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Gibberellin Metabolism and Signaling

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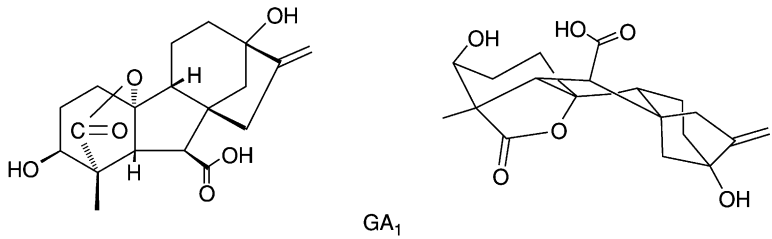
Gibberellins (GAs) are a family of plant hormones controlling many aspects of plant growth and development including stem elongation, germination, and the transition from vegetative growth to flowering. Cloning of the genes encoding GA biosynthetic and inactivating enzymes has led to numerous insights into the developmental regulation of GA hormone accumulation that is subject to both positive and negative feedback regulation. Genetic and biochemical analysis of GA-signaling genes has revealed that posttranslational regulation of DELLA protein accumulation is a key control point in GA response. The highly conserved DELLA proteins are a family of negative regulators of GA signaling that appear subject to GA-stimulated degradation through the ubiquitin-26S proteasome pathway. This review discusses the regulation of GA hormone accumulation and signaling in the context of its role in plant growth and development. © 2005 Elsevier Inc.

I. INTRODUCTION

A. HISTORICAL PERSPECTIVE

Unlike mammals, plants have evolved to be very plastic in their development. Every plant cell is ostensibly a ''stem cell'' capable of giving rise to a wide array of developmental fates in response to signals from plant hormones, also referred to as phytohormones. Also, unlike mammals, plants do not have clearly defined source and target organs for hormone signals.



GA_1

FIGURE 1. Example of the molecular structure of a gibberellin, GA_1, presented in 2D and 3D view. One-hundred and thirty-six naturally occurring GAs have been found in plants and fungi so far.

This has complicated the study of plant hormones. Numerous advances have been made in understanding the regulation of plant hormone accumulation, transport, and signaling through genetic, biochemical, and physiological approaches. This review is focused on the plant hormone gibberellin.

Gibberellins are a large family of tetracyclic diterpene plant hormones characterized by the *ent*-gibberellane ring system (Fig. 1). Gibberellins have been shown to promote many facets of plant growth and development including germination and stem elongation, and in most species transition to flowering, pollen tube elongation, and seed development (Olszewski *et al.*, 2002; Sun and Gubler, 2004). Every hormone signal transduction pathway is composed of two essential components, the control of hormone accumulation and reception of the hormone signal. This chapter will: (1) briefly review the history of GA research and the role of GA in regulating plant growth and development; (2) review the control of GA hormone accumulation through gene regulation; (3) review GA signal reception in the context of its role in plant growth and development; and (4) review the interaction of GA signaling with other hormone-signaling pathways.

Gibberellins were the first plant hormone identified (Phinney, 1983; Tamura, 1991). Ironically, the discovery of gibberellin by the Japanese scientist Eiichi Kurosawa in 1926 was based on its synthesis by the fungus *Gibberella fujikuroi*, the causative agent of *bakanae* disease in rice. The "foolish seedlings" infected by *bakanae* disease grew excessively tall and spindly. The rare infected seedlings that survived produced poor seed set. Kurosawa demonstrated that the fungal pathogen infecting these plants synthesized a chemical that could stimulate shoot elongation in rice and other grasses (Kurosawa, 1926). The structure of this chemical, gibberellin A_3 or GA_3, was proposed in 1956 and revised in 1961. The occurrence of gibberellins in higher plant species was discovered in the mid-1950s. This discovery marked the beginning of research on the role of GA in plant growth and development.

Since their discovery, over 136 GAs have been identified in plants and fungi; however, only a small fraction of these are biologically active in plants (Olszewski *et al.*, 2002). Each unique GA has a number ranging from GA_1 to GA_{136}. Gibberellins are divided into two classes based on the number of carbon atoms, C20-GAs and C19-GAs, in which C20 has been replaced by a gamma-lactone ring. The synthesis of bioactive GAs is essentially a three-step process involving: (1) the formation of *ent*-kaurene in the proplastid, (2) the formation of $GA_{12/53}$ in the ER, and (3) the formation of active GA in the cytoplasm by successive oxidation steps. In most plant species, GA_1 or GA_4 are the bioactive GA. GA_1 and GA_4 are formed by similar pathways differing only in early 13-hydroxylation in the case of GA_1.

