CHAPTER

20

Gibberellins: Regulators of Plant Height and Seed Germination

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, plant scientists tried to ascribe the regulation of all developmental phenomena in plants to the hormone auxin. However, as we will see in this and subsequent chapters, plant growth and development are regulated by many hormones acting both individually and in concert.

The second group of plant hormones to be characterized was the gibberellins (GAs). At least 136 naturally occurring GAs have been identified (MacMillan 2002), and their structures can be viewed at http://www.plant-hormones.info/ga1info.htm. This website is frequently updated as naturally occurring GAs are newly characterized and named. Unlike the auxins, which are defined by their biological properties, the GAs all share a similar chemical structure but relatively few of them have intrinsic biological activity. Many of the GAs that do not have intrinsic biological activity are either metabolic precursors of the bioactive GAs or their deactivation products. There are often only a few bioactive GAs in any given plant, and their levels are generally correlated with stem length. Gibberellins also play important roles in a variety of other physiological phenomena, such as seed germination, transition to flowering, and pollen development.

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

We begin this chapter by describing the discovery of GAs in a fungal pathogen (*Gibberella fujikuroi*) of rice plants (*Oryza sativa*) and discussing their chemical structures. We then provide an overview of the many physiological processes that are regulated by GAs—seed germination, shoot growth, transition to flowering, anther development, pollen tube growth, floral development, fruit set and subsequent growth, and seed development. We then examine biosynthesis of GAs and the roles of factors that regulate the levels of bioactive GA in tissues or organs at specific developmental stages.

The identification of a GA receptor in rice in 2005 has paved the way for recent findings that include the crystal structures of the GA receptors for both rice and Arabidopsis. The binding of bioactive GA to its receptor initiates a chain of events that leads eventually to the responses observed at the whole plant level.

Gibberellins: Their Discovery and Chemical Structure

A brief description of the groundbreaking discovery of gibberellins provides an explanation for the unusual terminology applied to GAs. After a consideration of their discovery, we will describe their chemical structures and numbering system. Finally, we will explore the relations of specific chemical structures to their biological activity.

Gibberellins were discovered by studying a disease of rice

Although GAs first came to the attention of Western scientists in the 1950s, they had been discovered much earlier in Japan. Rice farmers had long known of a fungal disease (termed *bakanae* or "foolish seedling" disease) that caused rice plants to grow too tall and eliminated seed production. Plant pathologists found that these symptoms in rice were caused by a pathogenic fungus, ***Gibberella fujikuroi***, that had infected the plants. Culturing this fungus in the laboratory and analyzing the culture filtrate enabled Japanese scientists in the 1930s to obtain impure crystals with plant growth–promoting activity. They named this mixture of compounds *gibberellin A*.

Gibberellic acid was first purified from Gibberella *culture filtrates*

In the 1950s two research groups, one in Britain and one in the United States, elucidated the chemical structure of a compound that both had purified from *Gibberella* culture filtrates and which they named *gibberellic acid*. At about the same time, Japanese scientists separated and characterized three different gibberellins from the original gibberellin A sample, naming them gibberellin A_1 (GA_1), gibberellin A_2 (GA_2), and gibberellin A_3 (GA_3). The numbering system for gibberellins builds on this initial nomenclature. The Japanese scientists' GA_3 was later shown to be identical to the gibberellic acid isolated by the U.S. and British scientists. Thus the name 'gibberellic acid' refers specifically to GA_3, whereas 'gibberellin' is a general name that can refer to the entire class of hormones.

It soon became evident that many different GAs were present in *Gibberella* cultures, although GA_3 was usually the principal component. (For this reason GA_3 is produced commercially in industrial-scale fermentations of *Gibberella*, for agronomic, horticultural, and other scientific use.) As GA_3 became available, scientists began to test it on a wide variety of plants. Spectacular responses were obtained in the stem elongation of dwarf and rosette plants, particularly in genetically dwarf peas (*Pisum sativum*), dwarf maize (corn; *Zea mays*) (**FIGURE 20.1**), and many rosette plants (**FIGURE 20.2**).

Because applications of fungus-derived GA_3 could increase the height of dwarf mutants, scientists asked whether wild-type plants contain their own endogenous GA. Bioassays of extracts from a variety of plant species showed that GA-like substances* were indeed present. Higher concentrations were found in immature seeds (approximately 1 part per million) than in vegetative tissue (1–10 parts per billion). This made immature seeds the plant material of choice for GA extraction, but chemical characterization still required tens of kilograms of seeds. The first identification of a GA from a plant extract was the 1958 discovery of GA_1 from immature seeds of runner bean (*Phaseolus coccineus*). Nowadays, the availability of very-sensitive spectroscopic methods permits the identification and quantitation of known GAs with less than a gram of plant material.

As more GAs from *Gibberella* and different plant sources were characterized, a scheme was adopted to number them (GA_1–GA_n) in chronological order of their discovery. Only GAs that are naturally occurring and whose chemical structures have been conclusively determined are assigned **A numbers** using the procedure described at http://www.plant-hormones.info/gibberellin_nomenclature.htm. (The

*The term *GA-like substance* refers to a compound that shows activity in a GA bioassay, but has not been chemically characterized.

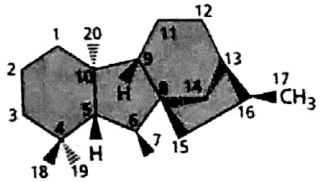
FIGURE 20.1 The effect of exogenous GA_1 on wild-type (labeled as "normal" in the photograph) and dwarf mutant (*d1*) maize. Gibberellin stimulates dramatic stem elongation in the dwarf mutant, but has little or no effect on the tall, wild-type plant. (Courtesy of B. Phinney.)

FIGURE 20.2 Cabbage, a long-day plant, remains a low-growing rosette in short days, but it can be induced to bolt (grow long internodes) and flower by applications of GA_3. In the case illustrated, giant flowering stalks were produced. (© Sylvan Wittwer/Visuals Unlimited.)

"number" of a GA is simply a cataloging convenience, and there is no implied metabolic relationship between GAs with adjacent numbers.)

All gibberellins are based on an ent*-gibberellane* skeleton

The GAs are diterpenoids that are formed from *four* isoprenoid units each consisting of five carbons (see Chapter 13). They possess a tetracyclic (four-ringed) *ent*-gibberellane skeleton (containing 20 carbon atoms), or a 20-nor-*ent*-gibberellane skeleton (containing only 19 carbon atoms because carbon 20 is missing)*.

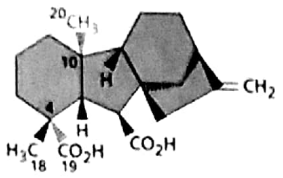


***ent*-gibberellane**

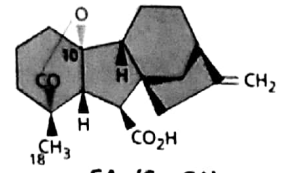
Gibberellins that have the full diterpenoid complement of 20 carbon atoms (e.g., GA_{12}) are referred to as C_{20}-GAs. Those that have only 19 carbons because carbon-20 (blue on next structure) has been lost by metabolism are referred to as C_{19}-

*The prefix *ent* refers to the fact that the skeleton is derived from *ent*-kaurene, a tetracyclic hydrocarbon that is enantiomeric to the naturally occurring compound, kaurene.

GAs (e.g., GA_9). In nearly all C_{19}-GAs, the carboxyl at C-4 forms a lactone at C-10 (red on structure below).

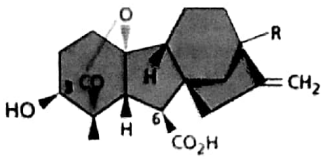


GA_{12} (C_{20}-GA)

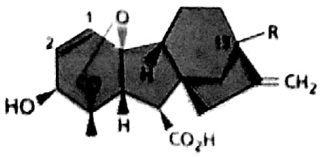


GA_9 (C_{19}-GA)

Other structural modifications include the insertion of additional features such as hydroxyl (—OH) groups or double bonds. The position and stereochemistry of these functional groups can have profound effects on biological activity. The GAs with highest intrinsic biological activity (GA_1, GA_3, GA_4, and GA_7) were among the first to be discovered: These GAs are all C_{19}-GAs. They all possess a 4,10-lactone (shown in red), a carboxylic acid (◄ COOH, shown in green on the structure below) at C-6, and a hydroxyl group at C-3 in β-orientation (◄ OH, shown in blue on the structure below). Elucidation of the tertiary structure of the GA receptor has identified the amino acid residues in the receptor that interact with each of these functional groups in a bioactive GA molecule, thereby holding the GA in the receptor pocket and allowing a "lid" to close in place (Murase et al. 2008; Shimada et al. 2008). This allosteric modification of the GA receptor upon binding an active GA facilitates subsequent interactions of the GA receptor with other proteins.



GA_4 R = H
GA_1 R = OH



GA_7 R = H
GA_3 R = OH

Although bioactive GAs *may* possess other features such as a double bond between C-1 and C-2 (as in GA_7 and GA_3) and/or an —OH group at C-13 (as in GA_1 and GA_3) these functional groups seem to neither help nor hinder GA binding in the receptor pocket. On the other hand, the presence of a 2β-OH group prevents the hydrophobic surface of the receptor lid from closing, and thus renders a GA inactive (Murase et al. 2008; Shimada et al. 2008). (A further discussion of GA structure can be found in WEB TOPIC 20.1.)

Effects of Gibberellins on Growth and Development

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

Gibberellins promote seed germination

Many seeds, particularly those of wild plant species, do not germinate immediately after dispersal from the mother plant, and may experience a period of dormancy. Dormant seeds will not germinate even if provided with water. Abscisic acid (ABA) and bioactive GA act in an antagonistic manner, and the relative amounts of the two hormones within the seed can, in many species, determine the degree of dormancy. Light or cold treatments of dormant seeds have been shown to lower the amount of ABA and increase the concentration of bioactive GA, ending dormancy and promoting germination (Piskurewicz et al. 2008; Seo et al. 2006). Treatment of seeds with bioactive GA can often substitute for the light or cold treatment needed to break dormancy.

During germination, GAs induce the synthesis of hydrolytic enzymes, such as amylases and proteases in cereal grains. These enzymes degrade the stored food reserves accumulated in the endosperm or embryo as the seed matured. This degradation of carbohydrates and storage proteins provides nourishment and energy to support seedling growth. The GA-induced synthesis of α-amylase in germinating cereal grains has been studied extensively and is considered in detail later in the chapter.

Gibberellins can stimulate stem and root growth

Applied GAs may not have dramatic effects on stem elongation in plants that are already "tall," since bioactive GA may *not* to be limiting in some tall plants. However, applied GAs can promote internode elongation very dramatically in genetically dwarf mutants, in "rosette" species, and in

various members of the Poaceae (grass family). Exogenous GA causes such extreme stem elongation in dwarf maize plants that they resemble the tallest varieties of the same species (see Figure 20.1).

Rosette species are plants in which the first-formed internodes do not elongate under certain growing conditions. This results in a compact cluster or rosette of leaves, as seen in members of the Brassicaceae (cabbage family). Rosette formation is frequently observed when long-day plants are grown in short-day conditions. Bolting (stem growth) and flowering will result if plants are treated with a bioactive GA, or are transferred to long days (see Figure 20.2).

Gibberellins are also important for root growth. Extreme dwarf mutants of pea and Arabidopsis, in which GA biosynthesis is blocked, have shorter roots than wild-type plants, and GA application to the shoot enhances both shoot *and* root elongation (Yaxley et al. 2001; Fu and Harberd 2003).

Gibberellins regulate the transition from juvenile to adult phases

Many woody perennials do not flower or produce cones until they reach a certain stage of maturity; up to that stage they are said to be juvenile (see Chapter 25). Applied GAs can regulate phase change, though whether GA hastens or retards the juvenile-to-adult transition will depend on the species. In many conifers, the juvenile phase, which may last up to 20 years, can be shortened by treatment with GA_3 or with a mixture of GA_4 and GA_7, and much younger plants can be induced to enter the reproductive, cone-producing phase precociously (**FIGURE 20.3**).

(A) White spruce

(B) White spruce

(C) Giant sequoia seedling

FIGURE 20.3 Gibberellins induce conebud formation in juvenile conifers. (A, B) These photographs show female cones developing on sapling-size, grafted plants of white spruce (*Picea glauca*). The stems of these plants had been injected the previous summer with a mixture of GA_4/GA_7 in aqueous ethanol. (C) A 14-week-old seedling of giant sequoia (*Sequoiadendron giganteum*) that had been sprayed with an aqueous solution of GA_3 some 8 weeks earlier, showing the development of a female conebud. (Courtesy of S. D. Ross and R. P. Pharis.)

Gibberellins influence floral initiation and sex determination

As already noted, GAs can substitute for the long-day requirement for flowering in many plants, especially rosette species. The interaction of photoperiod and GAs in flowering is complex, and this subject is discussed in Chapter 25.

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

Gibberellins promote pollen development and tube growth

Gibberellin-deficient dwarf mutants (e.g., in Arabidopsis and rice) have impaired anther development and pollen formation, and both these defects, which lead to male sterility, can be reversed by treatment with bioactive GA. In other mutants in which GA response (rather than GA biosynthesis) is blocked, the defects in anther and pollen development cannot be reversed by GA treatment, so these mutants are male-sterile (Aya et al. 2009). In addition, reducing the level of bioactive GA in Arabidopsis by overexpressing a GA deactivating enzyme severely inhibits pollen tube growth (Swain and Singh 2005). Thus GAs seem to be required for both the development of the pollen grain and the formation of the pollen tube. The regulation of anther development in rice by bioactive GA will be discussed in more detail later in the chapter.

Gibberellins promote fruit set and parthenocarpy

Gibberellin application can cause **fruit set** (the initiation of fruit growth following pollination) and growth of some fruits. For example, stimulation of fruit set by GA has been observed in pear (*Pyrus communis*). GA-induced fruit set may occur in the absence of pollination, resulting in parthenocarpic fruit (fruit without seeds). In grape (*Vitis vinifera*), the "Thompson Seedless" variety normally produces small fruits because of early seed abortion. Fruits can be stimulated to enlarge by treatment with GA_3 (FIGURE 20.4). This treatment also promotes growth of the pedicels (fruit-bearing stalks) and consequently reduces fungal infections that can be problematic in the compact clusters of untreated grapes. Both these effects of GAs on grapes are exploited commercially to produce large, seedless fruits.

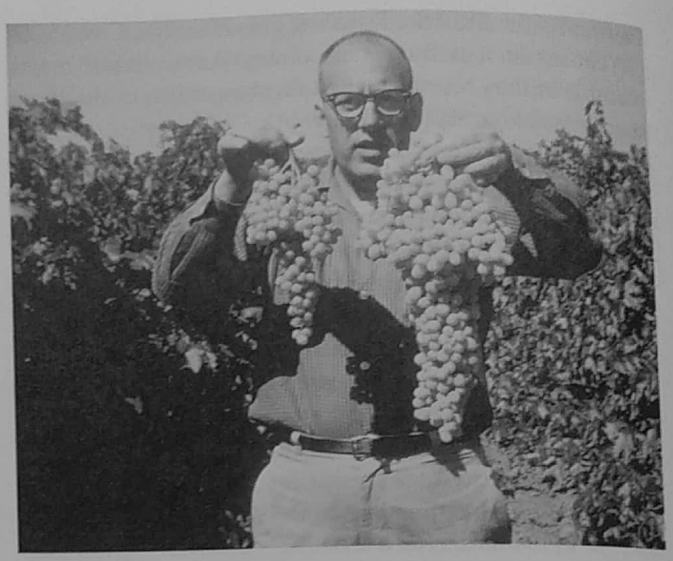


FIGURE 20.4 Gibberellin induces growth in "Thompson Seedless" grapes. Untreated grapes normally remain small because of natural seed abortion. The bunch on the left is untreated. The bunch on the right was sprayed with GA_3 during fruit development, leading to increased size of the fruits and elongation of the pedicels (fruit stalks). (© Sylvan Wittwer/Visuals Unlimited.)

Gibberellins promote early seed development

Some GA-deficient mutants, or transgenic plants with enhanced GA inactivation, have increased seed abortion. The failure of seeds to develop normally can be attributed to reduced levels of bioactive GAs in very young seeds. Treatment with GA will not restore normal seed development, because exogenous GA cannot enter the new seeds. However, the effect of GA deficiency on seed abortion can be negated by simultaneous expression of mutations that give a constitutive GA response (Swain and Singh 2005). Taken together, these results provide evidence for a role for GA in the early stages of seed development.

Commercial uses of gibberellins and GA biosynthesis inhibitors

The major commercial uses of GAs (typically GA_3) are to promote the growth of fruit crops, to stimulate the barley malting process in the beer-brewing industry, and to increase sugar yield in sugarcane. A description of the malting process can be found in WEB TOPIC 20.2.

Inhibitors of GA biosynthesis have been useful for crops in which a *reduction* in plant height is desirable.

For example, tallness is 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 wheat cultivars grown in Europe are sprayed with inhibitors of GA biosynthesis such as Cycocel to further reduce stem length and lodging.

In the field or greenhouse, tall plants are often difficult to manage. For floral crops such as lilies, chrysanthemums, and poinsettias, short, sturdy stems are desirable. Applications of GA biosynthesis inhibitors are often used to control the size of container-grown ornamental plants in nurseries, greenhouses, and shade houses.

The chemical structures of several commercially available inhibitors of GA biosynthesis can be found in WEB TOPIC 20.1, and there is further discussion of the uses of these inhibitors in WEB TOPIC 20.2.

Biosynthesis and Deactivation of Gibberellins

Gibberellins constitute a large family of tetracyclic diterpene acids synthesized via a terpenoid pathway. Early stages of that pathway are described in Chapter 13. In this section, we describe the later stages in the GA pathway. Additional details are discussed in WEB TOPIC 20.3 and in Yamaguchi (2008). We also discuss the regulatory enzymes in the pathway, as well as the genes that encode them. Knowledge of GA biosynthesis and deactivation is important, as it contributes to our understanding of *GA homeostasis*. By GA homeostasis, we mean the maintenance of appropriate levels of bioactive GA in plant cells and tissues throughout the life cycle. Homeostasis depends upon the regulation of GA biosynthesis, deactivation, and transport.

Fundamental to our understanding of how GAs control growth and development is the ability to identify and quantify the GAs present in our experimental plants. Highly sensitive physical techniques such as mass spectrometry are now used that allow precise identification and quantification of specific GAs from small amounts of tissue. GA identification and quantitation in plant extracts are discussed in WEB TOPIC 20.4.

