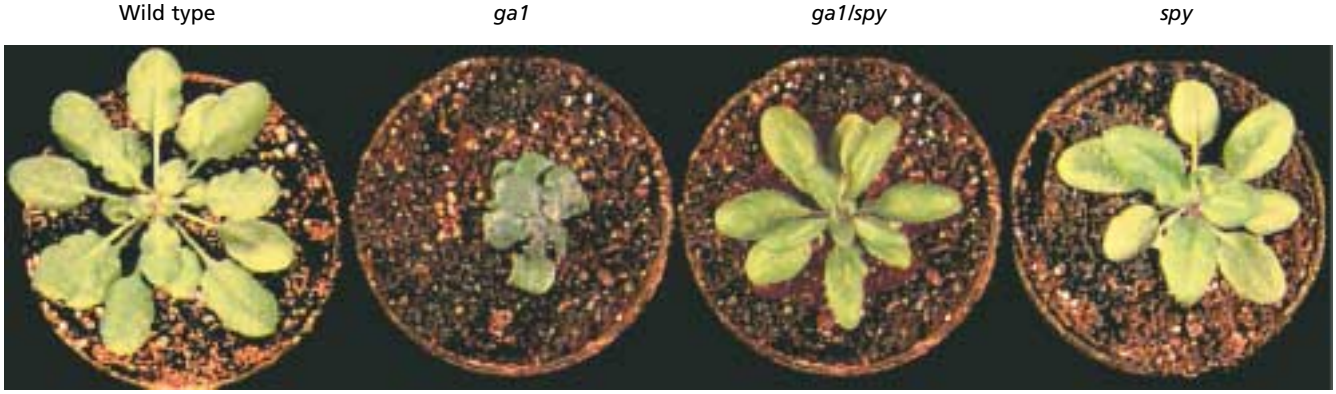


FIGURE 20.30 The *Arabidopsis spy* mutation causes the negation of a growth repressor, so the plants look as if they were treated with gibberellin. From left to right: wild type, *ga1* (GA-deficient), *ga1/spy* double mutant, and *spy*. (Courtesy of N. Olszewski.)

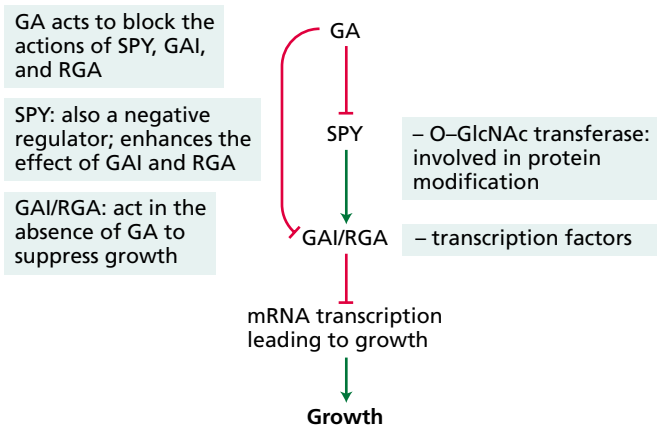
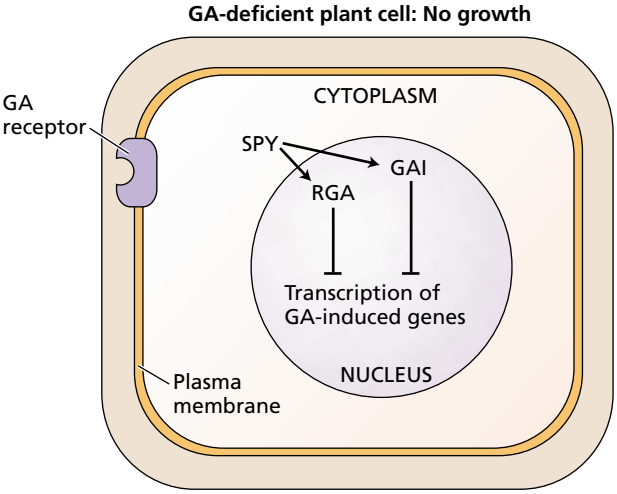


FIGURE 20.31 Interactions between gibberellin and the genes *SPY*, *GAI*, and *RGA* in the regulation of stem elongation.

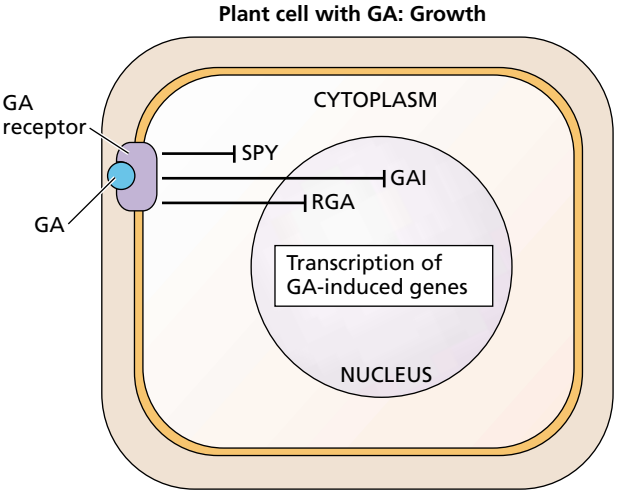
SPY Acts Upstream of *GAI* and *RGA* in the Gibberellin Signal Transduction Chain

On the basis of the evidence presented in the preceding sections and other studies on the expression of *SPY*, *GAI*, and *RGA* (Sun 2000; Dill et al. 2001), we can begin to sketch out the following elements of the gibberellin signal transduction chain (Figures 20.31 and 20.32):

- Two or more transcriptional regulators encoded by *GAI* and *RGA* act as inhibitors of the transcription of genes that directly or indirectly promote growth.
- SPY appears to be a signal transduction intermediate acting upstream of GAI and RGA that, itself, turns on or enhances the transcription or action of *GAI* and *RGA*, or another negative regulator.
- In the presence of gibberellin, *SPY*, *GAI*, and *RGA* are all negated or turned off.



