Annu. Rev. Plant Physiol. Plant Mol. Biol. 2001. 52:67–88

How Gibberellin Regulates Plant Growth and Development: A Molecular Genetic Analysis of Gibberellin Signaling

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Key Words signal transduction, genetics, aleurone layer, Arabidopsis, plant hormones

■ **Abstract** Gibberellins are hormones that control growth and a wide variety of other plant developmental processes. In recent years, significant progress has been made on the biochemistry of gibberellin biosynthesis and on the mechanisms by which gibberellin levels are regulated in plants. There have also been major advances in the understanding of gibberellin signaling, with several key genes being cloned. This review discusses our current understanding of gibberellin signaling, as seen from the perspective of molecular genetic analysis, and relates these observations to previous biochemical studies. In particular, we highlight an important conclusion of recent years: that GAI/RGA and orthologs play major roles in gibberellin signaling in diverse plant species, and that gibberellin probably stimulates growth by derepression of GAI/RGA.

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1040-2519/01/0601-0067$14.00

INTRODUCTION

Gibberellins (GAs) are tetracyclic diterpenoid growth factors that are essential for normal growth and that affect a wide variety of plant developmental processes (39). The number of identified gibberellins is now over a hundred (see http://www.plant-hormones.bbsrc.ac.uk), but only a few of these are known to have biological activity. One of the most important structural features determining biological activity is 3β-hydroxylation. For example, in *Arabidopsis*, the 3β-hydroxylated GA_4 is biologically active, whereas its immediate non-3β-hydroxylated precursor GA_9 is not (15). In addition, the active 3β-hydroxylated form can be inactivated by hydroxylation at the 2β-position. GAs are thought to elicit biological responses via a specific GA-receptor interaction, and the structural limitations on biological activity described above suggest that the interaction receptor:GAs must be highly specific.

GAs affect varied and complex processes within the plant cell, and an understanding of how they perform their function is a fundamental question in plant biology. This review is concerned mainly with our current understanding of GA signaling. There have been several recent reviews on this subject (4, 30, 47, 61, 64, 85, 94, 98). Here we show how recent developments are enabling us to integrate a number of strands in GA biology that had previously seemed unconnected.

During signal transduction, an intra- or extracellular effector (a "signal") interacts with a cell, and its "message" is then relayed through one or more steps within the cell. Finally this process elicits a change in the behavior of the cell (a "response"). If the initial signal is a hormone, such as GA, the first step in signaling involves the interaction of that hormone with a receptor. Although no GA receptor, intra- or extracellular, has been isolated, the accumulated evidence suggests that GA is perceived at the extracellular surface (plasma membrane) of cells (27, 42, but see 2). Thus it seems likely that the GA-receptor is located in the plasma membrane. In this review we concentrate primarily on the molecular genetic analysis of GA-signaling components that are thought to operate downstream of the GA:receptor interaction. Since the mutations that affect

these components have quite widespread effects on GA-regulated processes, it is likely that the components themselves are involved at a relatively early stage in GA signaling and precede the different individual processes controlled by GAs. Each of these processes presumably is controlled by a separate sub-branch of the GA signaling cascade. This concept is illustrated in Figure 1.

GA BIOSYNTHESIS MUTANTS REVEAL THE DEVELOPMENTAL ROLES OF GA

GA-deficient mutants are known in a wide range of plant species and exhibit a characteristic dwarf phenotype. One of the best studied of these, the *Arabidopsis ga1-3* mutant (50, 106), provides a good illustration of the multiple effects of GA-deficiency. The *ga1-3* mutation is a large deletion that abolishes the function of a gene (*GA1*) that encodes *ent*-CDP synthase, an enzyme involved in an early step of gibberellin biosynthesis (33, 50, 92, 93, 106). *ga1-3* mutant plants, when compared to the wild-type, are dwarfed, bushier (apical dominance is reduced), and have darker green leaves. In addition, *ga1-3* seeds fail to germinate, *ga1-3* flowering is delayed [particularly in short days (102)], and *ga1-3* flowers are male sterile (50). The addition of GA restores all the characteristics of the wild-type to the mutant (50). Although the precise phenotypic consequences of GA-deficiency vary slightly from species to species, the general effects are much the same. In particular, GA-deficiency results in a dwarfed plant phenotype, showing that GA is essential for normal plant growth.

Since GA-deficiency causes dwarfism, it might be expected that increased GA levels would have the opposite effect. Studies of a pea mutant that has reduced 2β-hydroxylase activity (56, 82) have shown this to be the case. This mutant contains elevated levels of bioactive GA, because the mutation prevents deactivation of bioactive GAs via 2β-hydroxylation. This mutant displays a phenotype similar to that of wild-type plants treated with exogenous GA: It is elongated compared to wild-type, and has light green leaves. Thus GA levels correlate directly with plant growth: Elevated GA levels are associated with taller plants, whereas reduced GA levels are associated with dwarfism.

GA LEVELS ARE AUTOREGULATED AND ARE ALSO CONTROLLED BY LIGHT

Many of the genes that regulate GA biosynthesis have been cloned. This subject has been recently reviewed (33), and here we highlight only a few points pertinent to the present topic. Many steps in the GA biosynthesis pathway are controlled by enzymes that are the products of small multigene families, with each gene family member having a specific pattern of expression (76, 81). The expression of genes encoding enzymes involved in the later steps of the GA biosynthesis pathway

is often subject to regulation by GA itself, and by environmental signals such as light. GA regulates its own biosynthesis via negative feedback regulation on the accumulation of transcripts encoding the 20-oxidase and the 3β-hydroxylase (15, 33). Depending on the developmental stage of the plant and the species under study, the accumulation of these transcripts was also found to be phytochrome-regulated (1, 100, 105). More recently it was shown that GA feed-forward regulates the level of transcripts encoding the deactivating enzyme 2β-hydroxylase (96). Taken together, these observations indicate that during plant development, there is a tight regulation of the levels of GAs via the control of GA biosynthesis gene expression.