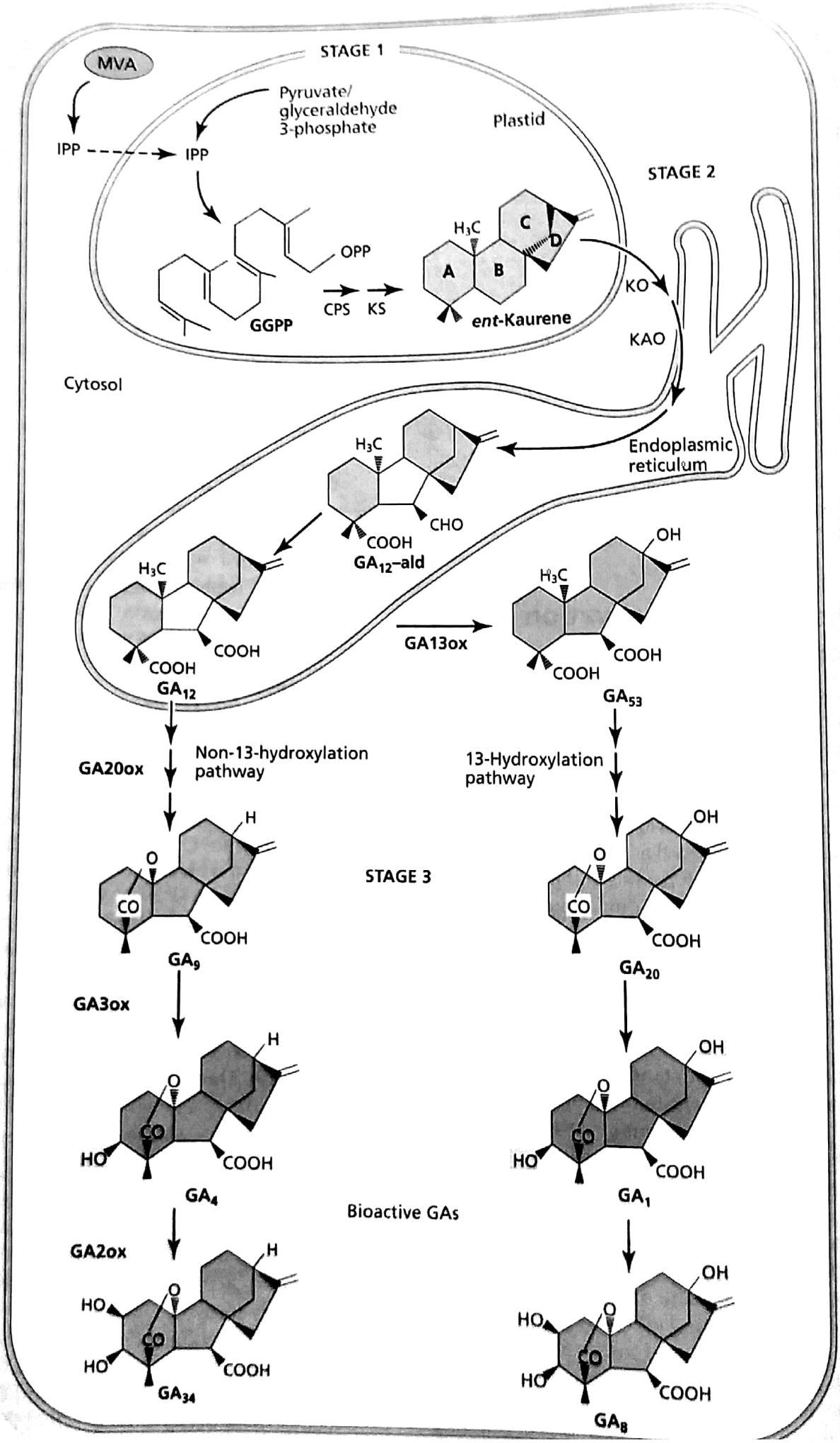
The isolation of mutants with altered stem length has made it possible to determine which of the many GAs present in a plant have intrinsic biological activity. The use of mutants has also facilitated the identification and cloning of genes encoding enzymes in the GA biosynthetic pathway. Sequencing the Arabidopsis and rice genomes has led to the development of comprehensive databases that facilitate the rapid identification of genes and proteins related to GA metabolism and its regulation.

Gibberellins are synthesized via the terpenoid pathway

Terpenoids are compounds made up of five-carbon **isoprenoid** building blocks. The GAs are diterpenoids that are formed from *four* such isoprenoid units (see Chapter 13). The GA biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment: plastid, ER, or cytosol. A simplified version of the pathway is shown in FIGURE 20.5. The complete pathway is presented in Appendix 3.

- In *stage 1*, which occurs in plastids, four isoprenoid units are assembled to give a 20-carbon linear molecule, geranylgeranyl diphosphate (GGPP). GGPP is then converted into a tetracyclic compound, *ent*-kaurene, in two steps, which are catalyzed by *ent*-copalyl-diphosphate synthase (CPS) and *ent*-kaurene synthase (KS).
- In *stage* 2, which occurs on the plastid envelope and in the endoplasmic reticulum, *ent*-kaurene is converted, in a stepwise manner, to the first-formed GA, which is GA_{12}. Two important enzymes in this part of the pathway are *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). The pathway to GA_{12} is essentially the same in all plant species studied so far.
- In *stage* 3, which occurs in the cytosol, GA_{12} is converted, through a series of oxidative reactions, first into other C_{20}-GAs, and then into C_{19}-GAs, including the bioactive GA(s). Two major stage 3 pathways have been identified. They both comprise the same series of oxidative reactions, except that the intermediates in one pathway all have a —OH group at C-13 (and so it is called the 13-hydroxylation pathway), whereas the intermediates in the other pathway do not (and so it is referred to as the non-13-hydroxylation pathway). The series of oxidative reactions occur in the A-ring, and are the same in both stage 3 pathways.

The 13-hydroxylation pathway is the major pathway in many plants, although in Arabidopsis and in some crop plants in the Cucurbitaceae (pumpkin family) the non-13-hydroxylation pathway predominates. In the following discussion the reactions leading to bioactive GA (which is GA_4 in the non-13-hydroxylation pathway and GA_1 in the 13-hydroxylation pathway) are referred to as "biosynthesis." Further metabolism of the bioactive GA is referred to as "deactivation." GA biosynthesis and deactivation are described in more detail in WEB TOPIC 20.3.



◀ **FIGURE 20.5** The three stages of GA biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene. In stage 2 in the endoplasmic reticulum, *ent*-kaurene is converted to GA_{12}-aldehyde and GA_{12}. GA_{12} is converted to GA_{53} by hydroxylation at C-13. In stage 3 in the cytosol, GA_{12} and GA_{53} are converted, via parallel pathways, to other GAs. This conversion proceeds with a series of oxidations at C-20, resulting in the eventual loss of C-20 and the formation of C_{19}-GAs. 3β-hydroxylation then produces GA_4 and GA_1 as the bioactive GAs in each pathway. Hydroxylation at C-2 then converts GA_4 and GA_1 to the inactive forms GA_{34} and GA_8, respectively. In most plants the 13-hydroxylation pathway predominates, although in Arabidopsis and some others, the non-13-OH-pathway is the main pathway. CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA 2-oxidase; GA13ox, GA 13-oxidase.

Some enzymes in the GA pathway are highly regulated

Work with biosynthetic mutants of Arabidopsis, pea, and maize (**FIGURES 20.6 AND 20.7**) facilitated the cloning of genes for many of the enzymes in GA biosynthesis and deactivation. Most notable from a regulatory standpoint are three enzymes in *stage 3* of the pathway. These are the GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox) enzymes, which catalyze the steps prior to bioactive GA, and the GA 2-oxidase (GA2ox), which is involved in GA deactivation. All three of these enzymes are classified as **dioxygenases** and utilize 2-oxoglutarate as a co-substrate and Fe^{2+} as a cofactor. For this reason they are referred to as 2-oxoglutarate-dependent dioxygenases (2ODDs). We shall discuss each of these enzymes, before describing some of what is known of their regulation.

- **GA 20-oxidase.** In Arabidopsis there is a small family of five GA 20-oxidases, named AtGA20ox1 through AtGA20ox5. These homologs (genes that have similar sequences to each other because they are derived from a single gene are said to be *homologous*) are expressed in different tissues and organs and at different developmental stages, although there may be some overlapping expression. The principal stem-expressed GA 20-oxidase is AtGA20ox1, which is encoded by a gene that was originally named *GA5* (Phillips et al. 1995; Xu et al. 1995). Mutation of the *GA5* gene results in a semidwarf, rather than extreme dwarf,

FIGURE 20.6 Phenotypes of wild-type and GA-deficient mutants of Arabidopsis, showing the position in the GA biosynthetic pathway that is blocked in each mutant. All mutant alleles (denoted by lower case notation of the wild-type alleles) are homozygous. Plants were grown in continuous light and are 7 weeks old. Note that the *ga1*, *ga2*, and *ga3* seedlings are sterile and have not produced seed pods (siliques). See Figure 20.5 for abbreviations. (Courtesy of V. Sponsel.)

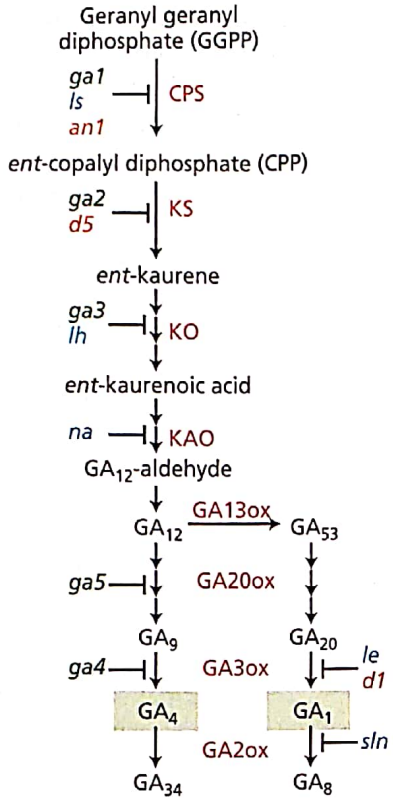


FIGURE 20.7 A portion of the GA biosynthetic pathway showing the metabolic steps that are blocked by known mutations (denoted by lower case notations of wild-type alleles) of Arabidopsis (green), pea (blue), and maize (orange). See Figure 20.5 for abbreviations. As a historical note, the names *GA1–GA5* for five nonallelic loci in Arabidopsis that encode enzymes in the GA biosynthetic pathway were assigned long before the nature of the enzymes or their genes were known (Koornneef and van der Veen 1980). After genetic tests, they were placed in sequence based on the anticipated order of action in the pathway.

phenotype (*ga5*) (see Figure 20.6). This is because of gene redundancy: one or more of the other GA 20-oxidases can partially compensate for the loss of AtGA20ox1. In contrast, the mutants *ga1*, *ga2*, and *ga3* are extreme dwarfs, since enzymes in stages 1 and 2 of the pathway (CPS, KS, and KO) are each encoded by only one gene (see Figure 20.6).

- **GA 3-oxidase.** The principal stem-expressed GA 3-oxidase in Arabidopsis (AtGA3ox1) is encoded by a gene originally named *GA4* (Chiang et al. 1995). Like *GA5*, *GA4* is one of a small gene family, and the *ga4* mutant, like *ga5*, is a semidwarf rather than an extreme dwarf (see Figure 20.6). In pea, this enzyme is encoded by the *LE* gene (see Figure 20.7 and later discussion). In maize this enzyme is encoded by *D1*, and a recessive mutation, *d1*, in this gene gives dwarf plants (see Figure 20.1).
- **GA 2-oxidase.** No mutant phenotype is known for the GA "deactivating" enzyme, GA 2-oxidase, in Arabidopsis. In contrast, a pea mutant identified because it grew taller than wild-type plants contains a mutation in a gene termed *SLENDER* (*SLN*), which encodes a GA 2-oxidase. In the *sln* mutant, GAs synthesized during seed maturation are not deactivated to the same extent as those in wild-type seeds, and some potentially bioactive GAs remain in the mature seed. During germination they are converted into bioactive GA_1, which enhances internode elongation in the young seedling and gives a "slender" phenotype (Reid et al. 1992).

Gibberellin regulates its own metabolism

As explained in Chapter 19 for auxin, many factors are important for maintaining hormone homeostasis, including the relative balance between synthesis and deactivation. Part of a plant's response to bioactive GA is to depress GA biosynthesis and stimulate deactivation, to prevent excessive stem elongation. Depression of biosynthesis is achieved through down-regulation (inhibition of expression) of some of the *GA20ox* and *GA3ox* genes encoding the last two enzymes in the formation of bioactive GA. This effect of GA on its own biosynthesis is termed **negative feedback regulation**. Enhanced GA deactivation is also important for maintaining GA homeostasis. It is achieved by up-regulating (stimulating) the expression of some of the *GA2ox* genes encoding the enzyme that deactivates GA. The ability of GA to promote the expression of genes involved in its own deactivation is termed **positive feed-forward regulation.**

The relative importance of each member of the gene family to feedback or feed-forward regulation varies with species and tissue (Hedden and Phillips 2000). The expression of one or more homologs that are not subject to feedback or feed-forward regulation may cause a "spike" in bioactive GA content, perhaps in response to a particular environmental signal; the subsequent expression of a regulated homolog provides a mechanism to reestablish GA concentrations to a normal level afterwards. In other circumstances, environmental regulation of expression can act on genes that are subject to feedback regulation by overriding homeostasis temporarily.

GA biosynthesis occurs at multiple plant organs and cellular sites

All reactions in GA biosynthesis leading from GGPP to a C_{19}-GA can be demonstrated in cell-free systems from seed or seed-parts, providing definitive evidence that developing seeds are sites of GA biosynthesis. In pea seeds, a surge in GA biosynthesis occurs soon after fertilization and is

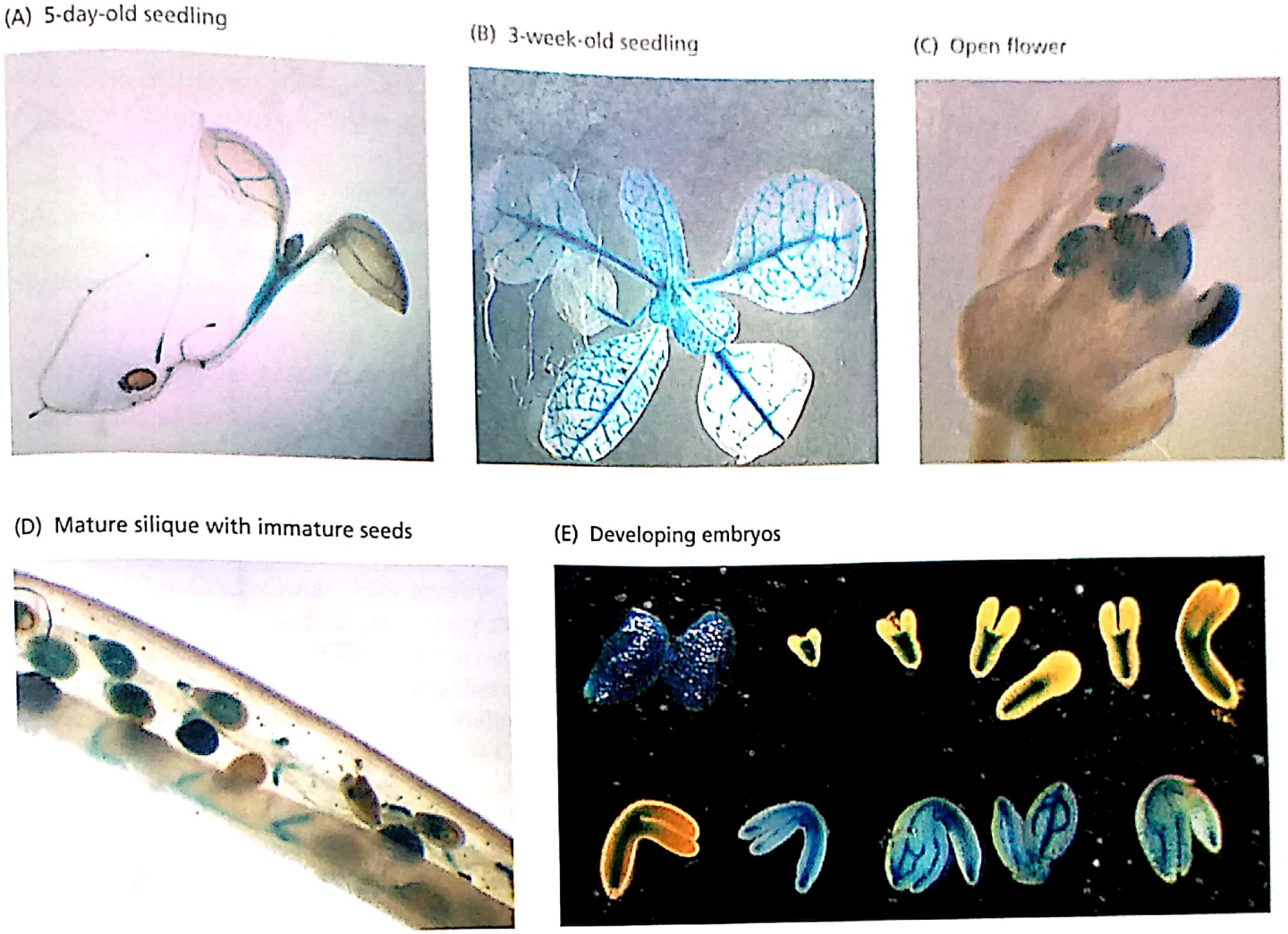


FIGURE 20.8 Histochemical analysis of Arabidopsis plants containing the *GA1* promoter:*GUS* gene fusion, at five different stages of development. *GA1* encodes CPS, the first committed enzyme in GA biosynthesis. The blue staining shows where there is *GA1* promoter activity. These panels indicate that this early step in GA biosynthesis occurs in numerous tissues and organs, and at several stages in the life cycle. (From Silverstone et al. 1997a; courtesy of T-p. Sun.)

necessary for early seed and fruit growth. In later stages of seed maturation in many species, the level of GA accumulation is quite high.

Reporter gene studies have shown that *GA1*, which encodes CPS, the first committed enzyme in GA biosynthesis, is expressed in immature seeds, shoot apices, root tips, and anthers of wild-type Arabidopsis plants (**FIGURE 20.8**) (Silverstone et al. 1997a). Not unexpectedly, these organs are affected in *ga1* mutants, which exhibit seed dormancy, an extreme-dwarf growth habit, and male sterility. This correlation between CPS activity and phenotype indicates that the early stages of GA biosynthesis and GA action can occur in the same organs. Other elegant studies by Yamaguchi et al. (2001) and Ogawa et al. (2003) compared the cellular locations of GA 3-oxidase (the enzyme that converts inactive GA_9 to bioactive GA_4) and the products of genes known to be up-regulated by GA_4. Some cells did not make GA_4 but could respond to it.