In a GA-deficient cell in a GA biosynthesis mutant, or a wild-type cell without the GA signal, the transmembrane GA receptor is inactive in the absence of GA signal. In this situation, SPY is an active O-GlcNAc transferase that catalyzes the addition of a signal GlcNAc residue (from UDP-GlcNAc) via an O linkage to specific serine and/or threonine residues of target proteins, possibly RGA and GAI. Active RGA and GAI function as repressors of transcription, and they indirectly or directly inhibit the expression of GA-induced genes.



In the presence of GA the GA receptor is activated by binding of bioactive GA. The GA signal inhibits RGA and GAI repressors both directly and by deactivating SPY. In the absence of repression by RGA and GAI, GA-induced genes are transcribed.

FIGURE 20.32 Proposed roles of the active SPY, GAI, and RGA proteins in the GA signaling pathway within a plant cell.

- The RGA protein is degraded, and it is likely that GAI is similarly destroyed.

Whether gibberellin negates *GAI* and *RGA* through *SPY*, or independently, or both, is currently under investigation. However, the basic message in this case and in the cases of other plant hormones, such as ethylene (see Chapter 22) and the photoreceptor phytochrome (see Chapter 17), is that the default developmental program is for the induced type of growth to occur, but the default pathway is prevented by the presence of various negative regulators. Rather than directly promoting an effect, the arrival of the developmental signal—in this case gibberellin—negates the growth repressor, enabling the default condition.

GIBBERELLIN SIGNAL TRANSDUCTION: CEREAL ALEURONE LAYERS

Genetic analyses of gibberellin-regulated growth, such as the studies described in the previous section, have identified some of the genes and their gene products, but not the biochemical pathways involved in gibberellin signal transduction. The biochemical and molecular mechanisms, which are probably common to all gibberellin responses, have been studied most extensively in relation to the gibberellin-stimulated synthesis and secretion of α-amylase in cereal aleurone layers (Jacobsen et al. 1995).

In this section we will describe how such studies have shed light on the location of the gibberellin receptor, the transcriptional regulation of the genes for α-amylase and other proteins, and the possible signal transduction pathways involved in the control of α-amylase synthesis and secretion by gibberellin.

Gibberellin from the Embryo Induces α-Amylase Production by Aleurone Layers

Cereal grains (*caryopses*; singular *caryopsis*) can be divided into three parts: the diploid embryo, the triploid endosperm, and the fused testa–pericarp (seed coat–fruit wall). The embryo part consists of the plant embryo proper, along with its specialized absorptive organ, the *scutellum* (plural *scutella*), which functions in absorbing the solubilized food reserves from the endosperm and transmitting them to the growing embryo. The endosperm is composed of two tissues: the centrally located starchy endosperm and the aleurone layer (Figure 20.33A).

The starchy endosperm, typically nonliving at maturity, consists of thin-walled cells filled with starch grains. The aleurone layer surrounds the starchy endosperm and is cytologically and biochemically distinct from it. Aleurone cells are enclosed in thick primary cell walls and contain large numbers of protein-storing vacuoles called *protein bodies* (Figures 20.33B–D), enclosed by a single membrane. The protein bodies also contain phytin, a mixed cation salt (mainly Mg^{2+} and K^{+}) of *myo*-inositolhexaphosphoric acid (phytic acid).

During germination and early seedling growth, the stored food reserves of the endosperm—chiefly starch and protein—are broken down by a variety of hydrolytic enzymes, and the solubilized sugars, amino acids, and other products are transported to the growing embryo. The two enzymes responsible for starch degradation are α- and β-amylase. α-Amylase hydrolyzes starch chains internally to produce oligosaccharides consisting of α-1,4-linked glucose residues. β-Amylase degrades these oligosaccharides from the ends to produce maltose, a disaccharide. Maltase then converts maltose to glucose.

α-Amylase is secreted into the starchy endosperm of cereal seeds by both the scutellum and the aleurone layer (see Figure 20.33A). The sole function of the aleurone layer of the seeds of graminaceous monocots (e.g., barley, wheat, rice, rye, and oats) appears to be the synthesis and release of hydrolytic enzymes. After completing this function, aleurone cells undergo programmed cell death.

Experiments carried out in the 1960s confirmed Gottlieb Haberlandt's original observation of 1890 that the secretion of starch-degrading enzymes by barley aleurone layers depends on the presence of the embryo. When the embryo was removed (i.e., the seed was de-embryonated), no starch was degraded. However, when the de-embryonated "half-seed" was incubated in close proximity to the excised embryo, starch was digested, demonstrating that the embryo produced a diffusible substance that triggered α-amylase release by the aleurone layer.

It was soon discovered that gibberellic acid (GA_3) could substitute for the embryo in stimulating starch degradation. When de-embryonated half-seeds were incubated in buffered solutions containing gibberellic acid, secretion of α-amylase into the medium was greatly stimulated after an 8-hour lag period (relative to the control half-seeds incubated in the absence of gibberellic acid).