IDENTIFICATION OF GA-SIGNALING MUTANTS

Since elevating or reducing the endogenous GA levels in plants causes characteristic changes in plant phenotype, it should be possible to find mutants that exhibit these changes, but in which alterations in GA perception or signaling, rather than in GA levels, are primarily responsible for the mutant phenotype. There are many such mutants known, some of which mimic the effects of GA-deficiency, and some mimic the effects of elevated endogenous GA levels. In many cases, the genes affected in these mutants have been cloned, giving some indication of the biochemical function of the products that they encode. These various mutants are discussed below, beginning with the *Arabidopsis gai* mutant. The *GAI* gene was the first described member of what is now known to be a group of genes, found in *Arabidopsis* and in other species, that encode the GAI/RGA family of GA signal-transduction components.

THE GAI/RGA FAMILY OF GA SIGNAL-TRANSDUCTION COMPONENTS

In the past few years it has become clear that a family of proteins defined initially by *Arabidopsis* GAI and RGA play key roles in GA signal-transduction. In the following sections we describe the cloning of the genes encoding GAI and RGA, the cloning of orthologous genes from species other than *Arabidopsis*, and we draw some general conclusions about the role of the GAI/RGA family in GA signaling. We end with a discussion of the possible biochemical function of these proteins.

Arabidopsis *gai:* An Altered Function Mutant That Mimics GA Deficiency

The *Arabidopsis gai* mutant shares many of the phenotypic characteristics of GA-deficient mutants (Figure 2): *gai* mutants are dwarfed (48, 69, 70), their leaves are darker green than wild-type (48, 71), and particularly in short

days, they flower later than wild-type (102). However, the *gai* mutant phenotype is not reversed by the addition of GA (48, 70, 103), and *gai* mutant plants contain higher levels of bioactive GAs than do wild-type plants (73, 95). Unlike the GA-deficiency mutations, *gai* is a semidominant mutation (48, 69) and appears to be so because it encodes a product that is structurally and functionally different from the product encoded by the wild-type allele (30, 68, 69). In addition, the levels of 20-oxidase and *GA4* transcripts (which encode enzymes that catalyze late steps in the biosynthesis of GAs) are increased in *gai* (15, 68), consistent with the idea that *gai* has elevated endogenous GA levels because of perturbed feedback regulation.

gai Encodes a Mutant Product That Lacks a Small Segment of N-Terminal Amino Acid Sequence

gai was cloned by insertional mutagenesis (68). *GAI* (the wild-type allele) encodes a protein (GAI) that displays extensive C-terminal homology with a previously cloned presumed transcription factor known as SCARECROW (SCR), and which is now classified as belonging to the GRAS family of proteins (see below; 30, 68, 80). This homology is restricted to a region towards the C termini of GAI and SCR (the "C" region; Figure 3), while the remainder of the GAI sequence (the "N" region, see Figure 3) is unrelated to that of SCR. The mutant *gai* allele contains a 51-base pair (in-frame) deletion mutation within the sequence that encodes GAI, resulting in the loss of a segment of 17-amino acid residues from the "N" region. This change in the structure of the protein confers reduced GA responses to the mutant. This suggests that the "N" region of GAI, the section that differs substantially from SCR, is crucial for normal GA responses.

Altered Function Mutations in *GAI Orthologs*: Maize *d8* and Wheat *Rht*

There are several examples of mutations in species other than *Arabidopsis* that confer phenotypes having similar properties to that conferred by *Arabidopsis gai*. For example, mutations at the maize *d8* locus, of which there are six dominant alleles of different severity, confer phenotypes that mimic that caused by GA deficiency (29, 77, 104). However, these phenotypes are unaffected by the addition of GAs (77, 104). The *D8-1* mutant is known to accumulate bioactive GAs to a higher level than do wild-type plants (22).

The mutant *Rht* genes of wheat confer a phenotype that has many characteristics in common with that conferred by the mutant *D8* alleles in maize. Wheat *Rht* mutants are dwarfed, dark green, and accumulate biologically active GAs to higher levels than are found in wild-type controls (24, 25, 38, 55, 101). There are 8 *Rht* homeoalleles, conferring different degrees of phenotypic severity (9), and these mutations behave genetically as dominant altered-function mutations (24). The *Rht* mutations are of great importance in agriculture, since they confer the higher

yields that contributed to the postwar increases in world wheat production that characterized the so-called green revolution (26).

Since the *D8* and *Rht* mutations confer maize and wheat phenotypes with characteristics that resemble that of the *Arabidopsis gai* mutant, it seemed possible that these mutations might identify related or orthologous genes in these three species. This was shown to be the case. A rice expressed sequence tag (EST) containing sequence that was closely related to that of *Arabidopsis GAI* was used to isolate wheat and maize cDNA and genomic DNA clones via low stringency hybridization. These DNAs were then shown to map to regions of the wheat and maize genomes to which *D8* and *Rht* were already known to map. Finally, the dominant mutant *D8* and *Rht* alleles were shown to carry mutations in the sequences of these cloned genes (72).