Thus, within germinating Arabidopsis embryos we see GA_4 action both in the cells in which it is synthesized and in different cells, implying that GA_4, or a component downstream of GA_4 in the response pathway, must move from cell to cell.

Environmental conditions can influence GA biosynthesis

Gibberellins play an important role in mediating the effects of environmental stimuli on plant development. Light and temperature can have profound effects on GA metabolism and GA response, as discussed in **WEB TOPIC 20.5**. In many cases the environment can alter the metabolism of, or response to, other hormones in addition to GAs. The ratio of bioactive GA to ABA is particularly important, and so is the relative responsiveness of different tissues to the two hormones.

GA_1 and GA_4 have intrinsic bioactivity for stem growth

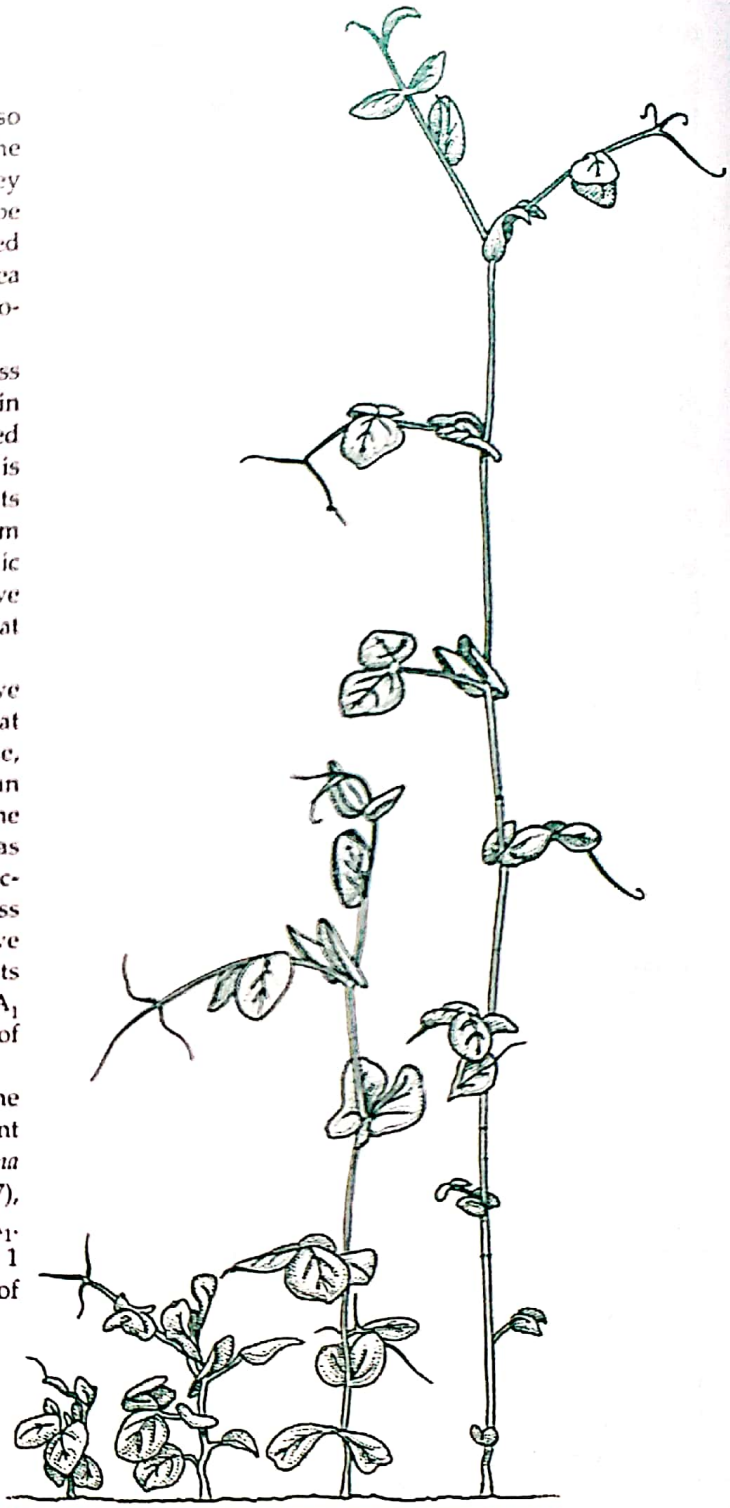
Seminal studies with GA biosynthetic mutants (also referred to as GA-deficient mutants) conducted in the 1980s achieved two important goals. Not only did they provide a way for the pathways of GA metabolism to be definitively established, but these studies also determined that GA_1 is the major bioactive GA for stem growth in pea and maize, and that its precursors have no intrinsic biological activity.

LE and *le* are two alleles of a gene that regulates tallness in peas, the genetic trait investigated by Gregor Mendel in his pioneering study published in 1866. If GA_{20} is applied to the *le* mutant of pea, it is not bioactive, whereas GA_1 is bioactive, and rescues the mutant phenotype (the plants grow tall). Gibberellin A_8 is also inactive. We can infer from this information, and from knowledge of the GA metabolic pathway in pea ($GA_{20} \rightarrow GA_1 \rightarrow GA_8$), that GA_{20} is inactive unless it can be converted to GA_1 within the plant, and that *GA_1 has intrinsic bioactivity* (Ingram et al. 1984).

Metabolic studies using isotopically labeled GAs have demonstrated that the *LE* gene encodes an enzyme that 3β-hydroxylates GA_{20} to produce GA_1. At the same time, it was confirmed that tall stems contain more GA_1 than dwarf stems (Reid and Howell 1995). Mendel's *LE* gene was eventually cloned, and the recessive *le* allele was shown to have a single base change leading to a defective enzyme (Lester et al. 1997; Martin et al. 1997). Less GA_1 is produced in plants homozygous for the recessive allele than in wild-type plants. However, since *le* plants still produce a partially active GA 3-oxidase, enough GA_1 is present for *le* seedlings to attain approximately 30% of the height of wild-type plants.

A study of other pea mutants has confirmed that the height of pea plants is directly correlated with the amount of endogenous GA_1 (Ross et al. 1989). For example, the *na* mutant of pea is deficient in KAO activity (see Figure 20.7), and mutant plants are almost completely devoid of GA_1. As a consequence they achieve a stature of only about 1 cm at maturity (**FIGURE 20.9**). In contrast, the seedlings of the *sln* mutant contain elevated levels of GA_1 because of impaired GA deactivation, and these mutant plants are actually taller than wild-type seedlings (see Figure 20.9). A personal account of this research on pea stem growth can be found in **WEB ESSAY 20.1**.

Work with dwarf mutants of other plants has confirmed that 3β-hydroxylated C_{19}-GAs have *intrinsic* biological activity for stem growth. Studies similar to those described above for pea determined that GA_1 is the major endogenous bioactive GA regulating stem growth in maize (Phinney 1984).



Phenotype	Ultradwarf	Dwarf	Tall	Slender
Genotype	*na/LE/SLN*	*NA/le/SLN*	*NA/LE/SLN*	*NA/LE/sln*
GA_1 content	None	Lower than level in wild type	Wild-type level	Higher than level in wild type

FIGURE 20.9 Phenotypes and genotypes of peas that differ in the GA_1 content of their vegetative tissue. (All alleles are homozygous.) (After Davies 1995.)

The situation is similar in rice, though a less abundant GA, GA_4 (non-13-hydroxylated GA_1), has higher affinity for the GA receptor than GA_1 when tested in vitro, and GA_4 may have an important role in, for example, reproductive growth of rice. In Arabidopsis and several members of the Cucurbitaceae (e.g., pumpkin and cucumber), applied GA_1 has less biological activity than GA_4. Therefore, in Arabidopsis and probably also in these cucurbits, GA_4 is assumed to be the main biologically active GA.

Plant height can be genetically engineered

The identity of the bioactive GAs in crop plants, together with the characterization of key enzymes in GA biosynthesis and deactivation, has enabled genetic engineers to alter the levels of bioactive GA in crops, and thus affect plant height (Hedden and Phillips 2000). The conserved function of the GA20ox, GA3ox, and GA2ox enzymes between species means that genes encoding these enzymes in one species can be introduced and expressed in another species, thus extending the range of crop plants that can be manipulated. Several examples are given by Phillips (2004). For instance, a gene encoding an Arabidopsis GA 20-oxidase can be introduced into quaking aspen (*Populus tremuloides* × *P. tremula* hybrid) and expressed constitutively (i.e., expressed at high levels throughout the plant). The constitutive expression of the GA 20-oxidase is achieved using a promoter from cauliflower mosaic virus (CaMV 35S). As a consequence of this **overexpression** of GA 20-oxidase activity, the transgenic poplar seedlings contain higher levels of bioactive GA_1, are taller, and have enhanced xylem fiber quantity and length, which are desirable for paper manufacture (Eriksson et al. 2000).

In some other agricultural crops the desired effect is often to *decrease* growth. Reductions in GA_1 levels have been achieved by the transformation of crop plants with antisense constructs of *GA20ox* or *GA3ox*, thereby lowering the transcript levels (and hence expression) of these genes and reducing GA_1 biosynthesis. Alternatively, overexpressing the *GA2ox* gene, which encodes the enzyme catalyzing GA_1 deactivation, can also lead to a reduction in GA_1 levels. These approaches have been used to introduce more extreme dwarfing into wheat (Appleford et al. 2007) (**FIGURE 20.10**) and rice (Sakamoto et al. 2003).

FIGURE 20.10 Genetically engineered dwarf wheat plants. The wild-type (untransformed) wheat is shown on the extreme left. The three plants on the right were transformed with a *GA 2-oxidase* cDNA from bean under the control of a constitutive promoter. Consequently, the endogenous bioactive GA_1 is deactivated more in the transformed plants than in the wild-type plants. The varying degrees of dwarfing reflect varying degrees of overexpression of *GA 2-oxidase*, with the highest expression on the extreme right. (From Hedden and Phillips 2000; courtesy of A. Phillips.)

Dwarf mutants often show other phenotypic defects

We have learned that bioactive GAs control many aspects of plant growth and development in addition to stem length. What else happens in dwarf plants in which GA biosynthesis in stems is blocked by mutation? Clearly, many of these mutations give rise to pleiotropic phenotypes. The severely dwarfed *ga1*, *ga2*, and *ga3* mutants of Arabidopsis have dormant seeds that cannot germinate unless treated with GA. Moreover, the plants are male-sterile unless treated with GA, because of a requirement for bioactive GA in anther and pollen development (see Figure 20.6).

In pea, too, one would expect that GA-deficient mutants would have short stems and reduced seed growth, as bioactive GA is required for both stem elongation and seed development. However, homozygous mutations in three genes encoding enzymes in the GA pathway all give dwarf plants and *normal* seed development because of gene redundancy. For instance, the *na* mutant of pea is blocked at the step catalyzed by the enzyme KAO (see Figure 20.7) and is an extreme dwarf, but these tiny plants are still able to produce pods containing viable seeds, because a second gene that encodes KAO is expressed in seeds (Davidson et al. 2003). In contrast, the *lh*-2 mutation in the *LH* gene, which encodes the enzyme KO (see Figure 20.7), reduces GA_1 levels in both stems *and* seeds (Davidson and Reid 2004). Thus this *lh*-2 mutant, in addition to being dwarf, has impaired seed development as well (**FIGURE 20.11**).

Auxins can regulate GA biosynthesis

There is a considerable body of evidence, initially from studies in pea and then in other dicots and in monocots,

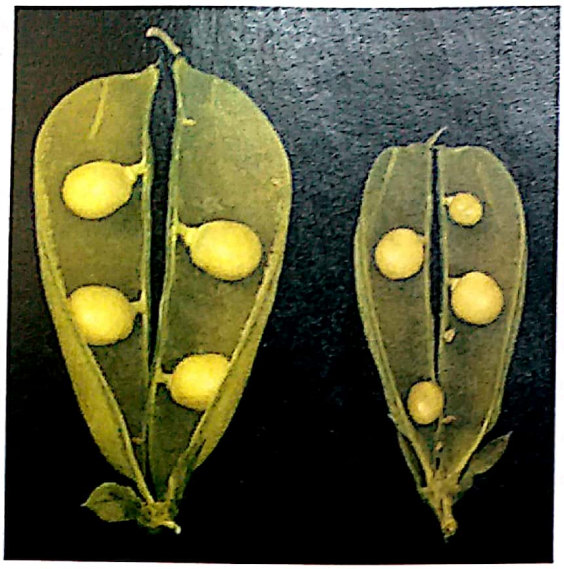


FIGURE 20.11 Impaired seed development in a GA-deficient mutant of pea. Pods of the wild type (left) and the *lh-2* mutant (right), showing impaired seed development in the mutant. (Courtesy of J. B. Reid.)

that auxins can regulate GA biosynthesis. The "targets" for auxin regulation—that is, which genes are up- or down-regulated—are different in different species, and even in different organs or tissues of the same species. Some of these studies are described in WEB TOPIC 20.6.

Gibberellin Signaling: Significance of Response Mutants

Single-gene mutants impaired in their response to GA have been valuable tools for identifying genes that encode possible GA receptors or components of their signal transduction pathways. In general terms, factors that affect signal transduction can be either *positive* or *negative* regulators. Three main classes of mutants can be distinguished:

1. A mutation that renders a **positive regulator** of GA signaling nonfunctional gives rise to a *dwarf* phenotype. These loss-of-function mutations are recessive, and the mutants do not respond to applied GA because of the deficiency in an essential component of the GA signal transduction pathway.
2. A mutation that renders a **negative regulator** of GA action nonfunctional gives rise to a *tall* phenotype. Again, these loss-of-function mutations are recessive.
3. A third class of mutants, in which a *negative regulator* is made *constitutively active*, also gives rise to GA-nonresponsive *dwarf* plants, but in these cases the mutations are "gain-of-function," and thus semidominant.

What makes these GA response mutants different from those plants with mutations that block enzymes in the GA biosynthetic pathway? The difference is that the height of these GA response mutants is *not* proportional to the amount of endogenous bioactive GA. We know this because GA-insensitive dwarf plants will *not* grow tall when treated with bioactive GA. Nor do the constitutive extra-tall mutants (the phenotype referred to as "slender") exhibit reduced growth in the presence of inhibitors of GA biosynthesis.

The response mutants have been extensively studied in Arabidopsis, starting with the *GA insensitive* (*gai-1*) mutant that was isolated in the mid-1980s (Koornneef et al. 1985). Sequencing some of the positive and negative regulators in Arabidopsis paved the way for their characterization in a number of important crop plants, including rice, barley (*Hordeum vulgare*), wheat, maize, and pea. The discussion of GA signaling in the following sections is not treated historically, but the citations indicate the order in which the work was conducted.

GID1 *encodes a soluble GA receptor*

A major breakthough in our understanding of GA signal transduction came with the characterization of a recessive dwarf mutant of rice, termed ***GA-insensitive dwarf1*** **(*gid1*)** (Ueguchi-Tanaka et al. 2005). (This mutant fits the description for type 1 mutants listed earlier.) The wild-type allele codes for a globular protein, GID1, which is a GA receptor. Research to identify GA receptor proteins has been going on for at least two decades, and several putative GA-binding proteins have been investigated. So far, however, only GID1 fulfils all the criteria for a GA receptor. (Scientists doing this work used a GST–GID1 "fusion protein." The GST-tag allows the GID1 to be purified more easily, and it is known that GST–GID1 and GID1 have similar properties with respect to GA binding. For simplicity in the following discussion, we will refer to GST–GID1 as GID1. Likewise, we will refer to radiolabeled 16, 17 dihydro-GA_4 as radiolabeled GA_4.)

To be identified as a receptor, a protein must fulfill the following criteria:

- The binding of the ligand (in this case, GA) to the protein must be *specific*. This means you would expect bioactive GAs to bind to GID1 with higher affinity than less-active GAs. When the binding of ten different GAs (of varying relative biological activities) to GID1 was tested, bioactive GAs were shown to bind with higher affinity. Thus, GID1 can discriminate between bioactive GAs and those that have less activity.

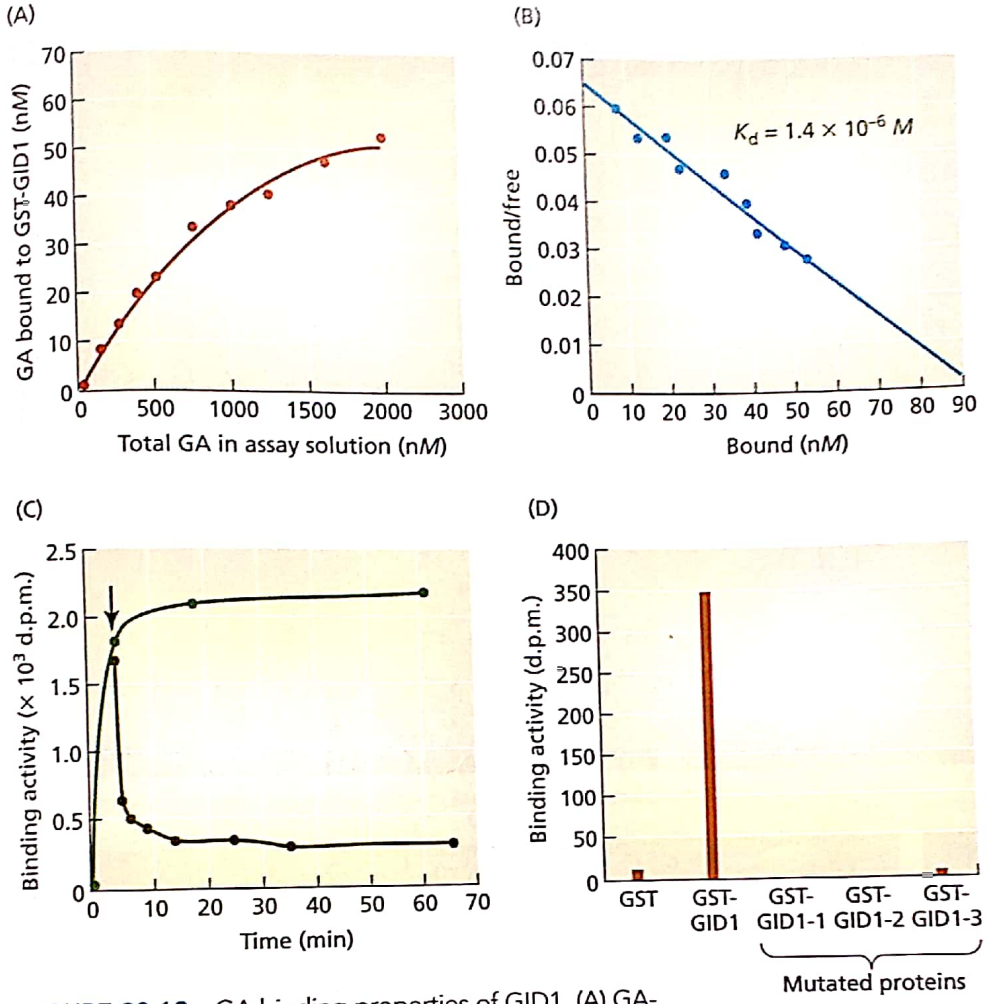


FIGURE 20.12 GA binding properties of GID1. (A) GA-binding to GID1 protein (expressed as a GST–GID1 fusion protein) shows saturability when GID1 is incubated with a constant amount of radiolabeled GA_4 and increasing concentrations of unlabeled GA_4. (B) A Scatchard plot of the data from A gives the dissociation constant (K_d). (C) Association/dissociation rates of radiolabeled GA_4 and GID1. Total binding of radiolabeled GA_4 reached one-half of the maximum within 5 min (light green line). Adding an excess of unlabeled GA_4 (arrow) reduced binding to less than 10% within 5 min (dark green line). (D) Three different mutated GID1 proteins (expressed as the fusion proteins GST–GID1-1, GST–GID1-2, and GST–GID1-3) did not bind radiolabeled GA_4, providing strong evidence that GA_4 binding to the wild-type protein is specific. (After Ueguchi-Takana et al. 2005.)