The significance of the gibberellin effect became clear when it was shown that the embryo synthesizes and releases gibberellins (chiefly GA_1) into the endosperm during germination. Thus the cereal embryo efficiently regulates the mobilization of its own food reserves through the secretion of gibberellins, which stimulate the digestive function of the aleurone layer (see Figure 20.33A).

Gibberellin has been found to promote the production and/or secretion of a variety of hydrolytic enzymes that are involved in the solubilization of endosperm reserves; principal among these is α-amylase. Since the 1960s, investigators have utilized isolated aleurone layers, or even aleurone cell protoplasts (see Figure 20.33C and D), rather than half-seeds (see Figure 20.33B). The isolated aleurone layer, consisting of a homogeneous population of target cells, provides a unique opportunity to study the molecular aspects of gibberellin action in the absence of nonresponding cell types.

In the following discussion of gibberellin-induced α-amylase production we focus on three questions:

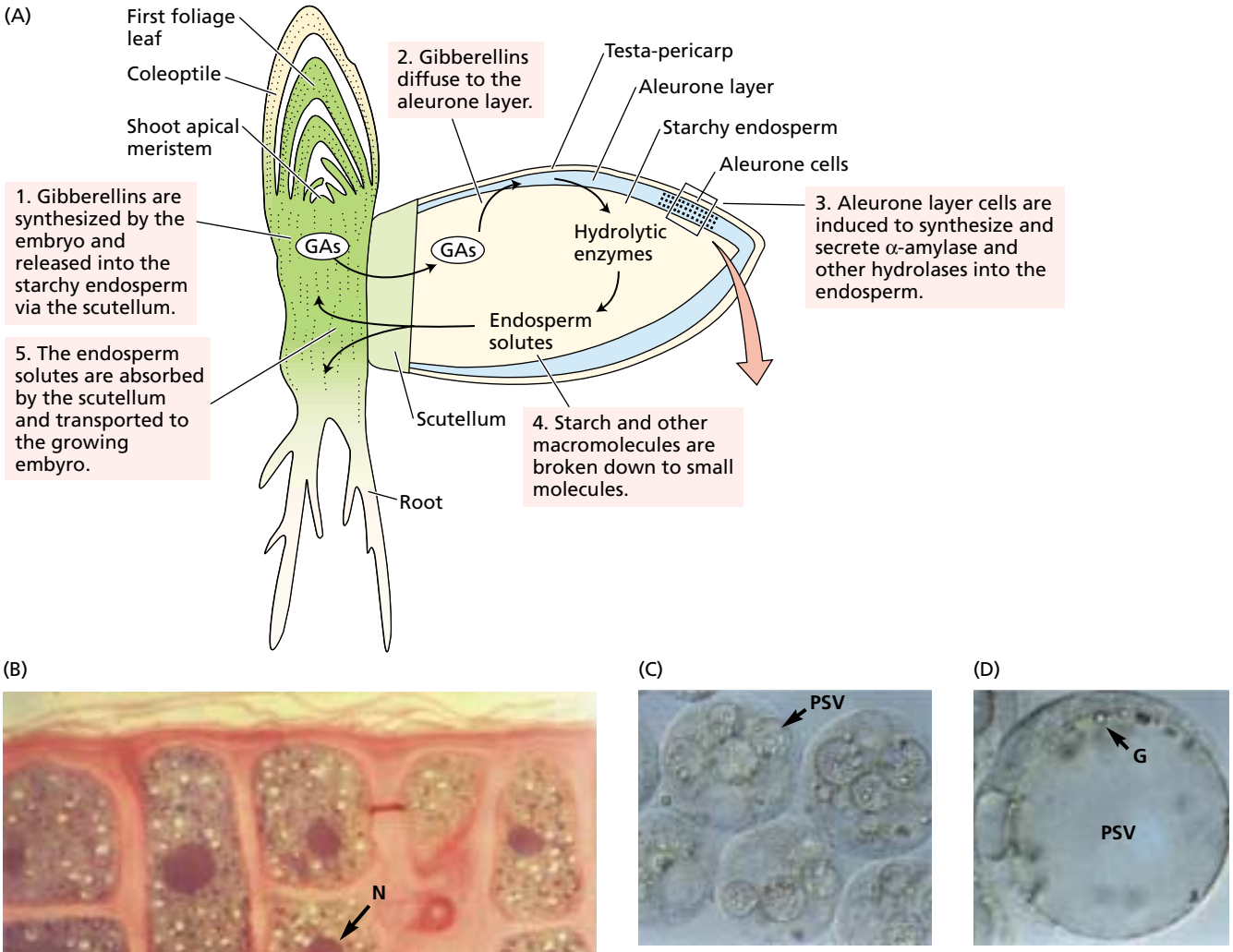


FIGURE 20.33 Structure of a barley grain and the functions of various tissues during germination (A). Microscope photos of the barley aleurone layer (B) and barley aleurone protoplasts at an early (C) and late stage (D) of amylase production. Protein storage vesicles (PSV) can be seen in each cell. G = phytin globoid; N = nucleus. (Photos from Bethke et al. 1997, courtesy of P. Bethke.)

1. How does gibberellin regulate the increase in a-amylase?
2. Where is the gibberellin receptor located in the cell?
3. What signal transduction pathways operate between the gibberellin receptor and a-amylase production?