Comparisons of the amino acid sequences of the Rht, D8, and GAI proteins identified two regions (domains I and II) of N-terminal sequence that are particularly well conserved (see 72). Interestingly, domain I is almost exactly coincident with the segment of protein that is deleted in the mutant gai protein. Furthermore, comparison of the sequences of the mutant alleles *D8-1, D8-Mpl*, and *D8-2023* in maize, and *Rht-B1b* and *Rht-D1b* in wheat with those of normal control alleles showed that each mutant allele encodes a protein that is altered in one or both of domains I and II. Taken together, these results show that deletions or truncations of the N terminus of the Rht/D8 proteins (equivalent to the "N" region of GAI, see Figure 3) result in reduced GA responses. This further emphasizes the importance of this region of the protein for GA signaling. In addition, these observations show that proteins related to GAI have a conserved function in GA signaling across a wide range of plant species, suggesting that this mechanism of GA signaling is of ancient origin.

Loss-of-Function Mutations in *GAI* and *RGA*

The *Arabidopsis RGA* gene was initially identified in an elegant screen for mutations that suppressed the phenotype conferred by *ga1-3* (89). As described above, *ga1-3* confers a severe dwarf phenotype, due to a dramatic reduction in endogenous GA levels. The screen involved a search for mutants that, although still homozygous for *ga1-3*, now grew taller than the *ga1-3* progenitor due to the presence of a new mutation. As a result of this screen, multiple alleles at a new genetic locus named *RGA* (for repressor of *ga1-3*) were identified. These recessive *rga* alleles partially restore the stem elongation of the *ga1-3* mutant, making *rga ga1-3* plants taller than *ga1-3* controls, but shorter than wild-type. However, *rga ga1-3* plants, like *ga1-3* plants, are sterile. The addition of GA restores the fertility of the mutant and further stimulates stem growth. The *rga* mutations suppress several of the phenotypic defects conferred by *ga1-3*: reduced stem growth, reduced leaf abaxial trichome initiation, delayed flowering time, and apical dominance. Preliminary measurement of GA levels showed that the double mutants have the same levels of GA as *ga1-3*, suggesting that the *rga* mutation is probably affecting gibberellin signal transduction and not the biosynthesis of gibberellins. The fact that the *rga* mutations suppress a broad spectrum of the phenotypes conferred

by *ga1-3* indicates that RGA plays a major role in GA signaling. Furthermore, since *ga1-3* reduces endogenous GA levels, the fact that a *rga ga1-3* plant is taller than a *ga1-3* plant shows that RGA opposes the effect of GA, and that plants lacking RGA require less GA for growth than do normal plants. Thus RGA acts as a negative regulator of GA signal transduction.

Once *RGA* was cloned, it was found to be a homologue of *GAI*, identical to a previously cloned gene called *GRS* (for *GAI Related Sequence*) (68, 88). This was an exciting finding, because it clearly implicated two very closely related proteins in GA signaling. GAI, RGA, Rht, and D8 all share substantial homology in the "N" region (see Figure 3), whereas the equivalent region of SCR and of other members of the GRAS family is substantially different in sequence. This suggests that the "N" region in the GAI/RGA/Rht/D8 proteins is important for GA-related functions.

Molecular analysis of the *rga* alleles showed that some contain deletions that would be expected to abolish or destroy the activity of RGA, and thus can be described as loss-of-function alleles (88). Since loss-of-function *rga* alleles partially suppress the effects of GA-deficiency, do loss-of-function mutant alleles of *GAI* have the same effect? While such alleles of *GAI* confer a visible phenotype indistinguishable from that of a wild-type plant (69, 103), detailed studies of the effects of paclobutrazol (PAC), a GA biosynthesis inhibitor, revealed that they have increased PAC resistance (68). This observation confirmed that *GAI* loss-of-function alleles do partially suppress the effects of GA-deficiency, and it suggests that GAI, like RGA, acts as a negative regulator of GA-responses. Taken together with the high degree of sequence identity between the two genes, these results indicate that GAI and RGA may have overlapping roles in GA signaling. However, the roles of GAI and RGA do not entirely overlap as, if GAI and RGA could completely substitute for one another, then neither loss-of-function allele would confer a detectable change in phenotype.

The GA-Derepressible Repressor Model

As discussed above, the *GAI* gene can be mutated in two distinct ways. First, mutations that cause N-terminal deletions or truncations of GAI confer a dominant reduced GA-response phenotype that is due to the mutant protein having an altered function. Second, mutations that would be expected to abolish GAI function confer a reduced requirement for GA. In order to reconcile these observations a hypothesis that accounted for the action of GAI as a GA-derepressible repressor of plant growth was proposed (30, 68). Here we expand that model to propose a mechanism by which GAI and RGA may control plant growth, based on the premise that GAI and RGA have overlapping roles in GA signaling (Figure 4).

Figure 4 outlines a hypothesis suggesting that GAI and RGA repress GA-mediated growth responses, and that GA derepresses growth by opposing the activity of GAI and RGA. According to this hypothesis, the altered structure of the gai mutant protein causes it to be less affected by GA. Thus, the gai protein

constitutively represses plant growth, and this effect is dominant over the wild-type RGA, which can still recognize the GA signal. The converse also applies—a mutant RGA protein that lacks the equivalent amino acid sequence as is missing in the gai mutant protein confers a dominant, dwarfed reduced GA-response phenotype very similar to that conferred by *gai* (A Dill & T-p Sun, personal communication). In *GAI* loss-of-function mutants the repressor function of GAI is lost. However, this does not result in complete independence from GA, as wild-type RGA requires the GA signal to release its repression of plant growth.

One prediction of this model is that if GAI and RGA functions were both missing, GA would no longer be required for normal stem growth. This prediction has been tested experimentally: *Arabidopsis* plants lacking the *GA1* gene (a key gene in gibberellin biosynthesis, see above), and also lacking GAI and RGA are not dwarfed, and grow as tall as the wild-type (KE King & NP Harberd, unpublished results; A Dill & T-p Sun, personal communication).