B. GIBBERELLINS AND PLANT DEVELOPMENT

The role of GA in plant growth and development has been elucidated through the physiological characterization of GA biosynthesis and signaling mutants and the characterization of GA-responsive genes. This section deals with the role of GA in seed development and germination, plant growth and elongation, flowering, and meristem cell identity.

1. GA in Seed Development and Germination

Our understanding of GA in seed development and germination is based on mutants or tissues with reduced accumulation of GAs (Bentsink and Koornneef, 2002; Ni and Bradford, 1993; Singh *et al.*, 2002). For example, the *ga1*, *ga2*, and *ga3* mutants of *Arabidopsis* were isolated in an elegant screen for GA-dependent germination by Koornneef and van der Veen (1980). These mutants cause marked reduction in endogenous GA and are unable to germinate unless GA is applied externally. While seeds are an excellent source of GA, the failure to synthesize GA in these mutants does not completely block seed development (Bentsink and Koornneef, 2002). Thus, it was originally thought that GA is not required for seed development. However, physiological characterization of *Arabidopsis* plants constitutively expressing the GA catalytic enzyme *GAox2* revealed that reduced accumulation of GA in seed leads to increased probability of seed abortion (Singh *et al.*, 2002). This suggests that GA is actually required in seed development. Moreover, reduced GA accumulation leads to reduced seed set by interfering with pollen tube elongation and silique expansion (Singh *et al.*, 2002; Swain *et al.*, 2004). How does GA stimulate germination? Germination and seedling growth require the production of hydrolytic enzymes to weaken the seed coat, mobilize seed nutrient storage reserves, stimulate plant embryo expansion and hypocotyl elongation, and activate the embryo meristem to produce new shoots and roots (Bewley and Black, 1994). Gibberellin has been implicated in all of these processes.

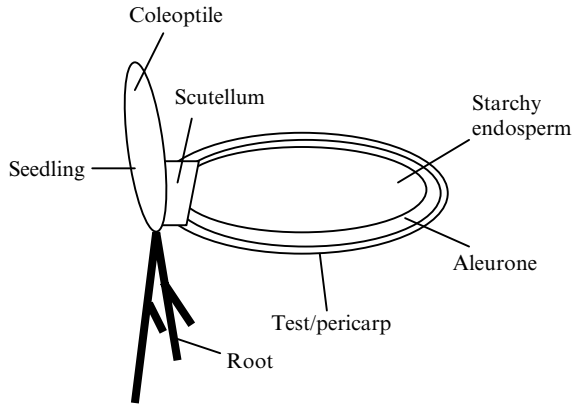


FIGURE 2. Schematic of a germinating cereal grain. Gibberellin produced in the germinating embryo stimulates production of α-amylase and other hydrolytic enzymes in the aleurone layer. These enzymes break down starch in the endosperm providing nutrition for the emerging seedling.

The germination process is considered complete when any part of the plant embryo emerges from the seed (Bewley and Black, 1994). Initial studies in tomato and muskmelon suggested that the decision to germinate results from the balance between the internal pressure of an expanding embryo and the external restraint of the endosperm cap or seed coat (Groot and Karssen, 1987; Ni and Bradford, 1993). Gibberellin-induced hydrolytic enzymes such as endo-[β]-mannase are apparently needed to weaken the endosperm cap in these species (Still and Bradford, 1997).

Gibberellin stimulation of seed nutrient storage mobilization is best illustrated by the cereal aleurone system (Jacobsen *et al.*, 1995). Gibberellin synthesized by the plant embryo stimulates secretion of the hydrolytic enzymes including α-amylase by the aleurone layer. Aleurone-derived hydrolases diffuse to the adjacent endosperm where they degrade starch for use by the embryo (Fig. 2). Because the aleurone layer itself secretes no GA, it can be isolated and used to assay α-amylase secretion in response to hormone (Bush and Jones, 1988; Varner *et al.*, 1965). α-Amylase is arguably the best characterized GA-responsive gene. Measurement of α-amylase enzyme activity and mRNA accumulation has been used to identify GA-responsive promoter elements and transcription factors (Sun and Gubler, 2004).