Gibberellic Acid Enhances the Transcription of α-Amylase mRNA

Before molecular biological approaches were developed, there was already physiological and biochemical evidence that gibberellic acid might enhance α-amylase production at the level of gene transcription (Jacobsen et al. 1995). The two main lines of evidence were as follows:

1. GA_3-stimulated α-amylase production was shown to be blocked by inhibitors of transcription and translation.
2. Heavy-isotope- and radioactive-isotope-labeling studies demonstrated that the stimulation of α-amylase activity by gibberellin involved de novo synthesis of the enzyme from amino acids, rather than activation of preexisting enzyme.

Definitive molecular evidence now shows that gibberellin acts primarily by inducing the expression of the

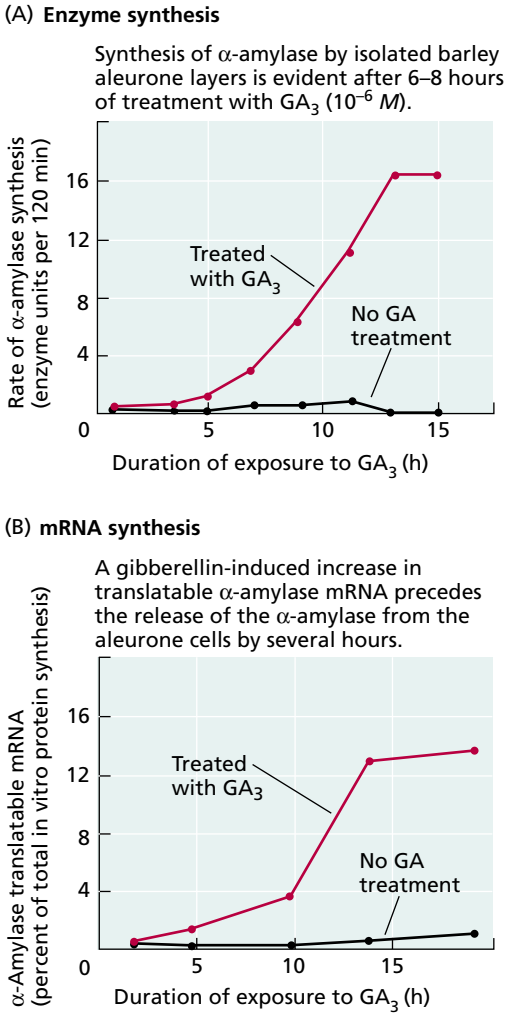


FIGURE 20.34 Gibberellin effects on enzyme synthesis and mRNA synthesis. The α-amylase mRNA in this case was measured by the in vitro production of α-amylase as a percentage of the protein produced by the translation of the bulk mRNA. (From Higgins et al. 1976.)

gene for α-amylase. It has been shown that GA_3 enhances the level of translatable mRNA for α-amylase in aleurone layers (Figure 20.34). Furthermore, by using isolated nuclei, investigators also demonstrated that there was an enhanced transcription of the α-amylase gene rather than a decrease in mRNA turnover (see **Web Topic 20.5**).

The purification of α-amylase mRNA, which is produced in relatively large amounts in aleurone cells, enabled the isolation of genomic clones containing both the structural gene for α-amylase and its upstream promoter sequences. These promoter sequences were then fused to the reporter gene that encodes the enzyme β-glucuronidase (GUS), which yields a blue color in the presence of an artificial substrate when the gene is expressed. The regulation of transcription by gibberellin was proved when such chimeric genes containing α-amylase promoters that were fused to reporter genes were introduced into aleurone protoplasts and the production of the blue color was shown to be stimulated by gibberellin (Jacobsen et al. 1995).

The partial deletion of known sequences of bases from α-amylase promoters from several cereals indicates that the sequences conferring gibberellin responsiveness, termed *gibberellin response elements*, are located 200 to 300 base pairs upstream of the transcription start site (see **Web Topic 20.6**).

A GA-MYB Transcription Factor Regulates α-Amylase Gene Expression

The stimulation of α-amylase gene expression by gibberellin is mediated by a specific transcription factor that binds to the promoter of the α-amylase gene (Lovegrove and Hooley 2000). To demonstrate such DNA-binding proteins in rice, a technique called a *mobility shift assay* was used (see **Web Topic 20.7**). This assay detects the increase in size that occurs when the α-amylase promoter binds to a protein isolated from gibberellin-treated aleurone cells (Ou-Lee et al. 1988). The mobility shift assay also allowed identification of the regulatory DNA sequences (**gibberellin response elements**) in the promoter that are involved in binding the protein.

Identical gibberellin response elements were found to occur in all cereal α-amylase promoters, and their presence was shown to be essential for the induction of α-amylase gene transcription by gibberellin. These studies demonstrated that gibberellin increases either the level or the activity of a transcription factor protein that switches on the production of α-amylase mRNA by binding to an upstream regulatory element in the α-amylase gene promoter.

The sequence of the gibberellin response element in the α-amylase gene promoter turned out to be similar to that of the binding sites for MYB transcription factors that are known to regulate growth and development in phytochrome responses (see Chapter 14 on the Web site and Chapter 17) (Jacobsen et al. 1995). This knowledge enabled the isolation of mRNA for a MYB transcription factor, named GA-MYB, associated with the gibberellin induction of α-amylase gene expression.