Loss-of-Function Mutations in *GAI/RGA* Orthologs in Barley, Rice, and Pea

Barley plants homozygous for the recessive *slender* mutations (20) display phenotypes characteristic of plants treated with saturating levels of exogenous GAs. *slender* mutant plants have long internodes, narrow leaves, and are male sterile. In the *slender* mutant, but not in the wild-type, the secretion of α-amylase and other hydrolytic enzymes is induced in the absence of added GAs (13, 53; see below for discussion of the cereal α-amylase response). This suggests that the *slender* mutation is causing a constitutive GA response. Several lines of evidence are in favor of this hypothesis. First, the concentration of GAs is lower in the *slender* mutant than in the wild-type (16); second, the *slender* mutant is relatively resistant to inhibitors of gibberellin biosynthesis (16, 53); and finally, the progeny of crosses of *slender* with dwarf mutants that are either GA-deficient mutants or GA-sensitivity mutants have the *slender* phenotype (14).

Recently, barley *slender* mutants have been shown to carry mutations in a barley *GAI/RGA* ortholog (P Chandler, F Gubler, A Marion-Poll & M Ellis, personal communication). These mutations would be expected to result in a loss of gene function, consistent with the recessive nature of the *slender* phenotype. Thus it seems that the barley *SLENDER* gene is the functional ortholog of *GAI/RGA* in *Arabidopsis*.

A similar story is emerging from studies of rice *slender* mutants. These mutants also resemble plants supplied with an excess of exogenous GAs, exhibiting rapid growth and elongated leaf sheaths. Furthermore, the *slender* mutant rice plants are resistant to GA biosynthesis inhibitors, and they contain endogenous GA levels lower than those found in wild-type plants. Recently, the rice *SLENDER* gene has been cloned and shown to be a the rice ortholog of *Arabidopsis GAI*/*RGA*. One rice *slender* mutant carries what would be expected to be a null mutation in this gene: A frameshift mutation near the first nuclear localization signal produces a (potentially) truncated protein (J Yamaguchi, personal communication).

Although genetically more complex than the *slender* mutants of barley and rice (see above), the pea *la cry*^{s} double mutant has a phenotype very similar to them, resembling a plant treated with excess GAs: long, thin internodes, pale green foliage, and parthenocarpic fruit development (18, 63, 79). However, following the (by now) familiar pattern of the *slender* mutants, the pea *la cry*^{s} mutant has reduced levels of endogenous GAs (59, 79) and increased resistance to inhibitors of gibberellin biosynthesis (60, 79). Furthermore, the triple mutant *la cry*^{s} *na* has the same phenotype as the *la cry*^{s} double mutant (79), although the *na* single mutant is a gibberellin-deficient mutant with a characteristic dwarf phenotype (78). Preliminary mapping experiments (X Cubells, A Grenell, N Ellis, J Carbonel, personal communication) have indicated that pea *GAI/RGA* homologues map close to the known genetic locations of *la* and *cry*^{s}, suggesting that *LA* and *CRY* may also encode proteins belonging to the GAI/RGA family.

What Do We Know of GAI/RGA Function at the Biochemical Level?

The above sections show that the cloning of *GAI* and *RGA* initiated some important developments in our understanding of the genetics of GA signaling. Many of the "classical" GA-signaling mutants were shown to carry mutations in *GAI/RGA* orthologs. Furthermore, two mutant phenotypes, which had previously been thought to be genetically unrelated, dominant reduced GA-response, and recessive increased GA-response, were shown to be conferred by distinct classes of mutation in *GAI/RGA* and orthologs.

Given the importance of the *GAI/RGA* family in GA signaling, what do we know of the biochemical function of the proteins that these genes encode? *GAI/RGA/Rht/d8* encode members of a recently discovered family of putative plant transcription factors, named GRAS (80), all of which share homology with the "C" region of GAI (see Figure 3). Other members of this family are *Arabidopsis SCR* (19), the first to be characterized, and a gene that regulates asymmetric cell divisions during root development; *LATERAL SUPPRESSOR* (86), a tomato gene involved in the control of production of lateral branches; *PAT1*, an *Arabidopsis* gene involved in phytochrome A signal transduction (10); and *SHORT-ROOT*, an *Arabidopsis* gene that is essential for both cell division and cell specification in root (36). The GRAS family members contain a number of characteristic features, including leucine heptad repeats and nuclear localization signals; they also contain LXXLL motifs (where L is a leucine residue and X any amino acid residue), which are necessary for the binding of transcriptional coactivators to nuclear receptors (34, 99). The nuclear localization signals of *Arabidopsis* RGA and GAI, and of rice GAI appear to be functional, since transiently expressed green fluorescent protein GFP-RGA or GFP-GAI (rice) fusion proteins localize in the nucleus of onion epidermal cells (67, 88); and in *Arabidopsis* plants transformed with *GAI::GFP* constructs, the GAI-GFP protein localizes to the nuclei of root and hypocotyl cells, as determined by confocal microscopy (B Fleck, personal communication).

Although the sequence comparisons and nuclear localization results are suggestive, the only direct evidence that the members of the GRAS family act as transcription factors is the report that the rice *GAI* homolog shows transactivation activity in a GAL4-dependent transactivation assay in spinach (67). As a further possible clue to the biochemical function of GAI/RGA/Rht/d8, visual analysis of the sequences of these proteins has led to the proposal that they contain an SH2-like domain and that they resemble the STAT proteins of metazoans and slime molds (17, 72, 83).