2. Gibberellin Stimulation of Growth and Elongation

Gibberellin stimulation of plant stem elongation was the basis for the hormone's discovery and remains a reliable assay for GA response. Research suggests that GA stimulates stem elongation through stimulation of cell elongation and cell division (Huttly and Phillips, 1995). Gibberellin treatment

causes microtubules to reorientate so as to encourage axial elongation (Shibaoka, 1994). It is thought that GA promotes cell elongation by induction of enzymes that promote cell wall loosening and expansion such as xyloglucan endotransglycosylase/hydrolase (XET or XTH), expansins, and pectin methylesterase (PME). Xyloglucan endotransglycosylases split cell wall xyloglucan polymers endolytically and then rejoin the free ends with another xyloglucan chain (Campbell and Braam, 1999). Xyloglucan endotransglycosylase activity has been associated with expanding regions and shown to be GA-induced in *Arabidopsis*, lettuce, and pea (Kauschmann *et al.*, *et al.*, 2003; Potter and Fry, 1993). Expansins disrupt hydrogen bonding in the cell wall and appear to be GA-induced in *Arabidopsis* and rice (Cosgrove, 2000; Lee and Kende, 2001; Ogawa *et al.*, 2003). Pectin methylesterase is thought to induce stem elongation by loosening the cell wall via pectin modification and is GA-induced in *Arabidopsis* (Ogawa *et al.*, 2003). Gibberellin was first shown to stimulate growth through induction of the cell cycle in rapidly growing deepwater rice (Sauter *et al.*, 1995). In rice, GA induces expression of the cyclin *cycA1;1* and the cyclin-dependent kinase *cdc2Os-3* in the G2/M phase transition (Fabian *et al.*, 2000). Microarray analysis in *Arabidopsis* has demonstrated GA induction of genes involved in the G1/S transition including *cyclinD*, *MCM*, and *replication protein A* (Ogawa *et al.*, 2003). Further research on the mechanism of GA induction of these genes and their exact mode of action is needed.

3. Gibberellins and Flowering

In most species, the transition to floral development is stimulated by gibberellins (Sun and Gubler, 2004). However, gibberellins are not the sole factor in determining transition to flowering. In *Arabidopsis*, a facilitative long-day (LD) plant, transition to flowering is controlled by the integration of signals from the GA pathway, the autonomous pathway, the vernalization pathway, and the light-dependent pathway (Komeda, 2004). It is clear that gibberellins are required for transition to flowering in short days (SD, 8-h light) because the strong GA biosynthesis mutant *ga1-3* cannot transition to flowering without application of GA under these conditions (Wilson *et al.*, 1992). The failure of *ga1-3* to flower under SD appears to be due to reduced expression of the *LEAFY* (*LFY*) gene (Blazquez *et al.*, 1998). The fact that the *ga1-3* mutant causes poor development of floral organs including petals and stamen shows that GA is also involved in the stimulation of floral development. Gibberellin has also been shown to induce expression of floral homeotic genes *APETELA3*, *PISTILLATA*, and *AGAMOUS* (Yu *et al.*, 2004).

Studies on *Lolium temulentum* have suggested that GA is an inducer of flowering or "florigen" in LD-responsive grasses (King and Evans, 2003). In *Lolium*, GA_1, GA_3, and GA_4 are more active for stem elongation, whereas

GA_5 and GA_6 are more active in triggering transition to flowering. It has been proposed that GA_5 and GA_6 are more active in the floral meristem because they have greater resistance to the expression of the GA catabolic enzyme GA2ox early in floral induction.

4. Gibberellin in Shoot Apical Meristem Development

Studies in *Arabidopsis* have indicated an emerging role for GA in shoot apical meristem (SAM) cell identity (Hay *et al.*, 2002, 2004). The SAM is a reservoir of undifferentiated cells that gives rise to the aerial leaves and stems of higher plants. Knotted-like homeobox (KNOX) transcription factors appear to control meristem versus leaf cell identity. The KNOX gene *SHOOTMERISTEMLESS* (*STM*) has been shown to prevent expression of the GA biosynthesis gene *GA20ox1* in the SAM (see Section II). The fact that ectopic GA signaling is detrimental to meristem maintenance suggests that GA signaling is antagonistic to meristem cell identity and may be involved in the transition from meristem to leaf cell fate.