The synthesis of *GA-MYB* mRNA in aleurone cells increases within 3 hours of gibberellin application, several hours before the increase in α-amylase mRNA (Gubler et al. 1995) (Figure 20.35). The inhibitor of translation, cycloheximide, has no effect on the production of *MYB* mRNA, indicating that *GA-MYB* is a *primary response gene*, or *early gene*. In contrast, the α-amylase gene is a *secondary response gene*, or *late gene*, as indicated by the fact that its transcription is blocked by cycloheximide.

How does gibberellin cause the *MYB* gene to be expressed? Because protein synthesis is not involved, gibberellin may bring about the activation of one or more *preexisting* transcription factors. The activation of transcription factors is typically mediated by protein phosphorylation events occurring at the end of a signal transduction pathway. We will now examine what is known about the signaling pathways involved in gibberellin-induced α-amylase production up to the point of GA-MYB production.

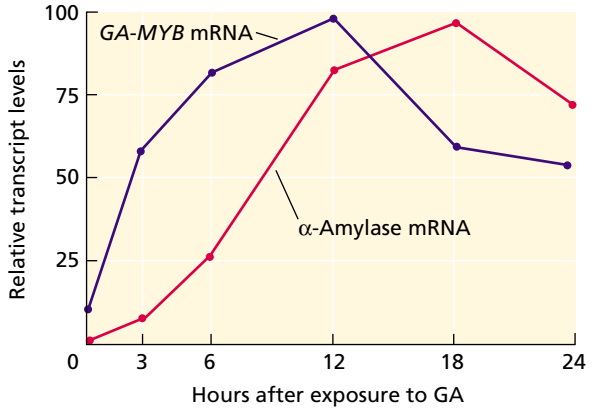


FIGURE 20.35 Time course for the induction of *GA-MYB* and α-amylase mRNA by gibberellic acid. The production of *GA-MYB* mRNA precedes α-amylase mRNA by about 5 hours. This result is consistent with the role of *GA-MYB* as an early *GA* response gene that regulates the transcription of the gene for α-amylase. In the absence of GA, the levels of both *GA-MYB* and α-amylase mRNAs are negligible. (After Gubler et al. 1995.)

Gibberellin Receptors May Interact with G-Proteins on the Plasma Membrane

A cell surface localization of the gibberellin receptor is suggested from the fact that gibberellin that has been bound to microbeads that are unable to cross the plasma membrane is still active in inducing α-amylase production in aleurone protoplasts (Hooley et al. 1991). In addition, microinjection of GA_3 into aleurone protoplasts had no effect, but when the protoplasts were immersed in GA_3 solution, they produced α-amylase (Gilroy and Jones 1994). These results suggest that gibberellin acts on the outer face of the plasma membrane.

Two gibberellin-binding plasma membrane proteins have been isolated through the use of purified plasma membrane and a radioactively labeled gibberellin that was chemically modified to permanently attach to protein to which it was weakly bound. Because the presence of excess gibberellin reduces binding, and these proteins from a semidwarf, gibberellin-insensitive sweet pea bind gibberellin less strongly, they may represent the gibberellin receptors (Lovegrove et al. 1998).

In animal cells, heterotrimeric GTP-binding proteins (G-proteins) in the cell membrane are often involved as first steps in a pathway between a hormone receptor and subsequent cytosolic signals. Evidence has been obtained that G-proteins are also involved in the early gibberellin signaling events in aleurone cells (Jones et al. 1998).

Treatment of oat aleurone protoplasts with a peptide called Mas7, which stimulates GTP/GDP exchange by G-proteins, was found to induce α-amylase gene expression and to stimulate α-amylase secretion, suggesting that such a GTP/GDP exchange on the cell membrane is a reaction en route to the induction of α-amylase biosynthesis by gibberellin. In addition, gibberellin-induced α-amylase gene expression and secretion were inhibited by a guanine nucleotide analog that binds to the α subunit of heterotrimeric G-proteins and inhibits GTP/GDP exchange, further supporting the preceding conclusion.

Recent genetic studies have provided further support for the role of G-proteins as intermediates in the gibberellin signal transduction pathway. The rice dwarf mutant *dwarf 1* (*d1*) has a defective gene encoding the α subunit. Besides being dwarf, the aleurone layers of the *d1* mutant synthesize less α-amylase in response to gibberellin than wild-type aleurone layers do. This reduction in α-amylase production by the *d1* mutant demonstrates that G-proteins are one of the components of the gibberellin signal transduction pathway involved in both the growth response and the production of α-amylase. However, the difference in α-amylase production between the mutant and the wild type goes away with increasing gibberellin concentration, suggesting that gibberellin can also stimulate α-amylase production by a G-protein-independent pathway (Ashikari et al. 1999; Ueguchi-Tanaka et al. 2000).

Cyclic GMP, Ca^{2+}, and Protein Kinases Are Possible Signaling Intermediates

In animal cells, G-proteins can activate the enzyme guanylyl cyclase, the enzyme that synthesizes cGMP from GTP, leading to an increase in cGMP concetration. Cyclic GMP, in turn, can regulate ion channels, Ca^{2+} levels, protein kinase activity, and gene transcription (see Chapter 14 on the Web site). Gibberellin has been reported to cause a transient rise in cGMP levels in barley aleurone layers, suggesting a possible role for cGMP in α-amylase production (Figure 20.36) (see **Web Topic 20.8**) (Pensen et al. 1996).