SPY: An *O*-GLcNAc Transferase Involved in GA Signaling

The first GA signaling component to be cloned was SPY, the product of the *SPINDLY (SPY)* gene of *Arabidopsis*. Recessive *spy* mutant alleles were isolated (44) in a screen where seeds were placed on medium containing paclobutrazol (PAC), an inhibitor of gibberellin biosynthesis. The concentration of PAC used prevented germination of wild-type seeds, but permitted germination of the PAC-resistant *spy* mutants. *spy* mutant plants resemble wild-type plants treated with exogenous GAs: Mutant plants have longer hypocotyls than wild-type, increased elongation of the main stem, light green leaves, and are early flowering. Addition of GAs to *spy* mutant seedlings caused a further increase in hypocotyl length, in a dose-dependent manner, indicating that *spy* mutants are not saturated in their responses to GAs (44). Double mutant studies showed that *spy* mutant alleles are partially epistatic to *ga1-2*, a gibberellin-deficiency mutation: *spy ga1-2* double mutant seeds germinated in the absence of exogenous GA and double mutant adult plants reached a height intermediate between that of wild-type and *ga1-2*. These observations suggest that the function of the SPY gene product is to act as a negative regulator of the GA signal transduction pathway.

SPY is a tetratricopeptide repeat (TPR) protein and exhibits extensive homology to *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) transferases (43, 84). The TPR is a 34-amino acid repeated sequence motif that may act in protein-protein interactions. *O*-GlcNAc transferases are enzymes that, in animals, play a role in signal transduction pathways in the dynamic modification of proteins, in a manner similar to protein phosphorylation (31, 32, 51). Recent data suggest that SPY is indeed an *O*-GlcNAc transferase: When the SPY protein is expressed using the baculovirus expression system, it shows *O*-GlcNAc transferase activity toward gp40, a tobacco nuclear pore protein known to be *O*-GlcNAc modified (97).

Double mutant analysis has suggested that *spy* mutant alleles are epistatic to *gai* (12, 43, 68). In these experiments *spy gai* double mutants are less severely dwarfed than the *gai* single mutant. Perhaps SPY acts upstream of GAI/RGA (68, 88) and affects their function by *O*-GlcNAc modification. One possibility, considering the functions that *O*-GlcNAc-transferases play in mammalian systems (31, 32), is that SPY influences the nuclear localization of GAI/RGA.

SLY1: A Positive Regulator of GA Signaling?

The effects of GA are often antagonized by the action of another plant hormone, abscisic acid (ABA). For example, ABA promotes the establishment of seed dormancy. GA opposes this effect of ABA, promoting the breaking of seed dormancy and triggering germination. As found for the GAs, some *Arabidopsis* mutants are ABA deficient, whereas others have altered ABA-responses. ABA-insensitive mutants have reduced seed dormancy, germinating at ABA concentrations that prevent germination of the wild-type plants (49). One such mutant, *ABI1-1*, was used as the genetic background for a screen to identify extragenic *ABI1-1* suppressors (90). Plants containing such suppressor mutations were expected to exhibit an enhanced ABA response. *ABI1-1* can germinate in the presence of 3 μM ABA, a concentration of ABA that suppresses the germination of wild-type seeds. This concentration of ABA was used to identify suppressed *ABI1-1* mutants: mutants that contained *ABI1-1*, but that were unable to germinate on 3 μM ABA (but able to germinate in the absence of exogenous ABA).

In the course of this screen, as expected, intragenic suppressors of *ABI1-1* were identified (as *ABI1-1* is a semidominant mutation, and these intragenic suppressors probably represent loss-of-function derivative alleles of *ABI1*). In addition, and again as expected, a number of mutations conferring GA-deficiency were identified, including several *ga1* alleles (GA-deficient mutants would not be expected to germinate in these conditions). However, unexpectedly, and most interestingly from the perspective of this review, this screen also identified a new class of mutant: dwarf, dark-green plants that could not be rescued by the addition of GAs (or brassinosteroids). The mutants resemble severely GA-deficient mutants, showing reduced male fertility, an increased number of buds per inflorescence, reduced apical dominance, and delayed senescence. The mutations conferring these phenotypes were all recessive, and they were found to fall into a single complementation group, the *SLEEPY1 (SLY1*) gene. It seems that the reduced dormancy of the *ABI1-1* background allows germination of these severely GA-insensitive mutants: *sly1* mutants fail to germinate in a wild-type background. The recessive nature of the *sly1* alleles suggests that they may be loss-of-function mutations, perhaps in a GA receptor or some other key positive GA response regulator. Answers to these questions await the cloning of the *SLEEPY* gene.

THE RICE *DWARF-1* MUTANT IMPLICATES THE α-SUBUNIT OF A GTP-BINDING PROTEIN IN GA SIGNALING

dwarf1 mutants of rice are dwarfed, have broad, dark green leaves, and make smaller than normal grains. These phenotypes cannot be reversed by GA treatments, suggesting that this mutant is defective in GA signaling. Furthermore, aleurone layers from *dwarf-1* grains produce no detectable α-amylase in response to concentrations of GA that activate α-amylase production in wild-type aleurones

(62; see below for further discussion of the cereal aleurone GA response). Recently, two groups (3, 23) reported the cloning of the *Dwarf1* gene. This gene encodes a protein displaying high homology with the α-subunit of heterotrimeric G-proteins. These proteins play a key role in signaling in animals (see below for further details). However, unlike in animals, this gene seems to exist as a single copy in the rice genome. One of the *dwarf-1* mutant alleles appears to be a complete loss-of-function allele, suggesting that, although the α-subunit encoded by *DWARF-1* is involved in GA signaling, it is not essential for it, since the *dwarf-1* mutant grows and is fertile (23).