- Ligand binding should be *saturable*; when all the receptor molecules have bound a GA molecule, no additional radiolabeled GA molecules will bind, and the curve flattens. This was shown to be the case for GID1 (see FIGURE 20.12A).
- Ligand binding should be of *high affinity*: the higher the affinity of GAs for the protein the more tightly bound they will be, and the less easily they will dissociate. The value for K_d (the dissociation constant) can be obtained from a Scatchard analysis, in which the amount of bound radiolabeled GA (x-axis) is plotted against the amount of bound radiolabeled GA divided by the amount of unbound radiolabeled GA (y-axis) (FIGURE 20.12B). The slope is $-1/K_d$, and the low value for K_d (1.4×10^{-6} M) indicates that GA is tightly bound to the protein.
- Ligand binding should be *rapid* and *reversible*. FIGURE 20.12C shows that total binding of radiolabeled GA to GID1 reached one-half of the maximal value in just 5 minutes. Also, the addition of unlabeled GA_4 showed that dissociation of radiolabeled GA from the protein is also very rapid. These features of a receptor allow it to accommodate rapid alterations in intracellular ligand concentration in a cell.

In addition to providing this compelling evidence that GID1 is a GA receptor, Ueguchi-Tanaka et al. (2005) also showed that single nucleotide substitutions in *gid1-1* and

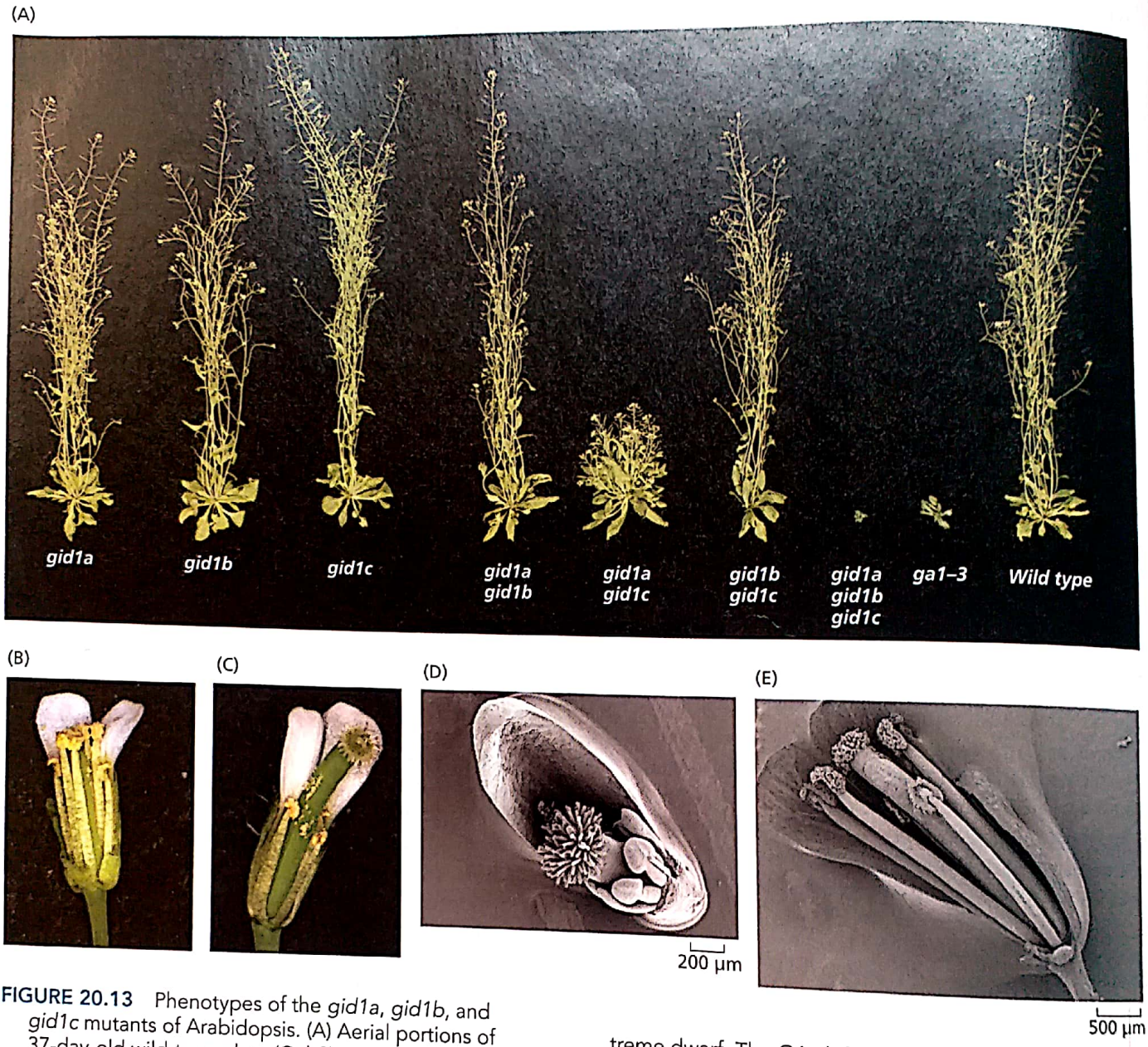


FIGURE 20.13 Phenotypes of the *gid1a*, *gid1b*, and *gid1c* mutants of Arabidopsis. (A) Aerial portions of 37-day-old wild-type plant (Col-0) and homozygous mutant plants showing that single mutants do not have a noticeably altered stem length phenotype. The *gid1a/gid1c* double mutant is dwarf, whereas the other two double mutants show some functional redundancy and are tall. The triple mutant (*gid1a/gid1b/gid1c*) is an extreme dwarf. The GA-deficient mutant ga1-3 is included for comparison. (B) Close-up view of a wild-type flower. (C) The *gid1a/gid1b* double mutant has defective anthers. (D, E) Scanning electron micrographs of flowers of *gid1a/gid1b/gid1c* (D), and wild type (E). In both panels, the sepals and petals were removed for clarity. (From Griffiths et al. 2006; courtesy of S. Thomas.)

gid1-2 and a deletion in *gid1-3* all produced GA-insensitive dwarf plants and prevented the mutant proteins from binding radiolabeled GA_4 (FIGURE 20.12D).

Shortly after the identification of GID1 in rice, three orthologs were discovered in Arabidopsis, namely GID1a, GID1b, and GID1c (Griffiths et al. 2006; Nakajima et al. 2006). (Genes in different species that have similar sequences to each other because they are derived from a single gene in their common ancestor are said to be *orthologous*.) Once the sequence of a gene, for example *GID1*, has been determined in one species (e.g., rice) it is relatively easy to search the genome databases of other species (e.g., Arabidopsis) in order to identify the orthologs. However in Arabidopsis there has been triplication of the original *GID1* gene, and each of the three genes, *GID1a*, *GID1b*, and *GID1c*, codes for a functional GA receptor. A mutation

in only one of these Arabidopsis genes will *not* be expressed as a noticeable difference in stem length, but if all three are mutated then the so-called "triple mutant" is an extreme dwarf (FIGURE 20.13). In addition, severe anther defects lead to male sterility in the triple mutant.

The tertiary protein structures of both rice (GID1) and Arabidopsis (GID1a) proteins have recently been established, shedding light on just why a bioactive GA must have certain functional groups (Murase et al. 2008; Shimada et al. 2008). High-resolution analysis focused on the Arabidopsis protein bound to GA_3 or GA_4 and the rice protein bound to GA_4. The Arabidopsis protein was also cocrystallized with the amino-terminal part of a DELLA protein, GAI. As we shall see later, DELLA proteins are negative regulators of GA response. In both rice and Arabidopsis, the bioactive GA is anchored in a central pocket within the receptor protein by the carboxylic acid at C-6 in the GA molecule making several hydrogen bonds with two serine residues at the base of the pocket and one water molecule. In addition, the 3β-OH group of the GA is hydrogen bonded to a tyrosine residue and a bridging water molecule. Some modifications of the GA molecule can reduce binding: GAs in which the C-6 carboxylic acid is methylated or that do not possess a 3β-OH bind GID1 with an affinity that is several orders of magnitude less than that of a bioactive GA. Even if a GA has a 3β-OH and a C-6 COOH, the additional presence of a 2β-OH group, which has been known for a long time to reduce GA bioactivity, leads to steric interference and thus reduces binding to the receptor protein.

The "top" part of a bioactive GA molecule is hydrophobic and interacts with the N-terminal helical extension switch region of the GID1 receptor protein. The extension folds over the top of the GA, like a lid closing, and completely buries the GA inside the receptor (Murase et al. 2008; Shimada et al. 2008). Once the "lid" has closed, residues on the outer surface of the lid of GID1 are able to interact with specific residues on the amino-terminal end of GAI, which, as we will learn in the next section, is

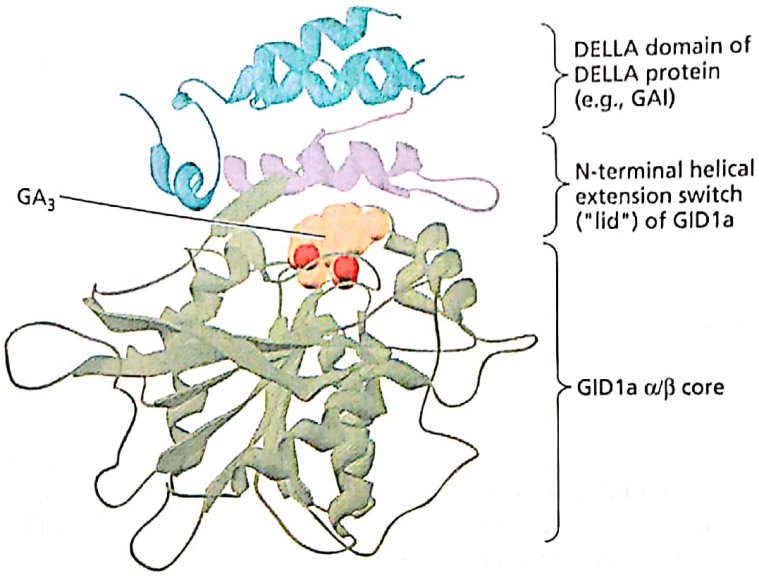
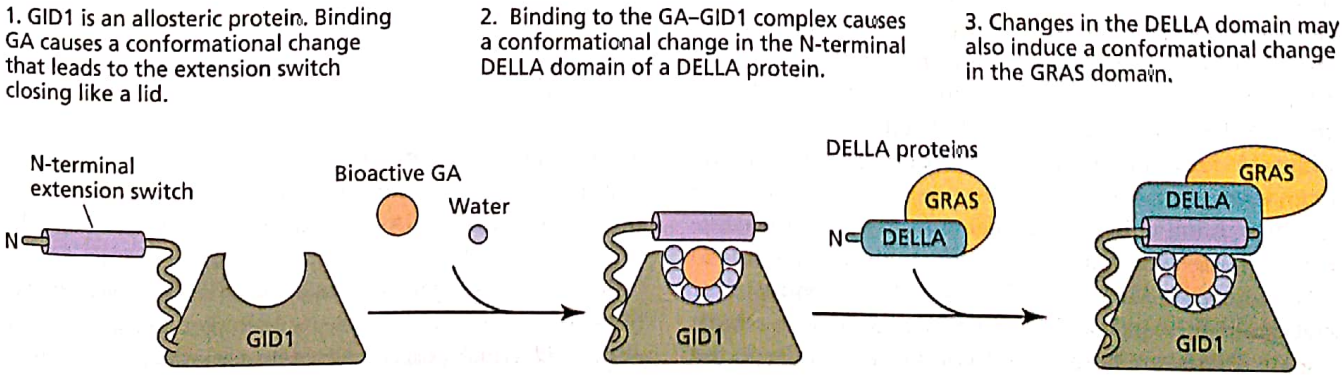


FIGURE 20.14 Structure of the GA_3-GID1a-DELLA complex. The bound GA_3 molecule is represented as a space-filling model with carbon in beige and oxygen in red. Binding of a bioactive GA (e.g., GA_3) within a pocket in the GID1a receptor allows an extension switch to close over the GA, rather like a "lid" closing. The lid closes over the hydrophobic "top" part of the GA, which has no exposed oxygen atoms. Once the extension switch has closed, the DELLA domain of a DELLA protein (e.g., GAI) can bind to the upper, outer surface of the switch. (After Murase et al. 2008.)

a DELLA protein (**FIGURE 20.14**). There is no *direct* interaction between the GA and the DELLA protein (the GA is buried within GID1), but bioactive GA is an **allosteric activator** of GID1, allowing a conformational change of shape in GID1 that facilitates its binding to a DELLA protein (**FIGURE 20.15**). In turn, the GA–GID1 complex appears to induce a coil-to-helix conformational change in the DELLA domain

FIGURE 20.15 A model of the GA-induced change in the GID1 protein, and the changes in the DELLA protein induced by the GA–GID1 complex. (After Murase et al. 2008.)



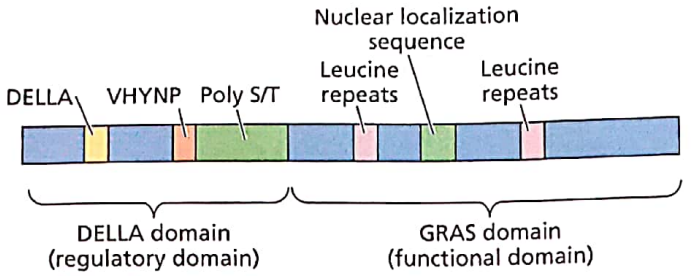


FIGURE 20.16 Domain structures of the RGA and GAI repressor proteins showing the regulatory DELLA domain and the functional GRAS domain.

of the DELLA protein, which may lead to further conformational changes in another domain (the so-called GRAS domain) of the DELLA protein.

DELLA-domain proteins are negative regulators of GA response

DELLA-domain proteins are a subclass of the GRAS family of transcriptional regulators. All GRAS proteins have homology at the C-terminal (GRAS) domain. Those involved in GA responses also have a domain at the N-terminal end in which the first five amino acids are aspartic acid (D), glutamic acid (E), leucine (L), leucine (L), and alanine (A), hence the notation "DELLA domain" (**FIGURE 20.16**). Rice and barley each possess only one DELLA-domain protein, but it is now known that Arabidopsis has five. From extensive studies in cereals and Arabidopsis it was concluded that DELLA proteins are negative regulators of GA response. When they are degraded by proteolysis a GA response can occur. The history of the isolation of DELLA protein and the elucidation of their function is covered in **WEB TOPIC 20.7**. The work is summarized briefly here.

Mutation of negative regulators of GA may produce slender or dwarf phenotypes

Mutations that prevent negative regulators of GA response from acting as negative regulators will produce tall plants. This is exactly what we see in "slender" mutants of rice (*slender rice 1* [*slr1*]) and barley (*slender 1* [*sln1*]). These mutants are excessively tall and, in fact, they look like plants that have been treated with high doses of bioactive GA (Ikeda et al. 2001; Chandler et al. 2002). But these plants do not contain high levels of endogenous GA, and even when they are grown in the presence of inhibitors of GA biosynthesis that can reduce the content of bioactive GA, *these plants are still tall*. Thus the tallness is *not* due to a high bioactive GA content, but to the GA response being constitutively expressed. (The *slender* mutations of cereals we are discussing here should not be confused with the *slender* mutation of pea, which inhibits GA deactivation. The tall phenotype of *slender* pea mutants *is* due to high levels of endogenous GA.)

It was hypothesized that in the *slender* mutants of rice and barley a negative regulator of GA signaling is lost or nonfunctional. Cloning the cereal *slender* genes revealed that they are orthologs of the genes that encode DELLA proteins in Arabidopsis (Peng et al. 1997; Silverstone et al. 1997b, 1998). Remember, orthologs are genes in different species that are derived from a single ancestral gene. Just as we saw with *GID1* (for which there are three genes in Arabidopsis but only one in rice), there has been multiplication of *DELLA* genes in Arabidopsis but not in the cereals. In fact, there are five *DELLA* genes in Arabidopsis that encode five DELLA proteins: GA-INSENSITIVE (GAI), REPRESSOR of ga1-3 (RGA), RGL1, RGA2, and RGL3, all of which are negative regulators of GA response.

RGA was characterized from work with a homozygous recessive mutation (*rga*) in Arabidopsis plants that were also homozygous for *ga1*. Remember, the *ga1* mutation blocks an early step in the GA biosynthetic pathway, so these plants are GA-deficient. The *rga* mutation allowed enough stem elongation that it partially overcame the extreme dwarfism caused by the GA-deficient status of *ga1*, which is why the mutant was called *repressor of ga1*. When a recessive mutation causes growth, the inference is that the mutation must be making a negative regulator nonfunctional (type 2 of the mutants described on page 596). But the original mutant of *GAI* (*gai-1*), which was isolated by Koornneef et al. (1985) and is now known to also encode a DELLA protein, was a semi-dominant, gain-of-function *GA-i*nsensitive dwarf (i.e., fitting the description of a type 3 mutant). Further work revealed that both *RGA* and *GAI* encode negative regulators of GA response, but that different mutations in these negative regulators of GA signaling could produce opposite phenotypes, either slender (tall) or dwarf, depending on where within the genes the mutations occurred. The production of several mutant forms of each of these genes clarified that:

- If a mutation was in the C-terminal GRAS domain, the mutant plant was often taller than expected. In contrast,
- If the mutation was in the N-terminal part of the protein that contained the DELLA domain, the mutant protein was an irreversible repressor, thus giving rise to the insensitive dwarf phenotype of *gai-1*.