Calcium and the calcium-binding protein calmodulin act as second messengers for many hormonal responses in

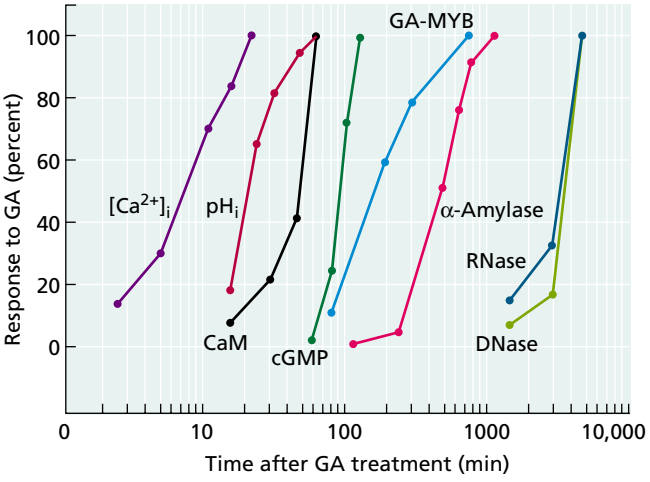


FIGURE 20.36 Following the addition of GA to barley aleurone protoplasts, a multiple signal transduction pathway is initiated. The timing of some of these events is shown. (From Bethke et al. 1997.)

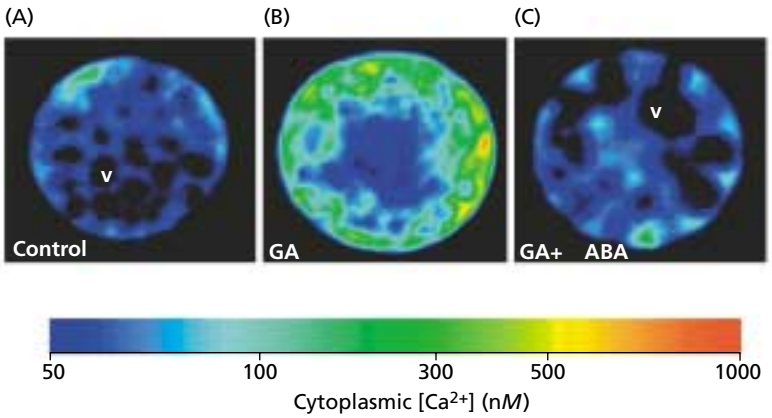


FIGURE 20.37 Increase in the calcium in barley aleurone protoplasts following GA addition can be seen from this false color image. The level of calcium corresponding to the colors is in the lower scale. (A) Untreated protoplast. (B) GA-treated protoplast. (C) Protoplast treated with both abscisic acid (AB) and GA. Abscisic acid opposes the effects of GA in aleurone cells. (From Ritchie and Gilroy 1998b.)

animal cells (see Chapter 14 on the Web site), and they have been implicated in various plant responses to environmental and hormonal stimuli. The earliest event in aleurone protoplasts after the application of gibberellin is a rise in the cytoplasmic calcium concentration that occurs well before the onset of α-amylase synthesis (see Figures 20.36 and 20.37) (Bethke et al. 1997). Without calcium, α-amylase secretion does not occur, though in barley aleurone protoplasts its synthesis goes ahead normally, so we have to conclude that, in barley, calcium is not on the signaling pathway to α-amylase gene transcription, though it does play a role in enzyme secretion.

Protein phosphorylation by protein kinases is another component in many signaling pathways, and gibberellin appears to be no exception. The injection of a protein kinase substrate into barley aleurone protoplasts to compete with endogenous protein phosphorylation inhibited α-amylase secretion, suggesting the involvement of protein phosphorylation in the α-amylase secretion pathway (Ritchie and Gilroy 1998a). This did not affect the gibberellin-stimulated increase in calcium, indicating that the protein kinase step is downstream of the calcium signaling event.

In conclusion, gibberellin signal transduction in aleurone cells seems to involve G-proteins as well as cyclic GMP, leading to production of the transcription factor GA-MYB, which induces α-amylase gene transcription. α-Amylase secretion has similar initial components but also involves an increase in cytoplasmic calcium and protein phosphorylation. The detailed signaling pathways remain to be worked out. A model of the known biochemical components of the gibberellin signal transduction pathways in aleurone cells is illustrated in Figure 20.38.

The Gibberellin Signal Transduction Pathway Is Similar for Stem Growth and α-Amylase Production

It is widely believed that gibberellin initially acts through a common pathway or pathways in all of its effects on development. As we have seen, the genetic approaches applied to the study of gibberellin-stimulated growth led to the identification of the *SPY/GAI/RGA* negative regulatory pathway. The proteins SPY, GAI, and RGA act as repressors of gibberellin responses. Gibberellin deactivates these repressors.

Because the aleurone layers of gibberellin-insensitive dwarf wheat are also insensitive to GA, the same signal transduction pathways that regulate growth appear to regulate gibberellin-induced α-amylase production. Indeed a *SPY*-type gene associated with α-amylase production has been isolated from barley (*HvSPY*), and its expression is able to inhibit gibberellin-induced α-amylase synthesis, while GA-MYB-type factors are also implicated in the gibberellin transduction chain regulating stem growth.

Rice with the *dwarf 1* mutation also produces little α-amylase in response to gibberellin. As noted earlier, the mutation causing *dwarf 1* is known to be in the α subunit of the G-protein complex, providing evidence that the action of gibberellin in both stem elongation and the production of α-amylase are regulated by plasma membrane heterotrimeric G-proteins.

As the entire elongation growth and α-amylase signaling pathways are worked out, it will be interesting to see how much they have in common and where they diverge.