II. GIBBERELLIN BIOSYNTHESIS

A. INTRODUCTION

In the last 50 years our understanding of GA metabolism has been advanced by using a variety of experimental systems, including most notably the characterization of GA metabolic enzymes and the reactions they catalyze using cell-free systems derived from immature seeds of *Cucurbita maxima* (pumpkin), *Pisum sativum* (pea), and *Phaseolus vulgaris* (bean) (Graebe, 1987). In several cases, expression of these enzymes in these immature seeds has served as a basis for cloning of their respective genes. Over the last two decades, *Arabidopsis* has become an experimental system of choice for studying GA metabolism. The power of *Arabidopsis* molecular genetic analyses was illustrated when the GA biosynthesis mutants *ga1*, *ga2*, *ga3*, *ga4*, and *ga5* served as a basis for cloning several of the biosynthetic genes (Koornneef and van der Veen, 1980). The characterizations of these genes are rapidly uncovering the complex regulatory mechanisms controlling GA metabolism. In addition, the *Arabidopsis* and rice genome sequences, together with convenient transformation procedures, have greatly improved our understanding of GA metabolism and the role of these phytohormones in regulating plant growth and development. The following sections describing GA metabolism will focus on advancements, including the discovery and regulation of GA biosynthetic and catabolic genes. The primary aim of this section is to review the steps in GA metabolism that are exclusive to this class of compounds.

B. INVOLVEMENT OF MVA AND MEP PATHWAYS IN GIBBERELLIN BIOSYNTHESIS

There are some excellent reviews that comprehensively describe the earlier steps in terpenoid biosynthesis (Goodwin, 1965; Rodriguez-Concepcion and Boronat, 2002; Sponsel, 2001). Although we will not discuss these steps in detail, it is necessary to mention some important findings that are relevant to GA biosynthesis.

Geranylgeranyl diphosphate (GGPP) is the precursor isoprenoid necessary for the synthesis of many terpenoid compounds, including GAs. The initial step in isoprenoid biosynthesis is the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). For many years, it was believed that the IPP destined for isoprenoid biosynthesis in plants was synthesized exclusively via the mevalonic acid (MVA) pathway. The incorporation of ^{14}C MVA into *ent*-kaurene in cell-free systems provided some initial support for this pathway in the biosynthesis of GAs. It is now known that another route for IPP biosynthesis, the plastidic methylerythritol 4-phosphate (MEP) pathway, exists in plants (Rodriguez-Concepcion and Boronat, 2002). Kasahara *et al.* (2002), has directly addressed the contribution of the MEP and MVA pathways to GA biosynthesis. Using ^{13}C feeding studies of *Arabidopsis* plants blocked in either of these pathways, they demonstrated that the MEP pathway has a predominant role in the biosynthesis of GAs, but it appears that the MVA pathway also contributes under certain conditions. Further studies are necessary to uncover the regulation of these two pathways controlling the production of IPP destined for isoprenoid biosynthesis.

C. *ENT*-COPALYL-DIPHOSPHATE SYNTHASE

The first committed step of GA biosynthesis is the cyclization of GGPP producing *ent*-copalyl diphosphate (CPP) (Fig. 3). In plants, this reaction is catalyzed by *ent*-copalyl-diphosphate synthase (CPS), a diterpene cyclase. The potential of *Arabidopsis* genetic analyses to identify genes encoding GA biosynthetic enzymes was illustrated when Sun and coworkers elegantly cloned the *GA1* gene using genomic subtraction and demonstrated that it encodes a functional CPS enzyme, AtCPS (Sun and Kamiya, 1994; Sun *et al.*, 1992). The authors subsequently provided evidence that AtCPS is localized in the plastids as a processed form (Sun and Kamiya, 1994). This is consistent with biochemical studies demonstrating CPS activity in the proplastids of several plant species (Aach *et al.*, 1995; Simcox *et al.*, 1975). Based on sequence homologies, there appears to be a single gene encoding a CPS enzyme in *Arabidopsis* (Hedden and Phillips, 2000); although it is interesting to note that *AtCPS* null mutants have detectable levels of GAs (Silverstone *et al.*, 2001; Zeevaart and Talon, 1992). This supports the existence of