OTHER *ARABIDOPSIS* GENES THAT MAY BE INVOLVED IN GA SIGNALING

In this section we discuss genes that may have a role in the GA signal transduction pathway, but which are either not cloned, or whose involvement in the GA pathway awaits further clarification.

The *GAR2* gene is currently represented by a single mutant allele, *gar2-1*, that was first identified as a dominant extragenic partial suppressor of the phenotype conferred by *gai* (103). When crossed into a wild-type background (recombined away from *gai*), *gar2-1* confers PAC-resistant seed germination, and a visible phenotype that is not obviously different from that of nonmutant controls (68, 73). As described above, *spy* mutant alleles also cause partial suppression of *gai* phenotype. The triple mutant *gai spy-7 gar2-1* homozygote displays complete suppression of the dwarf (*gai*) phenotype, increased PAC resistance, and earlier flowering than seen in wild-type controls, showing that *spy-7* and *gar2-1* act additively. The triple mutants are still sensitive to a reduction of GA concentration, and in the presence of PAC, they are sensitive to the addition of GA. The triple mutant *gai spy-7 gar2-1* can also reduce to normal the higher levels of 20-oxidase transcript found in *gai*, and reverse the increased content of GAs found in *gai*, so that the triple mutant contains levels of active GAs comparable to the wild type. The *gar2-1* mutation seems to modify the GA dose-response relationship in such a way that less GA is needed for a given effect. This fact, together with the increased PAC resistance of the mutant in a wild-type background, suggests that the *GAR2* gene acts as a negative regulator of GA responses. These observations suggest that the *gar2-1* mutation perturbs normal GA responses, and that the GAR2 gene product is involved in GA signaling.

shi (for short internodes) is a semidominant dwarfing mutation, isolated following two-component *Activator/Dissociation (Ac/Ds*) transposon-tagging mutagenesis (21). The phenotype of the mutant resembles that of weakly GA-deficient mutants: dwarfism, reduced apical dominance, narrow leaves that are darker green than normal, and late flowering in short days. The dwarfism of the *shi* mutant is due to reduced cell elongation in the bolting stem and cannot be reversed by the addition of GAs. The *shi* mutant is as late flowering in short days as is *gai*,

but with the addition of GAs the mutant flowered at the same time as wild type, although no effect on flowering time was seen in *gai*. As in *gai*, the levels of biologically active GAs are higher in the *shi* mutant than in wild-type controls. The *SHI* gene encodes a protein (SHI) that is likely to be a transcription factor: SHI contains a zinc-finger motif similar to the Zn_2Cys_6 cluster present in the DNA binding region of the yeast GAL4 transcriptional activator, two putative nuclear localization signals, and acidic and glutamine-rich stretches that are characteristic of transcriptional regulators. RNA gel-blot and RT-PCR analyses indicate that the *shi* phenotype can be attributed to overexpression of *SHI*, driven by the 35S promoter reading out of the transposon inserted in its promoter, leading to suggestions that SHI functions as a repressor of growth. However, *SHI* transcript is not detectable in *SHI* plants, so the *shi* phenotype could simply be due to ectopic expression of *SHI* or to a nonphysiologically high level of *SHI* transcripts. In the absence of loss-of-function mutant alleles, it is difficult to asses the role of SHI in GA signaling in normal plants.

Finally, the *pickle* mutant was obtained (65) in a screen for *Arabidopsis* mutants exhibiting abnormal root development, and further analysis of the *pickle* phenotype suggested that it may be involved in GA signaling. Recently, *pickle* has been cloned and found to be a CHD3 chromatin-remodeling factor conserved in eukaryotes (66). Further experiments are required to determine whether *pickle* is involved in the GA signaling pathway, and if so, what role it plays in it.

ANALYSIS OF DEVELOPMENTAL PROCESSES CONTROLLED BY GAs

GAs regulate many of the different processes that occur during the plant life cycle, from seed germination through fruit formation. In this section, we examine two processes that have received particular attention with respect to the controlling role of the GAs: the induction of hydrolytic enzymes in the cereal aleurone layer and the commitment of plants to flowering.

GAs Control the Production of α-Amylase by the Cereal Aleurone

During the germination of cereal grains, the aleurone layer (a layer of cells that surrounds the endosperm) secretes hydrolases (largely α-amylases) into the endosperm, thus releasing nutrients that feed the growing seedling. This process is controlled by GA. GA regulates α-amylase gene transcription and the secretion of α-amylase from the aleurone cells. This "cereal aleurone α-amylase response" has been the subject of intense investigation, resulting in many important advances in our understanding of GA signaling. Here, we review aspects of these studies that are particularly salient to the present discussion [for recent general reviews, see (4, 5)].

The addition of GAs to de-embryonated cereal grains, to isolated aleurone layers, or to aleurone protoplasts, stimulates the aleurone cells to produce enzymes, such as α-amylase, that are involved in the degradation of starch. Endogenous GAs are likely to be involved in the control of this process in intact plants, since some dwarf varieties of barley, which have reduced endogenous GA levels, also have reduced levels of α-amylase in the endosperm. Addition of exogenous GAs restores α-amylase activity to these varieties (107).

As mentioned above, studies using the cereal aleurone layer α-amylase response have indicated that GA is perceived by externally facing receptors located in the plasma membrane (27, 42). Following this initial GA perception event, there are several additional processes that are activated. There is an early increase of intracellular Ca^{2+} (11), and decrease of intracellular pH (35), followed by increases in the concentration of calmodulin (87) and cyclic GMP (74). After this, the transcription of a Myb-type protein GAmyb is activated, and this is followed by an increase in α-amylase activity (28). GAMyb may regulate the transcription of α-amylase, since in the absence of GA, the transient expression of GAMyb activates transcription of an α-amylase promoter fused to the reporter gene *GUS* (28). Protein phosphorylation also seems to be involved in the cereal aleurone α-amylase response, as the addition of okadaic acid, an inhibitor of protein phosphatases type 1 and 2A, prevented the GA-response of wheat aleurone layer cells (52).