SUMMARY

Gibberellins are a family of compounds defined by their structure. They now number over 125, some of which are found only in the fungus *Gibberella fujikuroi*. Gibberellins induce dramatic internode elongation in certain types of plants, such as dwarf and rosette species and grasses.

FIGURE 20.38 Composite model for the induction of α-amylase synthesis in barley aleurone layers by gibberellin. A calcium-independent pathway induces α-amylase gene transcription; a calcium-dependent pathway is involved in α-amylase secretion. (The SPY negative regulator was omitted for clarity.)

1. GA_1 from the embryo first binds to a cell surface receptor.

2. The cell surface GA receptor complex interacts with a heterotrimeric G-protein, initiating two separate signal transduction chains.

3. A calcium-independent pathway, involving cGMP, results in the activation of a signaling intermediate.

4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.

5. The DELLA repressors are degraded when bound to the GA signal.

6. The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as other genes, to proceed through transcription, processing, and translation.

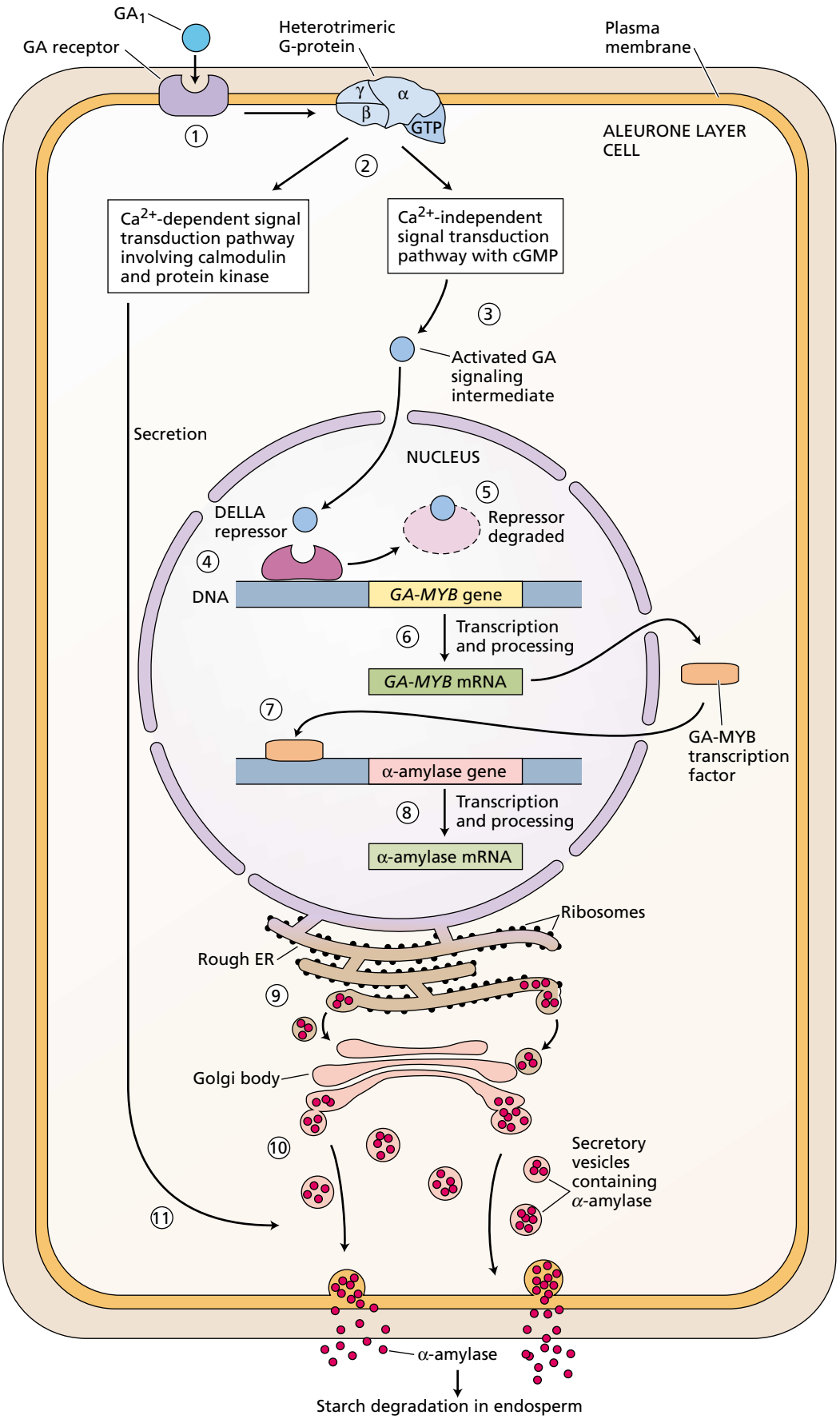
7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for α-amylase and other hydrolytic enzymes.

8. Transcription of α-amylase and other hydrolytic genes is activated.

9. α-Amylase and other hydrolases are synthesized on the rough ER.

10. Proteins are secreted via the Golgi.

11. The secretory pathway requires GA stimulation via a calcium–calmodulin-dependent signal transduction pathway.



Other physiological effects of gibberellin include changes in juvenility and flower sexuality, and the promotion of fruit set, fruit growth, and seed germination. Gibberellins have several commercial applications, mainly in enhancement of the size of seedless grapes and in the malting of barley. Gibberellin synthesis inhibitors are used as dwarfing agents.