Since Arabidopsis contains five homologs, mutations that result in loss of function of several of the homologs are necessary before a GA-constitutive phenotype (i.e., tallness) is manifest.

The alternative phenotypes are seen in the *slender* mutations in cereals. For example, wild-type rice transformed with *SLR1*, which encodes a protein containing a 17–amino

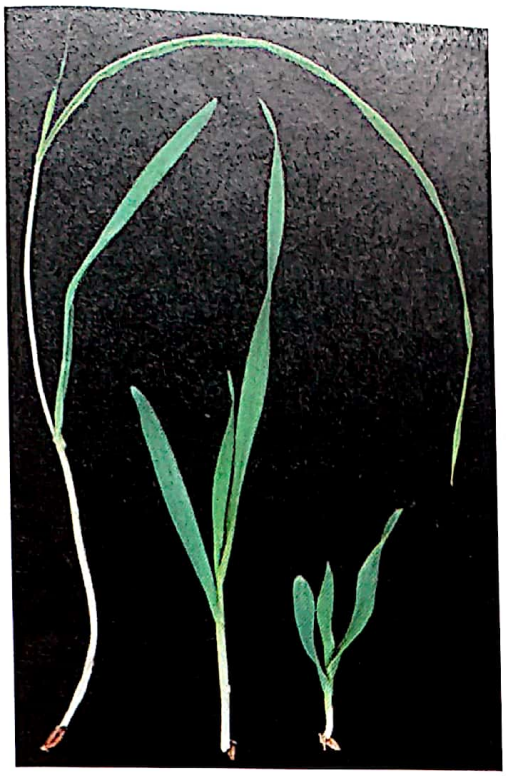


FIGURE 20.17 Demonstration of the opposite effects of two different mutations in the same *SLN1* repressor gene. Three shoots of 2-week-old barley seedlings are shown. Center: The wild-type seedling (*WT*). Left: The *sln1c* mutant has a GA-constitutive, slender phenotype associated with loss-of-function of the repressor protein due to a mutation in the GRAS repressor domain. Right: The *sln1d* mutant is a gain-of-function dominant dwarf, because a mutation in the DELLA domain prevents the repressor protein from being degraded. (From Chandler et al. 2002; courtesy of P. M. Chandler.)

acid deletion in the DELLA domain, gives rise to a GA-insensitive dwarf phenotype instead of the slender phenotype seen when mutations are in the GRAS domain (Ikeda et al. 2001). Similarly, as shown in **FIGURE 20.17**, the *sln1d* mutation in barley is a *d*ominant, insensitive dwarf, in contrast to the original slender *sln1c* mutant with a constitutive GA response (slender) phenotype (Chandler et al. 2002).

Gibberellins signal the degradation of negative regulators of GA response

DELLA-domain proteins are nuclear-localized negative regulators of GA action that must be degraded in order for GA response to occur. To demonstrate the nuclear localization of RGA, Arabidopsis plants were transformed with the *RGA* gene fused to the gene encoding green fluorescent protein (GFP), so that the transgenic plants produce an RGA–GFP fusion protein. Expression of *GFP* was controlled by the promoter for *RGA* so that detection of the RGA–GFP fusion protein would mimic that of native RGA (Silverstone et al. 2001). The effect of bioactive GA on levels and localization of the fusion protein was determined by immunoblotting and fluorescence microscopy. Roots were used because the autofluorescence of chlorophyll makes GFP analysis in shoots more difficult.

RGA–GFP was localized in nuclei of Arabidopsis root tips, and when the plants were treated with bioactive GA, the GFP fluorescence disappeared. In contrast, if the GA content of roots was depleted by treatment with the GA biosynthesis inhibitor paclobutrazol, the nuclei retained intense fluorescence (**FIGURE 20.18**) Since GFP reflects what is happening to RGA, we can conclude from these experiments that in the presence of bioactive GA the nuclear-localized RGA is degraded, whereas it is not degraded when GA levels are very low.

As we learned on page 599, bioactive GA is an allosteric activator of its receptor, GID1. In a GA-deficient plant, there will not be enough GA to bind GID1, and allow the conformational change in the GID1 protein that closes the "lid." Without this conformational change, a DELLA protein is unable to bind GID1, and as we shall now see, a DELLA protein needs to bind GID1 in order to be degraded. We can now also understand why a mutation in a DELLA protein that affects the N-terminal DELLA domain causes the mutated protein to be an irreversible repressor, since it is that part of the DELLA protein that binds the GA–GID1 receptor complex (see Figure 20.15)

F-box proteins target DELLA domain proteins for degradation

Dwarf mutants of rice and Arabidopsis have been identified that are defective in their abilities to degrade wild-type DELLA proteins in the presence of bioactive GA. The *GA-insensitive dwarf 2* (*gid2*) and *sleepy 1* (*sly1*) recessive mutations are in orthologous proteins in rice and Arabidopsis respectively, and give rise to GA-insensitive dwarf phenotypes (Sasaki et al. 2003; McGinnis et al. 2003). These phenotypes suggest that the wild-type *GID2* and *SLY1* genes encode *positive* regulators of GA signaling (i.e., these are type 1 mutants, see page 596). In fact, both genes encode proteins with conserved F-box domains that are components of **ubiquitin E3 ligase complexes**, also known as SCF complexes (Dill et al. 2004; Itoh et al. 2003) (see also Chapter 14). For a protein (such as a DELLA protein) to be degraded by the 26S proteasome, it must be "tagged" for degradation by the attachment of a string of ubiquitin residues. This tagging occurs when F-box components recruit proteins into the SCF complex, leading to the addition of many ubiquitin molecules to the protein and its subsequent degradation by the 26S proteasome. (Further information on this topic can be found in **WEB ESSAY 20.2** and in Chapters 2 and 14.)

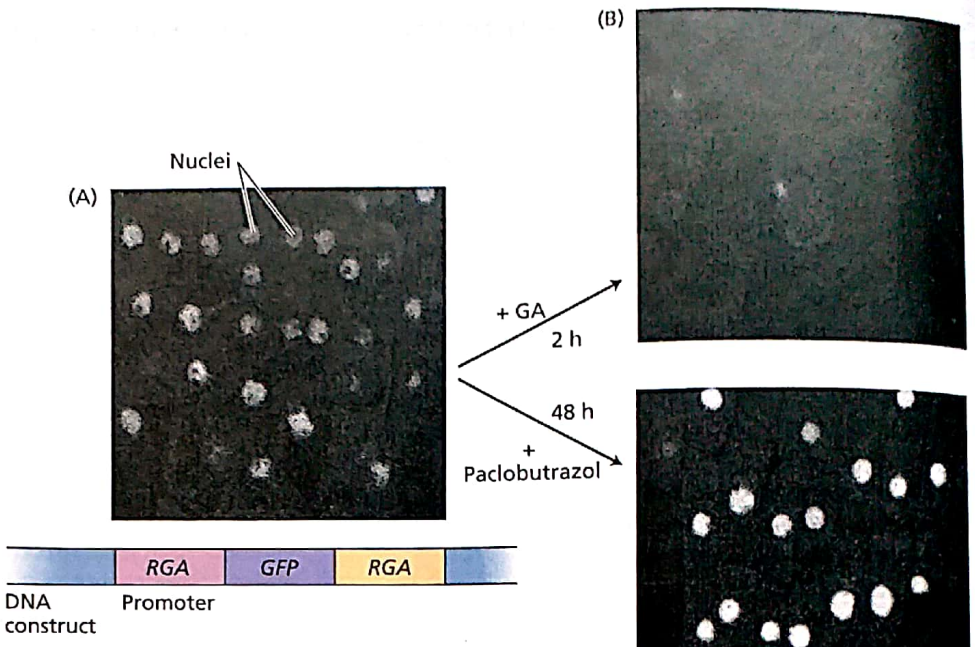


FIGURE 20.18 The RGA protein is found in the cell nucleus, consistent with its identity as a transcriptional regulator, and its level is affected by the level of GA. (A) Plant cells were transformed with the gene for RGA fused to the gene for green fluorescent protein (GFP), allowing detection of RGA in the nucleus by fluorescence microscopy. (B) A 2-hour pretreatment with GA causes the loss of RGA from the nucleus (top). When GA biosynthesis is inhibited by a 48-hour treatment with paclobutrazol (a GA biosynthesis inhibitor), the RGA content in the nucleus increases (bottom). These micrographs show that RGA is degraded in the presence of GA, but not in its absence. (From Silverstone et al. 2001.)

The orthologous proteins GID2 (in Arabidopsis) and SLY1 (in rice) target the DELLA proteins for degradation, and this targeting is enhanced when the DELLA protein is bound to the bioactive GA–GID1 complex (**FIGURE 20.19**). Just as binding bioactive GA causes a conformational change in GID1 so that it can bind the DELLA protein, the binding of the N-terminal DELLA domain to the GA–GID1 complex leads to a conformational (coil-to-helix) change in the DELLA domain. It has been hypothesized that this could, in turn, induce a conformational change in the GRAS domain that would favor binding to the F-box protein. For this reason the GA–GID1 complex has been termed a ubiquitinylation chaperone that stimulates recognition of the repressor protein by the SCF complex (Murase et al. 2008).

Negative regulators with DELLA domains have agricultural importance

The experiments described above provide a considerable body of evidence that the DELLA-domain proteins are important negative regulators of GA response in both dicots and monocots. Additionally, in wheat the semidominant *Reduced height* mutations (*Rht-B1b* and *Rht-D1b*), and in maize the *d8* mutation, are all in the DELLA domains of the respective *GAI* orthologs, and these mutations give rise to GA-insensitive dwarf plants (Peng et al. 1999).

Classical breeding of wheat and rice in the early and middle of the twentieth century paved the way for the "Green Revolution" of the 1960s—the introduction of high-yielding dwarf varieties of wheat and rice into Latin America and Southeast Asia to keep abreast of human population growth (Hedden 2003). Normal cereals grow too tall when close together in a field, especially with high levels of fertilizer. The result is lodging (collapse of plants in wind and rain), with unacceptably large losses in yield. The dissemination of wheat cultivars expressing *Rht* mutations, with reduced GA response and shortened stems, was pivotal to the success of the Green Revolution. (More details on Dr. Norman Borlaug's Nobel Prize–winning work can be found in **WEB ESSAY 20.3**.) In barley, new mutant alleles in orthologs of the Green Revolution genes of wheat give potentially useful agronomic traits, as described in **WEB ESSAY 20.4**.

Gibberellin Responses: Early Targets of DELLA Proteins

As you know from the earlier part of this chapter, GAs can affect many aspects of plant growth and development ranging from seed germination to flower and fruit development. The challenge, recently, has been to relate our newfound understanding of GA perception, at a molecular level, with downstream events that lead to changes in growth and

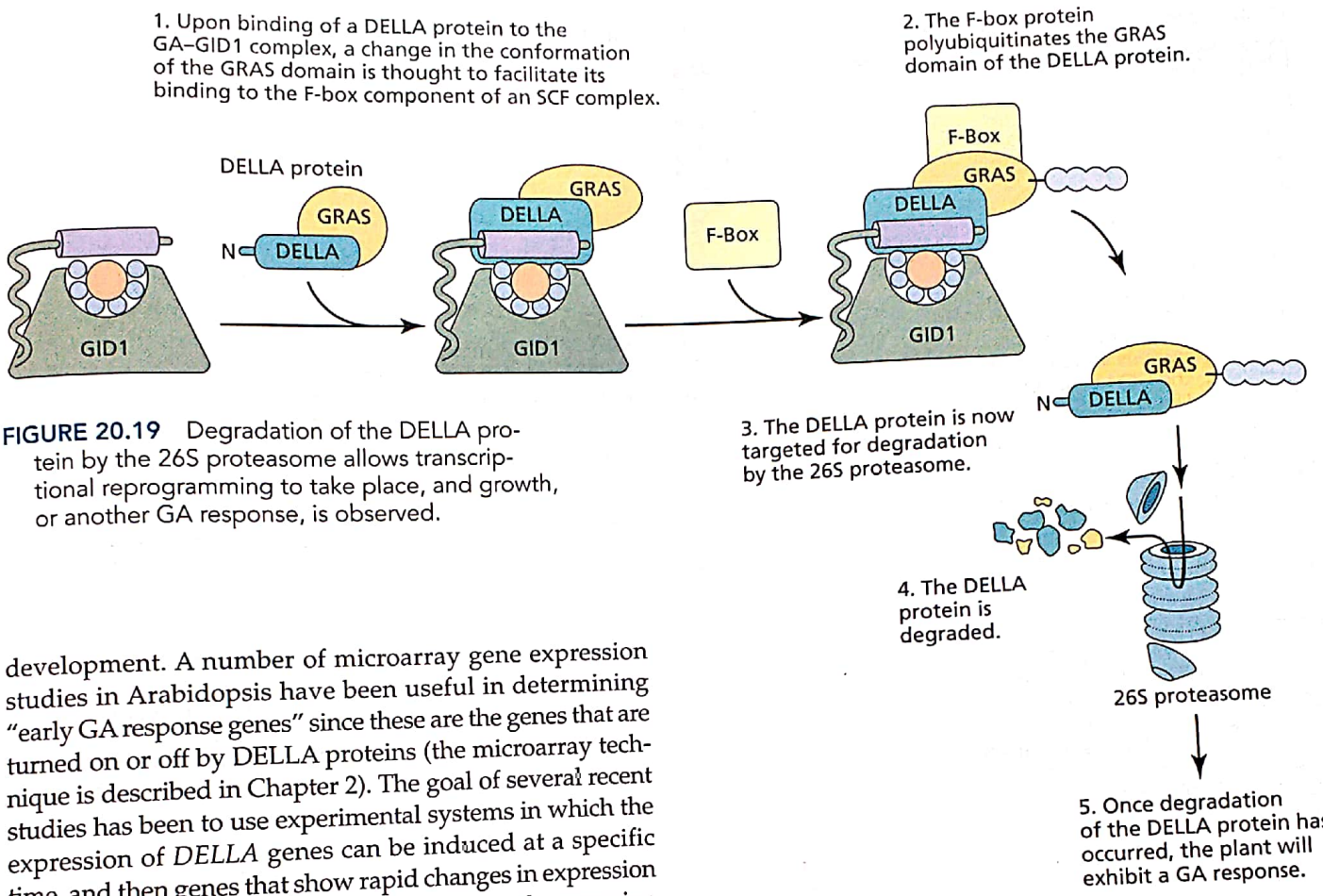


FIGURE 20.19 Degradation of the DELLA protein by the 26S proteasome allows transcriptional reprogramming to take place, and growth, or another GA response, is observed.

development. A number of microarray gene expression studies in Arabidopsis have been useful in determining "early GA response genes" since these are the genes that are turned on or off by DELLA proteins (the microarray technique is described in Chapter 2). The goal of several recent studies has been to use experimental systems in which the expression of *DELLA* genes can be induced at a specific time, and then genes that show rapid changes in expression can be identified. The genes that show altered expression within an hour or two of inducing DELLA expression are likely to be early GA response genes.

DELLA proteins can activate or suppress gene expression

Early GA response genes downstream of DELLA proteins have been identified in seedlings and inflorescences of Arabidopsis. In both experimental systems, several of the immediate targets of DELLA proteins are genes encoding GA biosynthetic enzymes or the GA receptor, implying strong homeostatic regulation to maintain GA levels and GA response within physiological limits. Other immediate targets of DELLA proteins are genes encoding transcription factors or transcriptional regulators. Although some of these DELLA targets in seedlings and in inflorescences belong to the same *classes* of transcriptional regulators—for example MYB and basic helix-loop-helix (bHLH) classes—what is remarkable is that there appears to be very little overlap in the individual genes regulated by DELLA proteins in seedlings and in inflorescences (Zentella et al. 2007; Hou et al. 2008). These observations may help to explain the specificity of GA action, and how a single hormone can lead to so many different types of responses depending on the developmental stage or target tissue.

DELLA proteins regulate transcription by interacting with other proteins such as phytochrome-interacting factors

Since DELLA proteins do not have any recognizable DNA-binding domains, how can they activate and repress genes? It is possible that additional factors may be necessary to allow DELLA proteins to bind DNA, or that DELLA proteins regulate transcription through interaction with other transcription factors, rather than binding DNA themselves. There is recent evidence for the latter scenario; DELLA proteins interact with **Phytochrome-*interacting* factors (PIFs)**, which are a type of bHLH transcription factor. When a DELLA protein binds to a PIF, it prevents the PIF from activating gene transcription; thus the target genes of a PIF are (indirectly) down-regulated by DELLA proteins.