How do these observations relate to the previous description of the genetics of GA signaling? Several of the recent discoveries described in previous sections establish a clear relationship between the α-amylase response and the GA-signaling components identified via the genetic approach. For example, both the wheat *Rht* mutants and the barley *sln* mutants are altered in their α-amylase responses: The *Rht* mutants have aleurone cells that are relatively insensitive to GA (24, 38), whereas *sln* mutant aleurones produce α-amylase constitutively and do not need GA to induce production (13, 53). These observations show that the GAI/RGA family of proteins are involved in the mediation of the cereal aleurone α-amylase response.

The cloning of the barley homologue of *Arabidopsis SPY* (*Hv-SPY*) allowed Robertson et al (84) to test whether the cereal *SPY* gene plays a role in the regulation of the α-amylase response. They co-bombarded aleurone layers with the barley high-pI α-amylase promoter-β glucoronidase (GUS) reporter gene construct, and the barley *SPY* gene in an overexpression effector construct. They showed that when the *SPY* overexpression effector construct was used, almost all of the increase in GUS activity seen in controls after the addition of GA disappeared. The results provide strong evidence that the *SPY* gene product is also a negative regulator of GA responses in cereal aleurones.

Several experiments have suggested that heterotrimeric G-proteins and the G-protein signaling pathway may be involved in mediating the GA regulation of cereal aleurone α-amylase production. The G-protein signaling pathway is well conserved among different species (91). These proteins transduce signals that arrive at extracellular receptors (G-protein-coupled receptors or GPCR), to downstream

signaling components. In plants [for reviews, see (40, 41, 58, 91)] the presence of G-proteins has been confirmed by the isolation of clones with homology to G-proteins. Concerning their possible role in GA signaling, indirect biochemical evidence has come from aleurone layer experiments (45). Adding GTP-γ-S, which mimics the activated state of G-proteins, slightly stimulated α-amylase expression, but the addition of GDP-β-S, which holds the α-subunit in its inactivated form, blocked completely the GA-induction of α-amylase. Further evidence comes from results obtained using a mastoparan analogue, Mas7. This compound stimulates GTP-GDP exchange by the heterotrimeric G-proteins, and is thought to mimic an activated G-protein-coupled receptor. When Mas7 was added to oat aleurone layers, it increased α-amylase secretion in a dose-dependent manner, and with a similar time course as adding GAs. ABA, which in the aleurone layer opposes the effects of GAs on α-amylase secretion, almost completely reverses the effect of Mas7. As mentioned by Fujisawa and co-workers (23), the interpretation of these results has to be tempered by the knowledge that Mas7 has effects on other signaling proteins, apart from G-proteins. However, the finding that the molecular defect in the *dwarf-1* mutant is in a protein with homology to the α-subunit of G-proteins (see above) suggests that G-proteins do indeed have a role to play in gibberellin signal transduction.

GAs Control Floral Initiation

The study of the role of GA in flowering is complicated because different species seem to respond differently to GA [for reviews see (57, 75)]. In *Arabidopsis*, a facultative long day plant, GAs have obvious effects on flowering, affecting both flowering time and flower morphology. The addition of exogenous GA makes the plants flower early, particularly in short days (54, 102). Mutants with reduced levels of endogenous GA, like *ga1-3* (see above), flower slightly later than wild type in long days (6, 89), and extremely late or not at all in short days (46, 89, 102). In long days, the mutant *ga1-3* is male sterile, with flowers that have poorly developed petals and stamens (50). All these flowering abnormalities seen in *ga1-3* can be restored to normal by the addition of exogenous GA (50, 54, 102). In addition, *spy* mutants, which behave as if the gibberellin signal transduction pathway is constitutively active, flower early (44). These observations show that GA have marked effects on flowering, but until recently, no details of the underlying molecular mechanisms were known.

Blázquez & Weigel (8) have argued that, because floral fate is specified by meristem-identity genes, the signals that regulate flowering must act through meristem-identity genes. One such meristem-identity gene is *LEAFY* (LFY) (7). In the wild-type plant, *LEAFY* is expressed in leaf primordia before the transition to flowering is made (7, 37).

The relation between GA and *LEAFY* expression has been studied in *Arabidopsis* by Weigel and colleagues. Application of GA to *Arabidopsis* in short days makes the plants flower early (54), and this effect is paralleled by an increase in

LEAFY promoter activity (7). As seen above, the exogenous application of GA makes *ga1-3* flower early, and again this effect is accompanied by an increase in *LEAFY* promoter activity (7). Further studies with a *LFY* promoter–*GUS* construct (*LFY::GUS*) (6) introduced into the *ga1-3* mutant showed that in long days, the initial levels of *LFY::GUS* were reduced, the up-regulation of *LFY::GUS* was delayed, and the maximum level of *LFY::GUS* was reduced, when compared to the wild type. The exogenous application of GA returned to normal both the flowering defect and *LFY::GUS* expression. In short days, where *ga1-3* plants flower extremely late, or never flower (46, 89, 102), the expression of *LFY::GUS* was undetectable. Also, the effects on *LFY::GUS* expression seen in *ga1-3* could be reproduced if wild-type plants carrying the *LFY::GUS* construct were treated with PAC, an inhibitor of gibberellin biosynthesis. Overexpression of *LFY* in *ga1-3* plants (6) restored the capacity of this mutant to flower in short days. However, the mutant still flowered later than the wild-type transformed with the same construct. Taken together, these results suggest that GA regulates the activity of the *LFY* promoter and also the competence to respond to *LFY* activity (6).