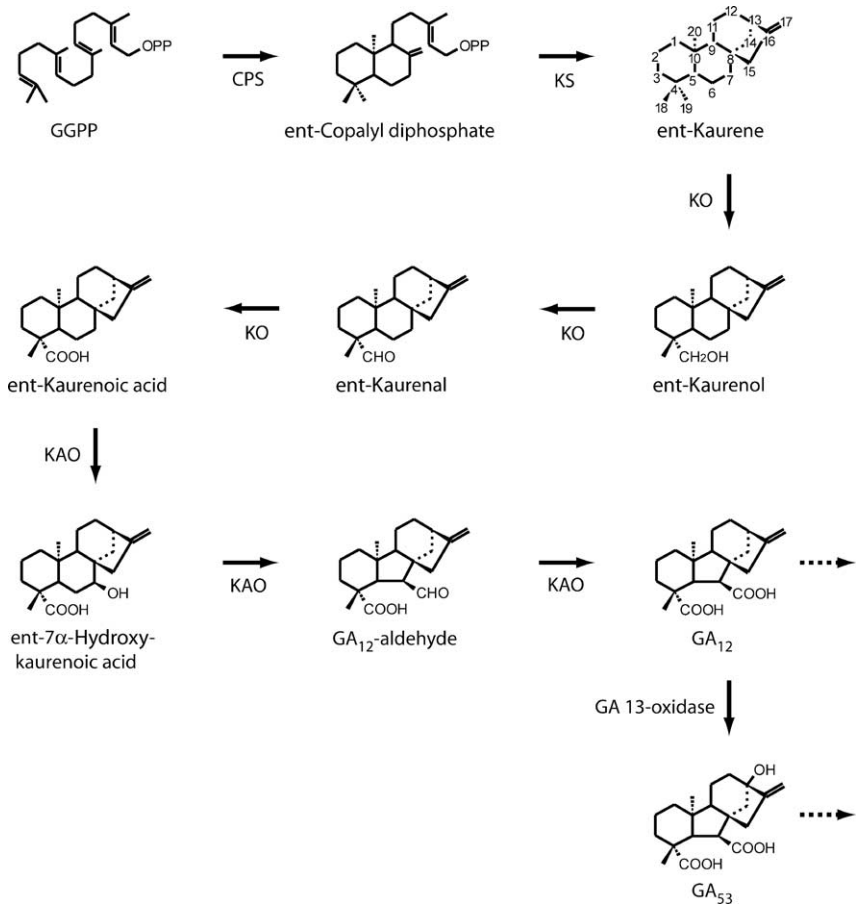


FIGURE 3. Early GA biosynthetic pathway showing conversions from geranylgeranyl diphosphate (GGPP) to GA_{12} and GA_{53}. Numbering of the C-atoms is shown for *ent*-kaurene.

another pathway capable of producing CPP or *ent*-kaurene. A study suggests that rice also contains a single gene encoding a CPS enzyme, *OsCPS1* (Sakamoto *et al.*, 2004). Null alleles of *OsCPS1* produce plants with a severe GA-deficient dwarf character reminiscent of the *Arabidopsis ga1* loss-of-function mutants.

The identity of the CPS-encoding genes has allowed the characterization of their spatial and temporal expression patterns with a view to determine the precise cellular sites of GA biosynthesis. In *Arabidopsis*, *AtCPS* demonstrates highly specific developmental and cell-specific expression patterns. Highest levels of promoter activity are localized to actively growing regions, consistent with GAs having a growth promoting role (Silverstone *et al.*, 1997b).

Interestingly, *AtCPS* expression is also observed in vascular tissue of expanded leaves, suggesting that these may be a source for GAs to be transported to a responsive tissue. Subsequent deletion analysis of the *AtCPS* promoter has identified *cis*-regulatory elements necessary for their tissue-specific expression (Chang and Sun, 2002).