There are at least five PIFs in Arabidopsis, which have some distinct and some overlapping functions. Several PIFs affect seedling growth, and are involved in the transition from skotomorphogenesis (growth and development in complete darkness) to photomorphogenesis (growth and development in light). In the dark, Arabidopsis seedlings are etiolated—the hypocotyl elongates and the cotyledons fail to open and expand. These effects are, in part, a conse-

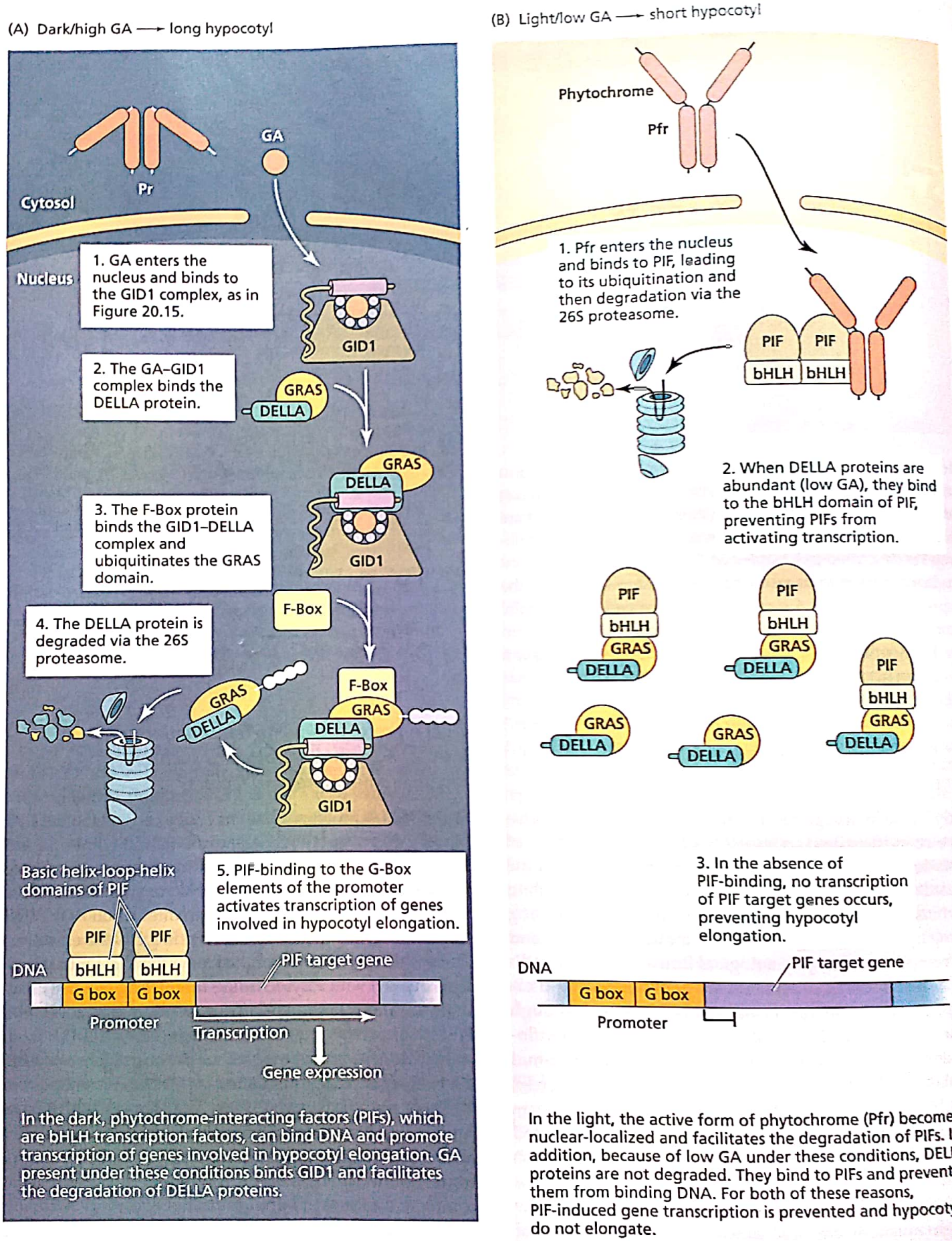


FIGURE 20.20 Integration of light and GA signaling in Arabidopsis seedlings controls hypocotyl length.

quence of PIFs activating the transcription of genes whose products lead to elongated hypocotyls (FIGURE 20.20A). For example, the bHLH domains of PIF3 and PIF4 bind to G-box elements in the promoters of target genes, such as the gene encoding expansin, which is a cell wall–loosening protein. In the light, photoactivated phytochrome moves into the nucleus and binds these PIFs, leading to their degradation. As a consequence their target genes (such as the one encoding expansin) are down-regulated and hypocotyl elongation is inhibited (FIGURE 20.20B).

Recently both PIF3 and PIF4 have been shown to bind DELLA proteins. This interaction is, in part, via the bHLH domain of the PIF protein, which is the domain needed for the PIF to bind DNA (de Lucas et al. 2008; Feng et al. 2008). When the PIF protein can no longer bind DNA, the PIF target genes are not transcribed (see Figure 20.20B), and hypocotyls do not elongate. This interaction of PIF and DELLA proteins occurs only when DELLA proteins are abundant. In the presence of bioactive GA, DELLA proteins are degraded so they cannot bind PIFs; the PIFs can activate transcription, and seedlings have long hypocotyls (see Figure 20.20A). Thus DELLA proteins have been shown to regulate gene transcription by binding directly to bHLH transcription factors, rather than to DNA, and the GA effect (long hypocotyls) is a consequence of PIF-regulated genes.

Gibberellin Responses: The Cereal Aleurone Layer

In this section and the following two sections, we discuss examples of GA action at three different stages in the life cycle of a plant. First, we discuss the classic model system, the cereal aleurone layer in germinating cereal grain. We then turn to the development of anthers, also in cereals. In both the aleurone and the anther systems, MYB transcription factors up-regulate genes known to encode enzymes that implement the GA response. Finally we will discuss internode elongation and its suppression in deep-water rice.

Cereal grains have three parts: the embryo, the endosperm, and the fused testa–pericarp (seed coat–fruit wall) (FIGURE 20.21). The embryo, which will grow into the new seedling, has a specialized absorptive organ, the scutellum. The endosperm is composed of two tissues: the centrally located starchy endosperm and the **aleurone layer** (see Figure 20.21). The nonliving starchy endosperm consists of thin-walled cells filled with starch grains. Living cells of the aleurone layer, which surrounds the endosperm, synthesize and release hydrolytic enzymes into the endosperm during germination. As a consequence, the stored food reserves of the endosperm are broken down, and the solubilized sugars, amino acids, and other products are transported to the growing embryo. The isolated aleurone layer, consisting of a homogeneous population of target cells for GA, provides a unique opportunity to study the molecular aspects of GA action in the absence of nonresponding cell types.

Two enzymes responsible for starch degradation are α- and β-amylase. α-Amylase (of which there are several isoforms) 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.

GA is synthesized in the embryo

Experiments carried out in the 1960s confirmed Gottlieb Haberlandt's original 1890 observation that the secretion of starch-degrading enzymes by barley aleurone layers depends on the presence of the embryo. It was soon discovered that GA_3 could substitute for the embryo in stimulating starch degradation. The significance of the GA effect became clear when it was shown that the embryo synthesizes and releases GAs into the endosperm during germination.

Aleurone cells may have two types of GA receptors

Rice mutants (*gid1*) that have a defective GA receptor are unable to synthesize α-amylase (Ueguchi-Tanaka et al. 2005), clearly implicating the soluble GA receptor in this classical GA response. Other evidence, which was obtained before the characterization of GID1, suggested that GA may bind to a protein in the plasma membrane of aleurone cells. Evidence for two types of hormone receptors has been obtained for auxin (see Chapter 19) and abscisic acid (see Chapter 23). Given the great diversity of GA responses, the existence of multiple receptors may not be too surprising, though at the present time there is no definitive identification of a plasma membrane–localized GA receptor. Within the aleurone cells there are both Ca^{2+}-independent and Ca^{2+}-dependent GA signaling pathways. The former leads to the production of α-amylase, while the latter regulates its secretion.

Gibberellins enhance the transcription of α-amylase mRNA

Even before molecular biological approaches were developed, there was already physiological and biochemical evidence that GA enhanced α-amylase production at the level of gene transcription (Jacobsen et al. 1995). The two main lines of evidence were:

- GA_3-stimulated α-amylase production was shown to be blocked by inhibitors of transcription and translation.

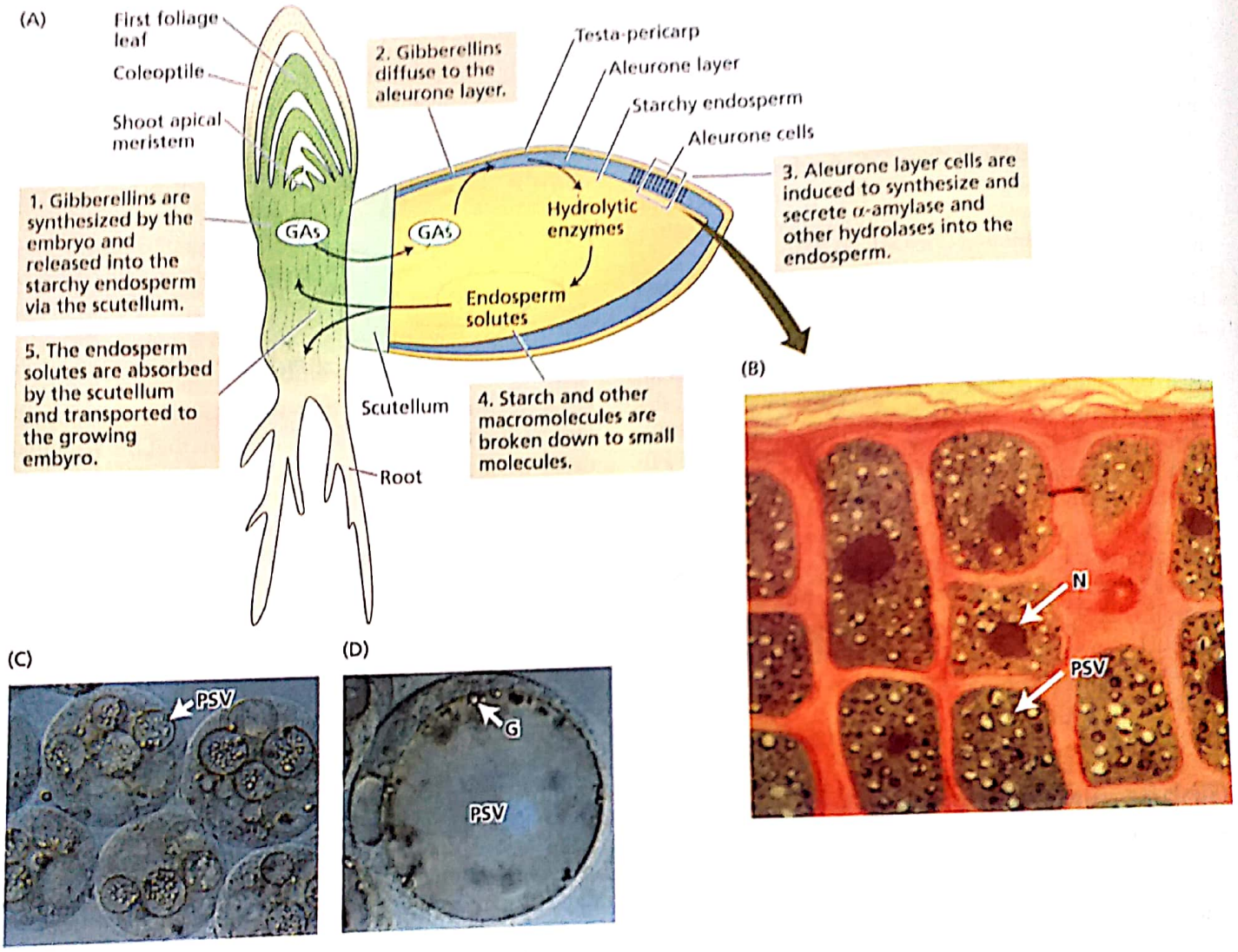


FIGURE 20.21 Structure of a barley grain and the functions of various tissues during germination. (A) Diagram of germination-initiated interactions. (B–D) Micrographs of the barley aleurone layer (B) and barley aleurone protoplasts at an early (C) and a late (D) stage of amylase production. Multiple protein storage vesicles (PSV) in (C) coalesce to form a large vesicle in (D), which will provide amino acids for α-amylase synthesis. G, phytin globoid that sequesters minerals; N, nucleus. (B–D from Bethke et al. 1997; courtesy of P. Bethke.)

- Isotope-labeling studies demonstrated that the stimulation of α-amylase activity by bioactive GA involved de novo synthesis of the enzyme from amino acids, rather than activation of preexisting enzyme.

Cereal grains can be cut in two, and half-grains that lack the embryo (the source of bioactive GA in intact grain) make a good experimental system for studying the action of applied GA. Microarray studies have confirmed the up-regulation of genes encoding several α-amylase isoforms in rice embryoless half-grains that have been treated for 8 hours with GA_3 (Bethke et al. 2006; Tsuji et al. 2006). In these half-grains the only living cells, and the only cells in which GA signaling occurs, are in the aleurone layer. Of all genes in the microarray analyses, those encoding α-amylase isoforms show the highest degree of up-regulation after GA treatment, followed closely by other hydrolases and proteases.

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. Sequences conferring GA responsiveness, termed **GA response elements** (**GAREs**), are located 200 to 300 base pairs upstream of the transcription start site (Gubler et al. 1995). Identical GAREs have been found in all cereal α-*amylase* promoters so far examined, and their presence has been shown to be necessary and sufficient for the induction of α-*amylase* gene transcription by GA.

GAMYB is a positive regulator of α-amylase transcription

The sequence of the GARE in the α-*amylase* gene promoter (TAACAAA) is similar to the DNA sequence to which **MYB proteins** bind. MYB proteins are a class of transcription factors in eukaryotes. In plants there is a large MYB family that is divided into subgroups based on structural features of the proteins. In barley and rice, one MYB protein in the R2R3 subgroup has been implicated in GA signaling and so has been given the name **GAMYB**. The sequence of GAMYB in barley is quite similar to that of three MYB proteins in Arabidopsis (AtMYB33, AtMYB65, and AtMYB101). In fact, the structures of these AtMYBs are so similar to the cereal GAMYB that a barley mutant lacking GAMYB can be "rescued" by any of these AtMYBs (Gocal et al. 2001). In rice two additional GAMYB-like proteins have been identified, but they are not known to function in GA signaling in aleurone cells.

The hypothesis that GAMYB turns on α-*amylase* gene expression (i.e., that GAMYB is a *positive regulator* of α-*amylase*) is supported by the following findings:

- Synthesis of *GAMYB* mRNA begins to increase as early as 1 hour after GA treatment, preceding the increase in α-*amylase* mRNA by several hours (**FIGURE 20.22**).
- A mutation in the GARE that prevents MYB binding also prevents α-*amylase* expression.
- In the absence of GA, constitutive expression of GAMYB can induce the same responses that GA induces in aleurone cells, showing that GAMYB is necessary and sufficient for the enhancement of α-*amylase* expression.

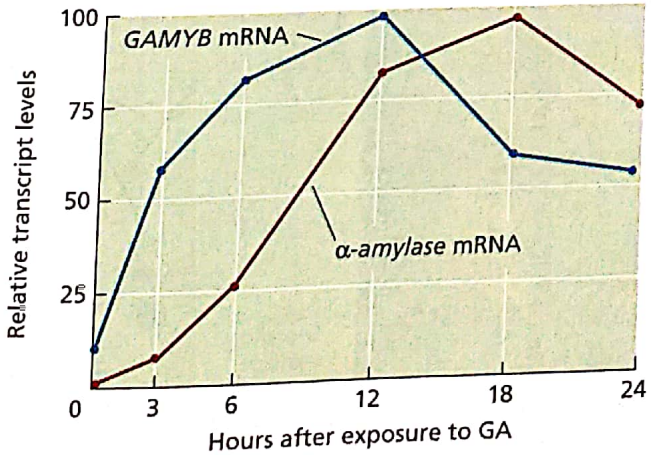


FIGURE 20.22 Time course for the induction of *GAMYB* and α-*amylase* mRNA by GA_3. The production of *GAMYB* mRNA precedes that of α-*amylase* mRNA by about 3 hours. These and other results indicate that *GAMYB* is an early GA response gene that regulates the transcription of the gene for α-amylase. In the absence of GA, the levels of both *GAMYB* and α-*amylase* mRNAs are negligible. (After Gubler et al. 1995.)

Cycloheximide, an inhibitor of translation, has no effect on the production of *GAMYB* mRNA, indicating that protein synthesis is not required for *GAMYB* expression. *GAMYB* can therefore be defined as a **primary** or **early response gene**. In contrast, similar experiments show that the α-*amylase* gene is a **secondary** or **late response gene**.

DELLA-domain proteins are rapidly degraded

How does GA application lead to the transcriptional activation of α-*amylase* by the GAMYB transcription factor? It is known that within 5 minutes of GA application to barley aleurone cells there is an effect on the DELLA protein, SLN1. Levels of a GFP-SLN1 fusion protein in nuclei decline, and indeed the protein is completely gone within 10 minutes of GA treatment (Gubler et al. 2002). However, GAMYB levels do not increase for 1–2 hours, suggesting that even though *GAMYB* is defined as an early response gene, it is probably not the *direct* target of the DELLA protein. We think that there are one or more unidentified steps between SLN1 degradation and *GAMYB* transcription.

Drawing together our information for the cereal aleurone system (**FIGURE 20.23**), we can hypothesize that the binding of bioactive GA to GID1 leads to degradation of the DELLA protein. As a consequence of DELLA degradation, and via some intermediary steps that have not yet been defined, the expression of *GAMYB* is up-regulated. Finally, the GAMYB protein binds to a highly conserved GARE in the promoter of the gene for α-amylase, activating its transcription. Alpha-amylase is secreted from aleurone cells by a pathway that requires Ca^{2+} accumulation. Starch breakdown occurs in cells of the endosperm by the action of α-amylase and other hydrolases, and sugars are transported to the growing embryo.

Some of the genes encoding other hydrolytic enzymes whose synthesis is promoted by GA also have GAMYB binding motifs in their promoters, indicating that this is a common pathway for GA response in aleurone layers.

Gibberellin Responses: Anther Development and Male Fertility

We have seen that the GAMYB transcription factor activates α-*amylase* gene expression during the GA response of cereal aleurone layers, leading to starch degradation in germinating grain. GAMYB is involved in other GA responses too. The effect of GA on flowering is discussed in **WEB TOPIC 20.8**, and, as we shall see here, the effect of bioactive GA on pollen development is also mediated by GAMYB.

1. GA_1 from the embryo enters an aleurone cell.

2. Once inside the cell, GA_1 may initiate a calcium-calmodulin–dependent pathway necessary for α-amylase secretion.

3. GA_1 binds to GID1 (a soluble GA receptor) in the nucleus.

4. Upon binding GA_1, the GID1 receptor undergoes an allosteric change that facilitates its binding to a DELLA protein.

5. Once the DELLA protein has bound the GA_1–GID complex, an F-box protein (part of an SCF complex) is now able to poly-ubiquitinate the GRAS domain of the DELLA protein.

6. The polyubiquitinated DELLA protein is degraded by the 26S proteasome.

7. Once the DELLA protein has been degraded, transcription of an early gene is activated. (GAMYB is shown, in this model, as an early gene, although there is evidence that transcriptional regulation of other early genes may occur first.) The mRNA for GAMYB is translated in the cytosol.