Recently, a study of deletions of the *LFY* promoter fused to the *GUS* reporter gene has been published (8). A minimum promoter, GOF9, behaved with the same temporal pattern as the full *LFY* promoter, with fast up-regulation in long days, and a slower, gradual increase in short days; and this slow increase was enhanced by the addition of GA. Mutation of an 8-base pair sequence in this construct (GOF9m) had very little effect on the activity of the LFY promoter in long days, but the promoter remained inactive in short days, and this lack of activity could not be overcome by the addition of GA. This 8-base pair sequence is potentially a GA response element, and has a sequence that agrees with the consensus binding site for MYB transcription factors of animals (8). This is reminiscent of the well-studied effect of GA in inducing expression of the α-amylase gene in the cereal aleurone layer, where a MYB protein also seems to be implicated (see above). Perhaps this suggests a general mechanism by which GAs exert their effects.

CONCLUSIONS

The past few years have seen major advances in our understanding of the signaling mechanisms by which the GAs control the growth of plants. Although the GA receptor has not been isolated, it is thought to be associated with the plasma membrane. Pharmacological and mutant data suggest that the early stages of the signaling process may involve G-protein-coupled receptors and/or α-subunits of G-proteins. Genetic analysis in a variety of plant species has highlighted the importance of the GAI/RGA family of nuclear proteins in the mediation of the GA response, and it may be that these proteins are responsible for transmitting the signal from cytoplasm to nucleus. In addition, the *SPY* gene appears to encode an *O*-GlcNAc activity that modulates GA signaling. Finally, the involvement of Myb-type transcription factors has been implicated in two downstream GA-responses

(the cereal α-amylase response and the initiation of flowering), suggesting that Myb-like proteins may be the point at which GA signaling diverges from the general pathway that is mediated by GAI/RGA and SPY to the specific branches that mediate specific responses.

Perhaps the most important consequence of all this exciting work has been to bring closer together the results of genetic studies with the wealth of biochemical and pharmacological information obtained from studies using the cereal aleurone layer. Although we are still far from a complete knowledge of GA signal transduction, these findings have opened many new avenues of research that will bring us nearer to that objective.

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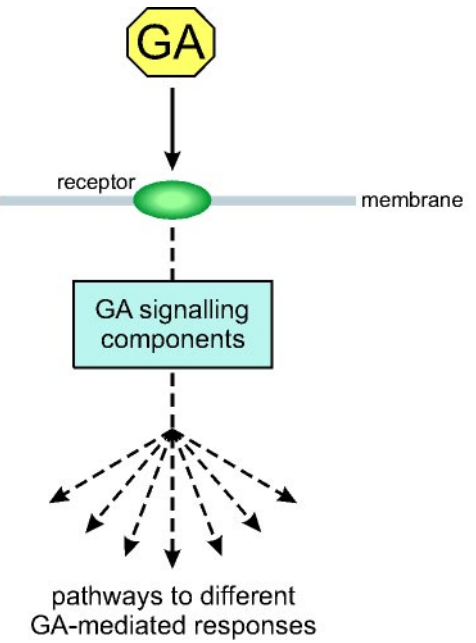

Figure 1 A generalized model for the pathway from GA perception, via a membrane-bound receptor, to the spectrum of GA-mediated plant growth responses.

Figure 2 *Arabidopsis* wild-type (*left*) and the *gai* mutant (*right*).

Figure 3 Representation of the GAI protein. The C terminus of GAI (the 'C' region) has homology to SCR. The N terminus (the 'N' region) is not related to SCR, and contains a region of 17 amino acids (the DELLA domain), which is missing in the *gai* mutant.

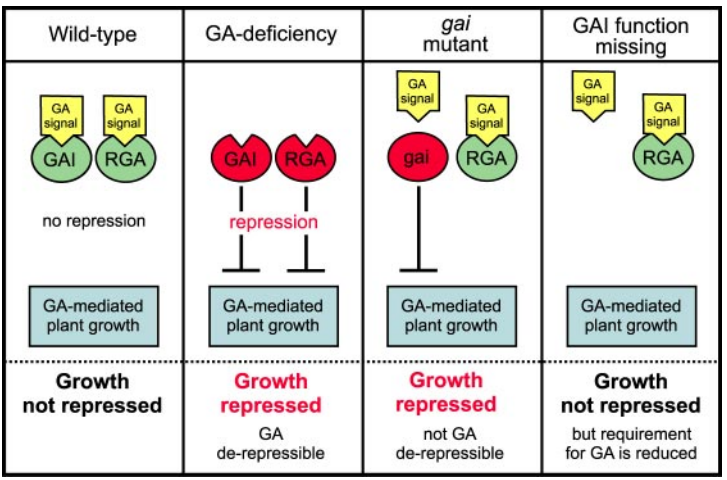

Figure 4 Derepression model for the regulation of plant growth responses by GA. The GAI and RGA proteins repress GA-mediated plant growth responses, and GA (via a signaling intermediate) derepresses growth by opposing this activity. The *gai* mutant no longer recognizes the GA signal and so constitutively represses growth. When the GAI repressor function is lost but RGA is active, GA is still required for derepression of growth, but the requirement is reduced. This model is an oversimplification for several reasons. First, it ignores the quantitative nature of GA responses. In addition, GAI and RGA do not have identical roles in GA signaling, although their functions overlap substantially. Finally, the representation of the interaction between GAI and RGA and the GA signal shown here is not meant to imply the nature of the interaction, as this may be transient or longer term.