D. *ent*-KAURENE SYNTHASE

The formation of *ent*-kaurene from CPP is catalyzed by another diterpene cyclase, *ent*-kaurene synthase (KS). This enzyme catalyzes the cyclization reaction needed to produce the characteristic tetracyclic backbone of GAs (Figs. 3 and 4). A gene encoding KS was first isolated from pumpkin (Yamaguchi *et al.*, 1996). The presence of high levels of KS activity in the developing cotyledons of immature pumpkin seeds allowed purification of the enzyme to homogeneity and amino acid sequencing. A cDNA clone was subsequently identified using a degenerate PCR strategy and demonstrated to encode a functional KS enzyme. This work led to the isolation of a gene encoding a KS from *Arabidopsis* (*AtKS*). The authors demonstrated that the *GA2* locus encodes AtKS (Yamaguchi *et al.*, 1998a).

Biochemical studies suggest that the KS enzymes are localized in proplastids (Aach *et al.*, 1995, 1997). This is supported by the presence of a putative N-terminal transit peptide in both CmKS and AtKS likely to direct targeting to the plastid (Yamaguchi *et al.*, 1996, 1998a). Furthermore, fusion of the first 100 amino acids of AtKS to GFP (TPKS-GFP) demonstrated plastid localization in transiently transformed tobacco epidermal cells (Helliwell *et al.*, 2001b). In the same study, the TPKS-GFP fusion protein was imported into isolated pea chloroplasts. The potential co-localization of CPS and KS raises the possibility that they may form a plastidic complex involved in *ent*-kaurene production.

The *Arabidopsis* genome appears to contain a single *AtKS* gene (Hedden and Phillips, 2000). This is consistent with the severity of the loss-of-function *ga2-1* allele that closely resembles the extreme dwarf *ga1* null mutants (Koornneef and van der Veen, 1980; Yamaguchi *et al.*, 1998a). Interestingly, there are differences in the expression profiles of *AtCPS* and *AtKS*, with *AtCPS* demonstrating a more localized pattern (Silverstone *et al.*, 1997b; Yamaguchi *et al.*, 1998a). In addition, differences in the expression profiles of *CmCPS1/2* and *CmKS* genes were also observed in pumpkin (Smith *et al.*, 1998; Yamaguchi *et al.*, 1996). It is conceivable that the more localized expression pattern of the *CPS* genes is indicative of the CPS enzymes catalyzing the rate-limiting step in the production of *ent*-kaurene. This is supported by studies showing that transgenic *Arabidopsis* plants over-expressing AtCPS have elevated *ent*-kaurene levels, whereas plants over-expressing AtKS have wild-type levels (Fleet *et al.*, 2003).

E. *ENT*-KAURENE OXIDASE

The biosynthesis of $GA_{12/53}$ from *ent*-kaurene is catalyzed by cytochrome-P450-dependent monooxygenase enzymes. The first of these steps is catalyzed by *ent*-kaurene oxidase (KO), a multifunctional enzyme that catalyzes the successive oxidation at the C-19 position (Fig. 3), producing *ent*-kaurenoic acid (KA) (Helliwell *et al.*, 1999; Swain *et al.*, 1997). Studies by Helliwell *et al.* (1998, 1999, 2001a) have proved instrumental in improving our understanding of the cytochrome-P450 monooxygenases involved in GA biosynthesis. This work initially involved the confirmation that the *ga3* mutants were deficient in KO activity. They subsequently confirmed that the *GA3* locus encoded a cytochrome-P450 monooxygenase that was capable of converting *ent*-kaurene to *ent*-kaurenoic acid when it was heterologously expressed in yeast (Helliwell *et al.*, 1998, 1999). This gene was designated as *AtKO* and appears to be present as a single copy in the *Arabidopsis* genome. RNAse protection analysis of *AtKO* gene expression demonstrated developmental regulation, with highest levels of transcripts in young seedlings, elongating stems, and inflorescences (Helliwell *et al.*, 1998). Gibberellin treatment did not affect the levels of *AtKO* mRNA.

Rice contains five KO-like genes (OsKOL1–5) that are arranged in tandem as a cluster of genes on chromosome 6 (Itoh *et al.*, 2004; Sakamoto *et al.*, 2004). One of these genes, OsKOL2, has been shown to correspond to the *D35* loci. Null mutations at *D35* produce a severe GA-deficient phenotype that is probably blocked at the GA biosynthetic step of *ent*-kaurene oxidation (Itoh *et al.*, 2004; Ogawa *et al.*, 1996). A weak allele of *D35*, $d35^{Tan\text{-}Ginbozu}$, produces a rice plant with a semidwarf character (Itoh *et al.*, 2004). The introduction of this allele in the 1950s, producing the Tan-Ginbozu cultivar, led to dramatic increases in rice crop yields. This is one of many examples where mutations affecting GA biosynthesis or response have been instrumental in producing crops with improved agronomic traits.