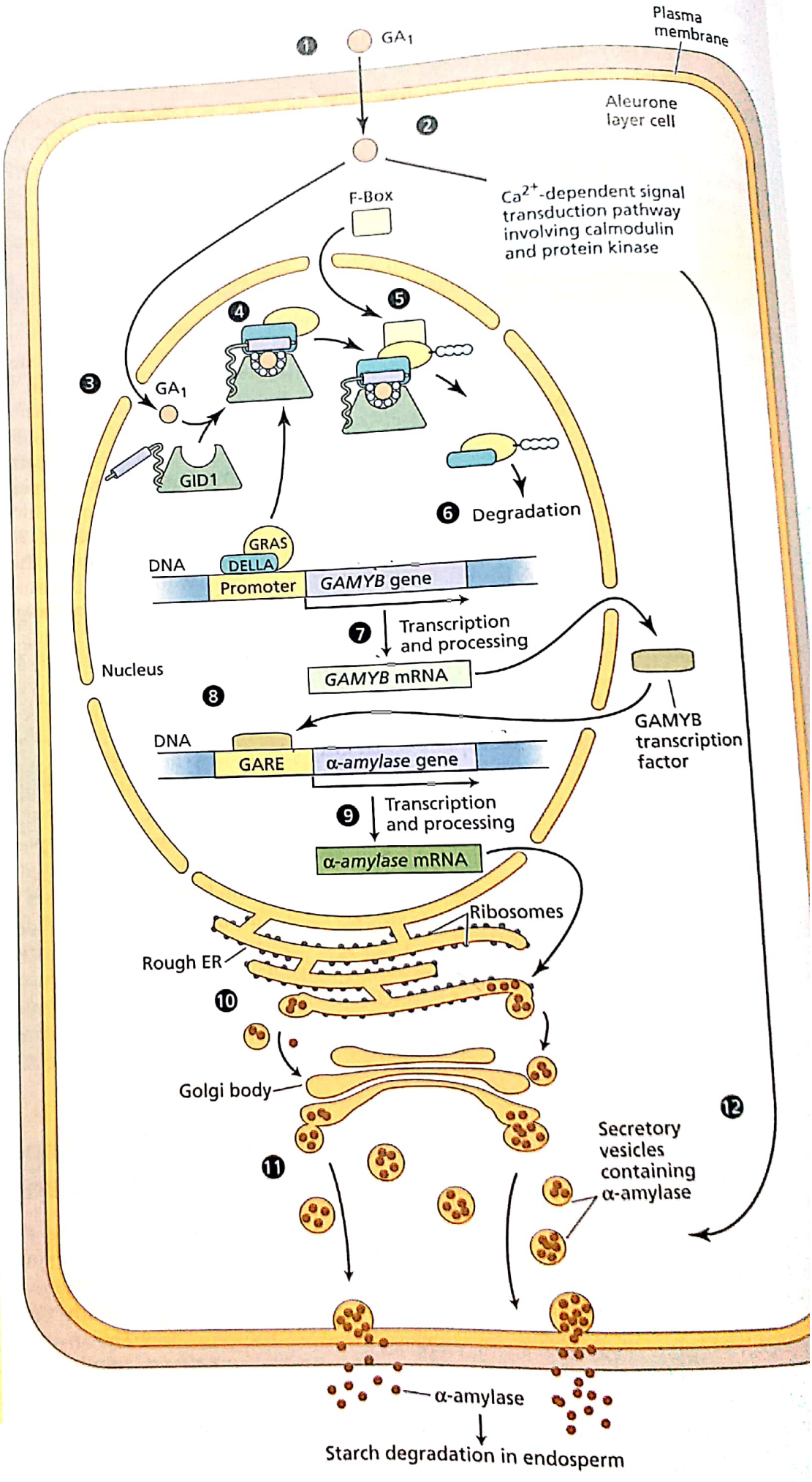
8. The newly synthesized GAMYB transcription factor enters the nucleus and binds the promoters of α- amylase and genes encoding other hydrolytic enzymes.

9. Transcription of these genes is activated.

10. α-amylase and other hydrolases are synthesized on the rough ER, processed, and packaged into secretion vesicles by the Golgi body.

11. Proteins are secreted by exocytosis.

12. The secretory pathway requires GA stimulation of the calcium-calmodulin–dependent pathway.



◀FIGURE 20.23 Composite model for the induction of α-amylase synthesis in barley aleurone layers by GA. A calcium-independent pathway induces *α-amylase* gene transcription; a calcium-dependent pathway is involved in α-amylase secretion.

GAMYB regulates male fertility

Loss-of-function mutants of *GAMYB* in rice have revealed the role of this transcription factor as a positive regulator of floral organ development (Kaneko et al. 2004). Rice plants lacking a functional *GAMYB* gene (*gamyb*) develop flowers with shrunken, white (instead of yellow) anthers that contain no pollen. In some flowers, even on the same plant, phenotypic abnormalities are more pronounced, including grossly malformed pistils in the most severe cases (**FIGURE 20.24**).

Rice pollen grains develop from the innermost layer of the anther and are nourished by a secretory tissue called the tapetum. Tapetal cells form protrusions called orbicules (also known as Ubisch bodies) that are thought to function in the release of *sporopollenin*. **Sporopollenin** is a complex polymer consisting of fatty acid derivatives and phenylpropanoids that eventually forms the resilient outer wall of the pollen grains. As we shall see, bioactive GAs up-regulate the biosynthesis of some of these compounds. Wild-type tapetal cells normally undergo programmed cell death when microspores (which develop into pollen grains) are at the tetrad stage of development (**FIGURE 20.25**). If tapetal cell death occurs too soon, or if it does not occur at all, then viable pollen may not be produced. In *gamyb* mutants tapetal cells do not degenerate at the tetrad stage but continue to enlarge until they fill the anther cavity at the stage that is referred to in wild-type plants as the mature pollen stage. There are no mature pollen grains in the *gamyb* anthers because, in addition to this proliferation of tapetal cells, the microspores collapse between the tetrad and vacuolated pollen stages (see Figure 20.25) (Aya et al. 2009).

In wild-type plants, bioactive GA is involved in regulating programmed cell death of tapetal cells. In addition, GA regulates the biosynthesis of sporopollenin components that provide a tough outer coat for pollen grains. There are several lines of evidence:

- GA up-regulates the expression of genes that encode two enzymes in lipid metabolism during pollen development. The genes, *CYP703A3* and *KAR*, have GAMYB binding sites in their promoters, and alteration of these binding sites by mutation prevents their expression.
- Reporter line studies showed that GAMYB–GUS (a translational fusion to measure GAMYB protein levels) and CYP703A3:GUS (a promoter:GUS fusion to measure the transcription of *CYP703A3*) are co-expressed in wild-type anthers, but CYP703A3:GUS is not expressed in the *gamyb* mutant, in which there is no GAMYB protein (**FIGURE 20.26**).

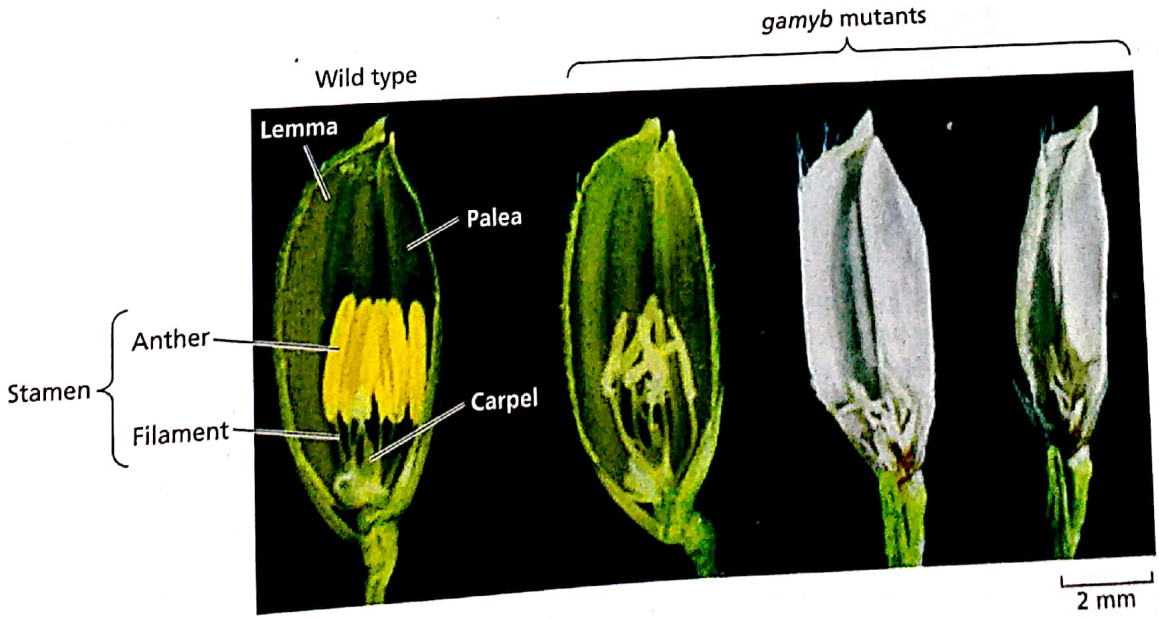


FIGURE 20.24 Phenotypes of floral organs of the *gamyb* mutants of rice, showing that normal pollen development requires the GAMYB transcription factor to be present and functional. A wild-type flower is shown on the far left, and progressing to the right are shown increasing severities of the *gamyb* mutant phenotype. In the absence of a functional GAMYB gene, the anthers are white instead of yellow, and lack pollen. More extreme phenotypes display white, shriveled bracts (lemma and palea) surrounding the flower and malformed carpels. (From Kaneko et al. 2004; courtesy of M. Matsuoka.)

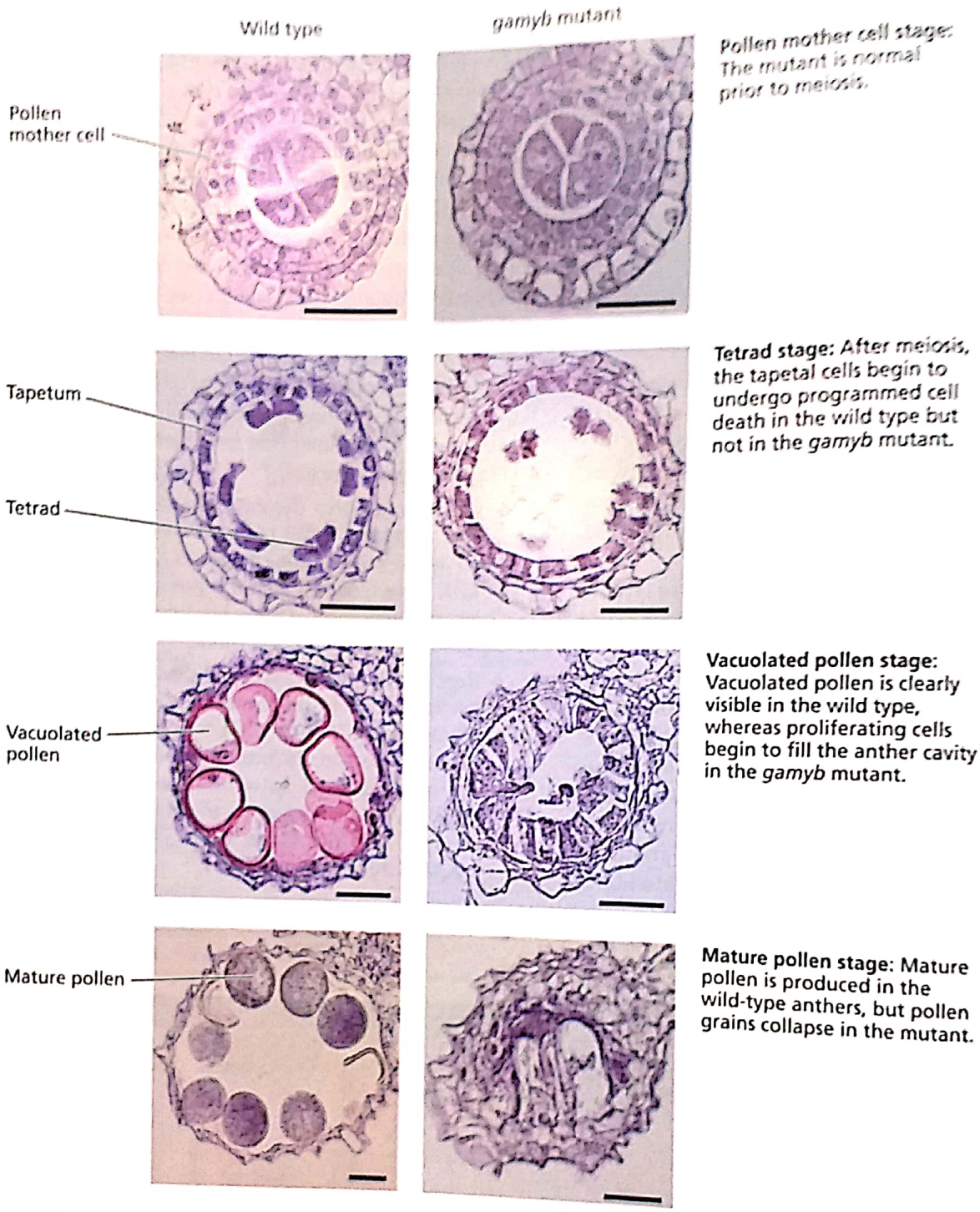


FIGURE 20.25 Histochemical analysis of anther development in wild-type and *gamyb* mutant rice plants at four different stages. Transverse sections of anthers show that tapetal cells in the mutant fail to undergo programmed cell death at the tetrad stage of development. In addition the resilient outer wall of pollen grains fails to develop in the mutant, and pollen grains collapse. Bars = 25 μm. (From Aya et al. 2009, courtesy of M. Matsuoka.)

These and other findings provide compelling evidence that in rice the formation of functional pollen grains requires GA-induced expression of GAMYB, which turns on transcription of genes involved in the biosynthesis of pollen wall components. In the absence of bioactive GA, or of any of the components of its signal transduction pathway, rice is male sterile.

In Arabidopsis, as in rice, GA-deficient mutants or those with defects in GA signaling are male sterile. Both *AtMYB33* and *AtMYB65* must be defective for male sterility to occur, because of functional redundancy between these two genes (Millar and Gubler 2005). The ortholog of *CYP703A3* in Arabidopsis also contains a MYB binding motif in its promoter, suggesting that in Arabidopsis as

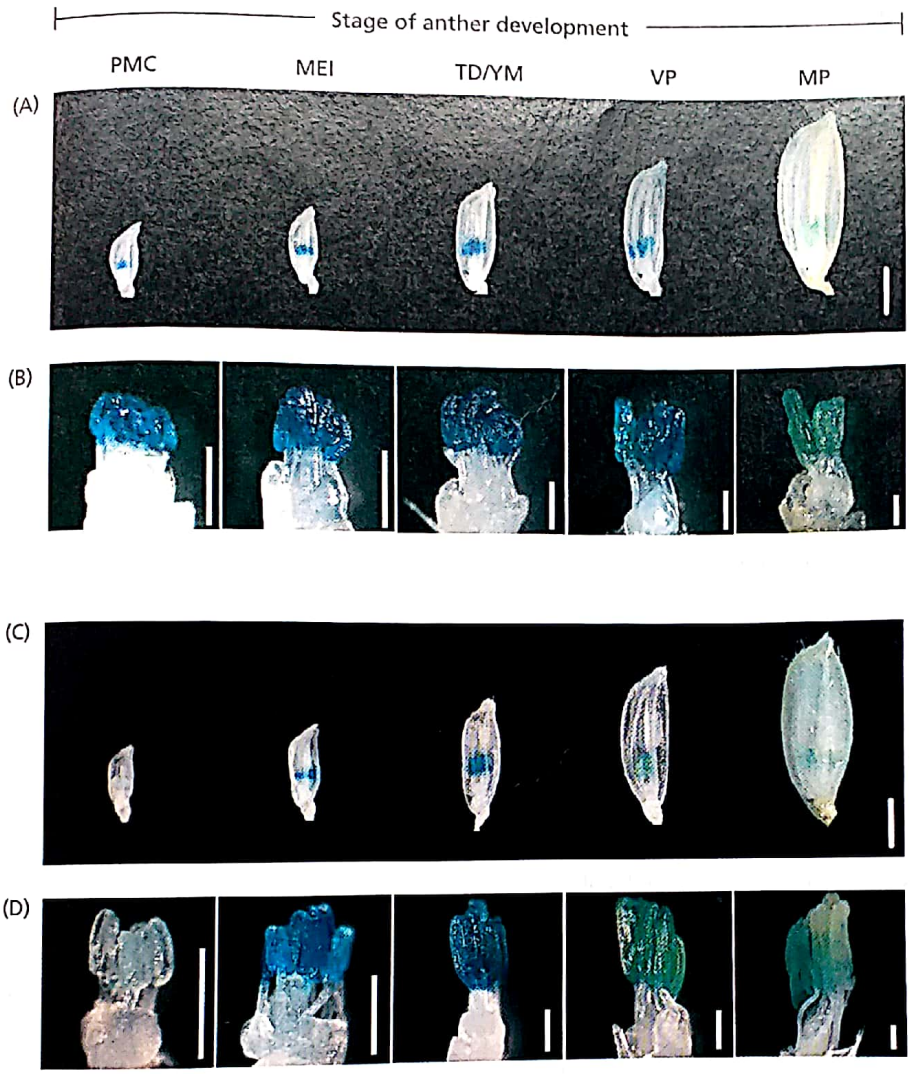


FIGURE 20.26 GUS expression under the control of the promoters of GA-inducible genes in rice anthers. (A and B) Expression of GAMYB–GUS in the wild type. GAMYB–GUS staining in flowers is localized in the anthers (B). (C and D) Expression of CYP703A3:GUS in the wild type. CYP703A3:GUS staining in the flowers is also localized to the anthers (D). (E) CYP703A3:GUS in the *gamyb* mutant at MEI stage of anther development. (A and C) Whole flowers at each developmental stage (bars = 1 mm). (B, D, and E) Close-up views of stamens. PMC, pollen mother cell stage; MEI, meiosis; TD/YM, tetrad/young microspore; VP, vacuolated pollen stage; MP, mature pollen stage. (From Aya et al. 2009; courtesy of M. Matsuoka.)

CYP703A3:GUS staining is shown to be in the same location as GAMYB–GUS. But, unlike GAMYB–GUS, it is not visible at the earliest developmental stage. This result indicates that expression of GAMYB may be required before CYP703A3 expression.

In a *gamyb* mutant there is no expression of CYP703A3:GUS, again indicating that GAMYB may be necessary for the expression of CYP703A4.

well as in rice GA regulates the synthesis of pollen wall components.

Events downstream of GAMYB in rice aleurone and anthers are quite different

A comparison of GAMYB-induced gene expression as determined by microarray analyses of embryoless half-grains and anthers of rice has revealed some very surprising results: Although GAMYB regulates many genes in both aleurone layers and in anthers, the GAMYB-regulated genes in the two systems are quite different (Tsuji et al. 2006). Although many of the GAMYB-dependent genes in anthers do contain the conserved GA response element (GARE) first characterized in GAMYB-regulated genes in the aleurone, it appears that this GARE may not be sufficient for GAMYB-induced expression in anthers, and that additional regulatory factors are involved. In fact, the promoters of GAMYB-regulated genes in anthers lack some of the *cis*-acting elements present in the promoters of GAMYB-regulated genes in aleurone cells. One can postulate that other elements in the promoters of GAMYB-regulated genes in anthers may provide an additional level of specificity in GA-induced, GAMYB-regulated gene expression in the two systems.