Gibberellins are identified and quantified by gas chromatography combined with mass spectrometry, following separation by high-performance liquid chromatography. Bioassays may be used to give an initial idea of the gibberellins present in a sample. Only certain GAs, notably GA_1 and GA_4, are responsible for the effects in plants; the others are precursors or metabolites.

Gibberellins are terpenoid compounds, made up of isoprene units. The first compound in the isoprenoid pathway committed to gibberellin biosynthesis is *ent*-kaurene. The biosynthesis up to *ent*-kaurene occurs in plastids. *ent*-Kaurene is converted to GA_{12}—the precursor of all the other gibberellins—on the plastid envelope and then on the endoplasmic reticulum via cytochrome P450 monooxygenases. Commonly a hydroxylation at C-13 also takes place to give GA_{53}.

GA_{53} or GA_{12}, each of which has 20 carbon atoms, is converted to other gibberellins by sequential oxidation of carbon 20, followed by the loss of this carbon to give 19-carbon gibberellins. This process is followed by hydroxylation at carbon 3 to give the growth-active GA_1 or GA_4. A subsequent hydroxylation at carbon 2 eliminates biological activity.

The steps after GA_{53} or GA_{12} occur in the cytoplasm. The genes for GA 20-oxidase (*GA20ox*), which catalyzes the steps between GA_{53} and GA_{20}, GA 3β-hydroxylase (or GA 3-oxidase; *GA3ox*), which converts GA_{20} into GA_1, and GA 2-oxidase (*GA2ox*), which converts active GA_1 to inactive GA_8, have been isolated. Dwarf plants have been genetically engineered by the use of antisense *GA20ox* or *GA3ox*, or overexpression of *GA2ox*. Gibberellins may also be glycosylated to give either an inactivated form or a storage form.

The endogenous level of active gibberellin regulates its own synthesis by switching on or inhibiting the transcription of the genes for the enzymes of gibberellin biosynthesis and degradation. Environmental factors such as photoperiod (e.g., leading to bolting and potato tuberization) and temperature (vernalization), and the presence of auxin from the stem apex, also modulate gibberellin biosynthesis through the transcription of the genes for the gibberellin biosynthetic enzymes. Light regulates both GA_1 biosynthesis through regulation of the transcription of the gibberellin degradation gene and also causes a decrease in the responsiveness of stem elongation to the presence of gibberellin.

The most pronounced effect of applied gibberellins is stem elongation in dwarf and rosette plants. Gibberellins stimulate stem growth by promoting both cell elongation and cell division. The activity of some wall enzymes has been correlated with gibberellin-induced growth and cell wall loosening. Gibberellin-stimulated cell divisions in deep-water rice are regulated at the transition between DNA replication and cell division.

Three types of gibberellin response mutants have been useful in the identification of genes involved in the gibberellin signaling pathway involved in stem growth: (1) gibberellin-insensitive dwarfs (e.g., *gai-1*), (2) gibberellin deficiency reversion mutants (e.g., *rga*), and (3) constitutive gibberellin responders (slender mutants) (e.g., *spy*).

GAI and RGA are related nuclear transcription factors that repress growth. In the presence of gibberellin they are degraded. The mutant *gai-1*, and the related wheat dwarfing gene mutant *rht*, have lost the ability to respond to gibberellin. *SPY* encodes a glycosyl transferase that is a member of a signal transduction chain prior to GAI/RGA. When a mutation interferes with the repressor function of any of these, the plants grow tall.

Gibberellin induces transcription of the gene for α-amylase biosynthesis in cereal grain aleurone cells. This process is mediated by the transcription of a specific transcription factor, GA-MYB, which binds to the upstream region of the α-amylase gene, thus switching it on. The gibberellin receptor is located on the surface of aleurone cells. G-proteins and cyclic GMP have been implicated as members of the signal transduction chain on the way to GA-MYB. Calcium is not on the route to α-amylase gene transcription, though it does play a role in α-amylase secretion via protein phosphorylation.

The gibberellin signal transduction pathway is probably similar for stem elongation and α-amylase production. Dwarf wheat and rice also have impaired α-amylase gene transcription. Gibberellin acts by deactivating repressors, such as SPY, GAI, and RGA en route to both an increase in cell elongation and the production of α-amylase.

Web Material

Web Topics

20.1 Structures of Some Important Gibberellins, Their Precursors and Derivatives, and Inhibitors of Gibberellin Biosynthesis

The chemical structures of various gibberellins and inhibitors of gibberellin biosynthesis are presented.

20.2 Gibberellin Detection

Gibberellin quantitation is now routine thanks to sensitive modern physical methods of detection.

20.3 Gibberellin-Induced Stem Elongation
Various mechanisms of gibberellin-induced cell wall loosening are discussed.

20.4 CDKs and Gibberellin-Induced Cell Division
Additional information on the mechanism of gibberellin regulation of the cell cycle is given.

20.5 Gibberellin-Induction of α-amylase mRNA
Evidence is provided for gibberellin-induced transcription of α-amylase mRNA.

20.6 Promoter Elements and Gibberellin Responsiveness
Gibberellin response elements mediate the effects of gibberellin on α-amylase transcription.

20.7 Regulation of α-amylase Gene Expression by Transcription Factors
Experiments identifying MYB transcription factors as mediators of gibberellin-induced gene transcription are described.

20.8 Gibberellin Signal Transduction
Various signaling intermediates have been implicated in gibberellin responsiveness.

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