MicroRNAs regulate MYBs after transcription in anthers but not in aleurone

MicroRNAs (miRNAs) are small gene-encoded RNAs containing about 20 to 25 nucleotides that regulate the expres-

sion of other genes posttranscriptionally. MiRNAs bind to ribonuclease complexes and serve as guides to target the ribonuclease to specific mRNA molecules. The mRNAs that hybridize to these miRNA guides are quickly digested by the ribonuclease complex, thus effectively silencing the expression of the target genes. (For a more detailed discussion of miRNAs, see Chapter 2.)

Three miRNAs in Arabidopsis have been shown to be complementary to a conserved motif in the coding regions of *GAMYB-like* genes (Park et al. 2002; Rhoades et al. 2002). One of them, *miR159a*, can silence *AtMYB33* in Arabidopsis shoots, and overexpressing or mutating *miR159a* can cause striking defects in plants. *AtMYB33* is involved in floral organ development and flowering in Arabidopsis. Thus *miR159a* overexpressers, in which *AtMYB33* is silenced, are male-sterile and have delayed flowering (Achard et al. 2004). MicroRNAs can alter *GAMYB* expression in rice anthers, but not in aleurone (Tsuji et al. 2006). This shows another difference between GA-induced, GAMYB-regulated gene expression in the aleurone and in anthers.

Taken together, the work on both GA-induced α-amylase production and GA-induced floral organ development all support a role for MYB transcription factors as important intermediates of GA signaling in cereals and in Arabidopsis. Confirmation of their importance in other dicotyledonous plants in addition to Arabidopsis can be anticipated. There are intriguing differences in the signaling downstream from GAMYBs in different tissues and organs that may determine how it is that a single GA can induce different biological responses in different target tissues.

Gibberellin Responses: Stem Growth

The effects of GAs on stem growth can be so dramatic that one might imagine it should be simple to determine how they act (see Figures 20.1 and 20.2). Unfortunately, this is not the case, because—as we have seen with auxin—much about plant cell growth is poorly understood. However, we do know some basic characteristics of GA-induced stem elongation. In the final section of this chapter we examine studies aimed at elucidating the physiological and biochemical mechanisms for GA-stimulated cell elongation and cell division in rice.

Rapid stem elongation occurs in rice in response to flash flooding, when the water level rises very quickly. However this tends to deplete carbohydrate reserves in the plants, and decreases their survival after the flooding subsides. Interestingly, submergence-tolerant lines of rice, which elongate less when underwater, have enhanced survival rates after flooding. This submergence-tolerance is directly linked to the persistence of DELLA proteins, which reduces GA sensitivity.

Gibberellins stimulate cell elongation and cell division

Gibberellins stimulate both cell elongation and cell division, as evidenced by increases in cell length and cell number, in response to applications of bioactive GAs. Certain plants or plant organs associated with GA-induced growth display greater cell numbers than their respective control plants.

- Internodes of tall peas have more cells than those of dwarf peas, and the cells are longer.
- Mitosis increases markedly in the rib or ground meristem of rosette long-day plants growing in short days, after treatment with bioactive GA.
- The dramatic stimulation of internode elongation in deep-water rice when submerged, or when treated with GA, is due in part to increased cell division activity in the intercalary meristem found in specific monocots.

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

As discussed in Chapter 15, the rate of cell elongation can be influenced by both cell wall extensibility and the osmotically driven rate of water uptake. Gibberellin has no effect 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 GA_1 content or sensitivity showed that GA decreases the *wall yield threshold* (the minimum force that will cause wall extension). Thus, both GA 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 GA action, since GA-stimulated increase in proton extrusion has not been demonstrated. On the other hand, GA is never present in tissues in the complete absence of auxin, and the effects of GA on growth may depend on auxin-induced wall acidification.

The typical lag time before GA-stimulated growth begins is longer for GA than it is for auxin; in deep-water rice it is about 40 minutes, 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 GA-specific wall-loosening mechanism, the growth responses to applied GA and auxin are additive.

Various hypotheses have been explored regarding the mechanism of GA-stimulated stem elongation, and all have some experimental support; as yet, however, none provides a clear-cut answer. For example, there is evidence

FIGURE 20.27 Transgenic rice plants harboring sense and antisense constructs of *OsEXP4*. Shown are antisense (A), control (i.e., not transgenic) (B), and sense (C). Height of the plants is related to the amount of expansin 4. Although the gene is transcribed in the antisense line, it is not translated, diminishing the level of expansin 4 in the plants relative to control. In the sense line the *OsEXP4* gene is overexpressed leading to more expansin 4. (From Choi et al. 2003; courtesy of H. Kende.)

that the enzyme **xyloglucan endotransglucosylase/hydrolase (XTH)** is involved in GA-promoted wall extension (Xu et al. 1996). The function of XTH may be to facilitate the penetration of expansins into the cell wall. Expansins are cell wall proteins that cause wall loosening in acidic conditions by weakening hydrogen bonds between wall polysaccharides (see Chapter 15). Transcript levels of one particular expansin in rice, encoded by *OsEXP4*, increase in deep-water rice within 30 minutes of GA treatment, or in response to rapid submergence, both of which induce growth. Moreover, plants expressing an antisense version of *OsEXP4* are shorter and do not grow in submerged conditions; also, overexpression of *OsEXP4* leads to taller rice plants (**FIGURE 20.27**) (Choi et al. 2003). Taken together, these results indicate that GA-induced cell elongation is at least in part mediated by the expansins.

GAs regulate the transcription of cell cycle kinases

The dramatic increase in growth rate of deep-water rice internodes with submergence is due partly to increased cell divisions in the intercalary meristem. To study the effect of GA on the cell cycle, researchers isolated nuclei from the intercalary meristem of deep-water rice and quantified the amount of DNA per nucleus (Sauter and Kende 1992). In submergence-induced plants, GA activates the transition from G_1 to S phase, leading to an increase in mitotic activity. The stimulation of cell division results from a GA-induced expression of the genes for several **cyclin-dependent protein kinases (CDKs)** (see Chapter 1). The transcription of these genes—first, those regulating the transition from G_1 to S phase, then those regulating the transition from G_2 to M phase—is induced in the intercalary meristem by GA (Fabian et al. 2000).

Reducing GA sensitivity may prevent crop losses

Deep-water rice plants respond to gradual flooding with accelerated internode elongation, which keeps some aerial parts of the shoot above the water. But flash flooding prompts very rapid growth, which causes severe depletion of nutrients and often the death of the plants. *Submergence-tolerant* cultivars of lowland rice do not respond to flash flooding with such extreme shoot elongation; consequently they are better able to conserve carbohydrate resources and recover once the flooding subsides. These cultivars possess a *Sub1A-1* gene that is highly induced during submergence and when introduced into intolerant cultivars gives flash flood tolerance. This is because *Sub1A* is associated with increased expression of *SLR1* leading to the accumulation of this DELLA protein, and of *SLR-like1* (*SLRL1*), which encodes another closely related GRAS protein. Both transgenic plants which overexpress *Sub1A* constitutively, or plants in which *Sub1A* is highly induced in submerged leaves, have reduced GA sensitivity and do not experience suicidal levels of growth in response to rapid submergence (Fukao and Bailey-Serres 2008) (**FIGURE 20.28**).

The introduction of cultivars expressing *Sub1A-1* is expected to reduce devastating flash flood–induced losses of rice in flash flood–prone areas of Laos, India, and Bangladesh. Like the Green Revolution cultivars of wheat that were developed to prevent crop losses from lodging, these flash flood–tolerant lines of rice also rely on DELLA proteins to modulate shoot growth.

Over the past few years it has become apparent that DELLA proteins are important in integrating a plant's

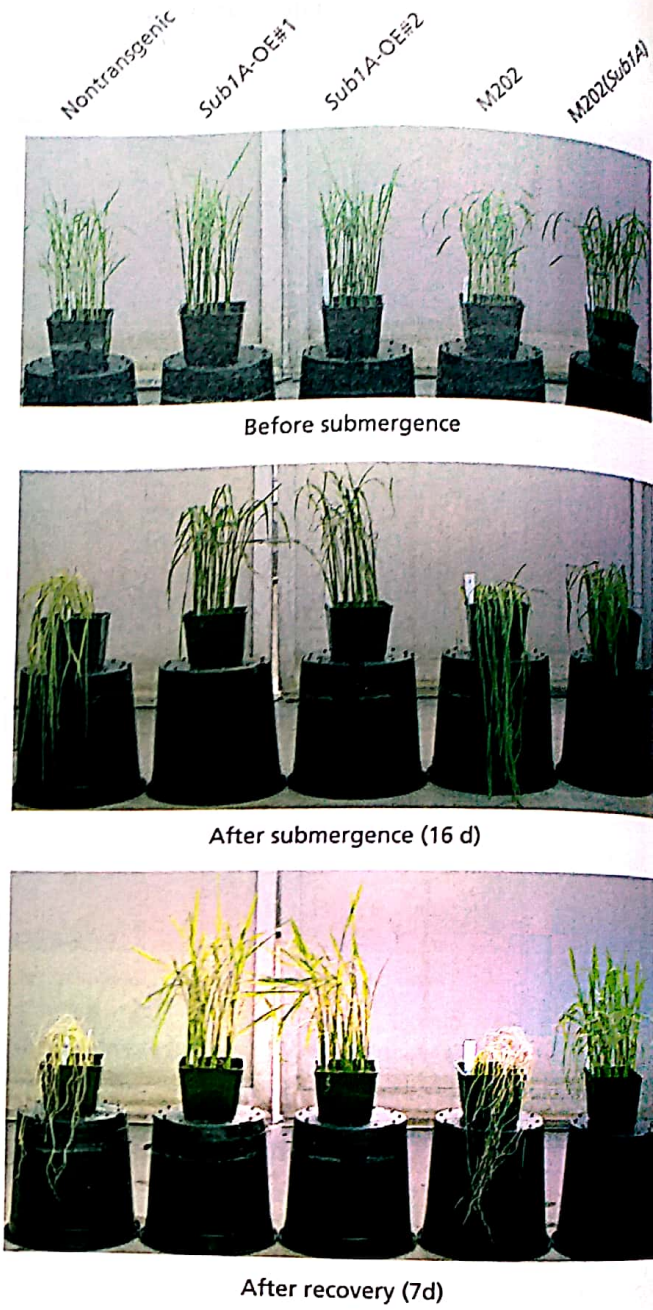


FIGURE 20.28 Either constitutive or submergence-induced expression of *Sub1A* gives rice plants that are better able to survive very rapid flooding. Photographs show constitutive (*Sub1A*-OE#1 and *Sub1A*-OE#2), submergence-induced [M202(*Sub1A*)], and submergence intolerant (Nontransgenic and M202) plants before submergence (top), after 16 days of submergence (middle) and 7 days after the end of submergence (bottom). All plants were at the same developmental stage at the beginning of the experiment. The submergence-intolerant lines show very marked leaf and internode elongation during submergence (middle photograph, first and fourth plants), which depletes resources and prevents the plants from recovering afterwards (bottom photograph). The other plants show reduced GA sensitivity and survive the flooding. (From Fukao and Bailey-Serres 2008; courtesy of J. Bailey-Serres).

response to several hormones in addition to GA, and certain environmental factors. Ethylene, auxins, ABA, light, and temperature are all implicated in the DELLA signaling pathway, though some affect the pathway upstream of the DELLA protein, and some downstream. This aspect of hormone cross talk is discussed further in **WEB TOPIC 20.9**.

SUMMARY

Gibberellins play important roles in regulating seed germination, shoot growth, transition to flowering, anther development, pollen tube growth, floral development, fruit set and subsequent growth, and seed development. All GAs share a similar chemical structure but relatively few of them have intrinsic biological activity.

Gibberellins: Their Discovery and Chemical Structure

- Gibberellins are best known for their dramatic effects on internode elongation in grasses and in dwarf and rosette species (**Figures 20.1, 20.2**).
- Gibberellins are tetracyclic diterpenoid acids made up of four 5-carbon isoprenoid units. In most plants GA_1 and/or GA_4 are the GAs with highest biological activity.

Effects of Gibberellins on Growth and Development

- GAs are necessary throughout the entire plant life cycle to promote seed germination, floral initiation, sex determination, pollen development, pollen tube growth, fruit set, and promotion of fruit growth (**Figure 20.3**).

- Gibberellin A_3 (gibberellic acid), which is obtained from the fungus *Gibberella fujikuroi*, is used commercially on several different types of crops and in the brewing industry (**Figure 20.4**).
- Inhibitors of GA biosynthesis are important dwarfing agents used in cereal crop production.
- Mutations that affect GA biosynthesis and response to active GA also produce dwarf plants.

Biosynthesis and Deactivation of Gibberellins

- GA biosynthesis occurs in multiple plant organs and at multiple cellular sites.
- GAs are mobile and may act either locally or distant from their sites of synthesis, which are in germinating embryos, young seedlings, shoot apices, and developing seeds.
- GA biosynthesis proceeds in plastids with the conversion of geranylgeranyl diphosphate to *ent*-kaurene, continues in the ER for the production of GA_{12}, and is completed in the cytosol where active GA_1 and GA_4 are produced (**Figure 20.5**).
- Research using GA-deficient mutants has helped to establish the biosynthetic pathway (**Figures 20.6, 20.7**).
- Endogenous bioactive GA regulates its own synthesis by inhibiting or enhancing the transcription of the genes for the enzymes of GA biosynthesis (GA 20-oxidases, and GA 3-oxidases) or GA deactivation (GA 2-oxidases).
- Photoperiod and temperature can modify gene transcription of GA biosynthetic enzymes.
- GA biosynthesis occurs at multiple plant organs and cellular sites (**Figure 20.8**).
- A study of GA-deficient pea mutants confirms that plant height is directly correlated with the amount of endogenous GA_1 (**Figure 20.9**).
- Genetic engineering to decrease GA_1 biosynthesis or increase its degradation can lead to decreases in stem growth that can enhance grain yield in cereal crops (**Figure 20.10**). However, some GA-deficient mutants may show defects in fruit or seed production (**Figure 20.11**).

Gibberellin Signaling: Significance of Response Mutants

- The GA response for some mutants is *not* proportional to the amount of endogenous bioactive GA, indicating that the mutated genes encode GA receptors or components of the signal pathway.
- *GID1* encodes a soluble protein that meets the criteria to be a GA receptor in rice (**Figure 20.12**).
- In Arabidopsis, three genes each code for a functional GA receptor, and a "triple mutant" is required to produce an extreme dwarf phenotype (**Figure 20.13**).
- Active GA is an allosteric activator of the receptor protein GID1, producing a conformational change of shape in GID1 and facilitating binding to a DELLA-domain protein, which is a negative regulator of GA response (**Figures 20.14–20.16**).
- Binding of a DELLA protein to the GA–receptor complex results in polyubiquitination and degradation of the DELLA protein.
- Mutation of negative regulators of GA may produce either slender or dwarf phenotypes. The *sln1c* mutation in a barley DELLA protein (SLN) results in constitutive expression of the GA response (tallness). In contrast, the *sln1d* mutation blocks the response to GA (dwarf phenotype) (**Figure 20.17**).
- Gibberellins stimulate the degradation of DELLA-domain proteins located in the nucleus. An Arabidopsis DELLA protein, RGA, is degraded in the presence of GA, leading to a growth response (**Figures 20.18, 20.19**).

Gibberellin Responses: Early targets of DELLA proteins

- DELLA proteins regulate transcription indirectly by interacting with other proteins.
- Phytochrome-interacting factors (PIFs) are transcription factors that in seedlings up-regulate gene expression in the dark and in the presence of bioactive GA, leading to hypocotyl elongation. Light signals PIF degradation and absence of GA response (**Figure 20.20**).

Gibberellin Responses: The Cereal Aleurone Layer

- During germination, the GA produced by the embryo stimulates synthesis of hydrolytic enzymes and their release from the aleurone layer into the endosperm (**Figure 20.21**).
- Active GA binds to GID1 protein, leading to degradation of DELLA proteins; loss of DELLA up-regulates an early response gene that encodes a GAMYB transcription factor. Binding of this factor to a GARE sequence in promoters of α-*amylase* genes enhances their transcription (**Figures 20.22, 20.23**).

SUMMARY continued

Gibberellin Responses: Anther Development and Male Fertility

- The action of GA on pollen development and male fertility is also mediated by GAMYB transcription factors (**Figure 20.24**).
- GAMYB transcription factors are necessary for expression of genes involved in normal pollen development, and they are regulated in anthers by microRNAs (**Figures 20.25, 20.26**).
- Differences in GAMYB signaling in different tissues may help account for GA inducing different biological responses in different target tissues.

Gibberellin Responses: Stem Growth

- Gibberellins stimulate both cell elongation and cell division, in some cases permitting rapid but unsustainable stem elongation in rice under stress (flooded) conditions.
- GA-induced cell elongation is partially mediated by a class of proteins named expansins. Transcription of one expansin gene, *OsEXP4*, increases in response to growth-stimulating GA treatment or rapid submergence. Seedlings expressing antisense *OsEXP4* do not grow in submerged conditions; overexpression of *OsEXP4* leads to taller rice plants (**Figure 20.27**).
- In rice, the *SUB1A-1* gene up-regulates DELLA proteins, reducing the sensitivity of stem tissues to GA, and preventing unsustainable stem elongation (**Figure 20.28**).
- DELLA proteins are important for integrating the effects of GAs, several other hormones, and environmental factors.

WEB MATERIAL

Web Topics

20.1 Structures of Some Important Gibberellins and Their Precursors, Derivatives, and Biosynthetic Inhibitors
The chemical structures of various gibberellins and the inhibitors of their biosynthesis are presented.

20.2 Commercial Uses of Gibberellins
Gibberellins have roles in agronomy, horticulture, and the brewing industry.

20.3 Gibberellin Biosynthesis
The GA biosynthetic pathways and GA conjugates in plants are described.

20.4 Gas Chromatography–Mass Spectrometry of Gibberellins
Identification and quantitation of individual GAs are accomplished by gas chromatography–mass spectrometry.

20.5 Environmental Control of Gibberellin Biosynthesis
The antagonistic relationship between gibberellins and abscisic acid is often mediated by environmental factors.

20.6 Auxin Can Regulate Gibberellin Biosynthesis
Studies in both monocot and dicot species have shown that auxin can up-regulate gibberellin biosynthesis and down-regulate gibberellin inactivation.

20.7 Negative Regulators of GA Response
The DELLA proteins are important regulators of GA response.

20.8 Effects of GA on Flowering
Bioactive GAs are involved in the transition to flowering, and interact with the floral meristem identity gene *Leafy*.

20.9 DELLA Proteins as Integrators of Multiple Signals
These proteins are involved in integrating signals from several hormones and multiple environmental factors.