The cytochrome-P450-dependent monooxygenases involved in GA biosynthesis have generally been considered as being localized to the endoplasmic reticulum (ER). This is based on studies showing that the enzymatic activity co-purifies with a microsomal fraction (Graebe, 1979). The availability of the *AtKO* gene has provided the opportunity to investigate the localization of these enzymes using more sensitive cell biology-based approaches. Interestingly, Helliwell *et al.* (2001b) found that an AtKO-GFP fusion protein was localized to the outer plastid membrane of transiently transformed tobacco epidermal cells. They have hypothesized that AtKO provides a link between the plastid and ER located steps of the GA biosynthetic pathway (Helliwell *et al.*, 2001b). In a somewhat conflicting study by Yamaguchi and coworkers, aimed at understanding the localization of enzymes involved in the biosynthesis of GAs in germinating *Arabidopsis* seeds, it was found that *AtCPS* and *AtKO* display distinctly different cell-specific

expression patterns (Yamaguchi *et al.*, 2001). Based on these studies, they proposed that intercellular transport of GA intermediates, possibly *ent*-kaurene, is occurring between the provasculature and the cortex/endodermis. Further studies aimed at detecting the localization of the endogenous proteins will be necessary to establish the precise subcellular distribution of these enzymes.

Repression of shoot growth (RSG) was identified, rather fortuitously, in a screen designed to isolate *trans*-acting factors that bind to an auxin-responsive *cis*-regulatory element in tobacco (Fukazawa *et al.*, 2000). It was demonstrated that RSG did not bind the auxin-responsive element but instead bound to the *AtKO* promoter *in vitro*. Furthermore, expression of a dominant-negative form of RSG in transgenic tobacco produced a GA-responsive dwarf phenotype with lower levels of bioactive GAs and reduced expression of the *AtKO* homologue. Studies have shown that GA signaling promotes RSG disappearance from the nucleus through its binding to a cytoplasmic 14-3-3 protein (Igarashi *et al.*, 2001; Ishida *et al.*, 2004). The interaction of RSG with the 14-3-3 protein appears to be dependent on phosphorylation of a serine residue. The authors propose a model in which RSG is negatively regulated by GAs and has a role in the maintenance of GA levels (Ishida *et al.*, 2004). Further studies are necessary to confirm whether RSG is a direct regulator of GA biosynthesis.

F. *ENT*-KAURENOIC ACID OXIDASE

The conversion of *ent*-kaurenoic acid to GA_{12} is catalyzed by another cytochrome-P450 monooxygenase, *ent*-kaurenoic acid oxidase (KAO). The multifunctional KAO enzyme oxidizes the C-7 of *ent*-kaurenoic acid to produce *ent*-7α-hydroxy-kaurenoic acid, which is then oxidized by this enzyme on C-6 to form GA_{12}-aldehyde. Finally, KAO oxidizes GA_{12}-aldehyde on C-7 to produce GA_{12} (Fig. 3). Gibberellin-deficient mutants blocked at this step in the biosynthetic pathway have not been identified in *Arabidopsis*. In contrast, the barley *grd5* and pea *na* mutants, both of which display a GA-responsive dwarf character, demonstrate reduced KAO activity (Helliwell *et al.*, 2001a; Ingram and Reid, 1987). The maize *dwarf3* (*d3*) mutants have a similar GA-deficient phenotype. Although the precise GA biosynthetic step blocked in the *d3* mutants was unknown, the identity of the *D3* gene proved instrumental in the identification of a *KAO* gene. The *D3* gene was cloned using a transposon tagging strategy and demonstrated to encode a cytochrome-P450–dependent monooxygenase (Winkler and Helentjaris, 1995) belonging to the CYP88A subfamily (Helliwell *et al.*, 2001a). Helliwell and coworkers isolated a *Grd5* cDNA clone based on its homology to *D3* and confirmed that it encoded a cytochrome-P450 monooxygenase, also belonging to the CYP88A subfamily (Helliwell *et al.*, 2001a). Furthermore, they identified two *Arabidopsis* genes encoding CYP88A enzymes. Using a

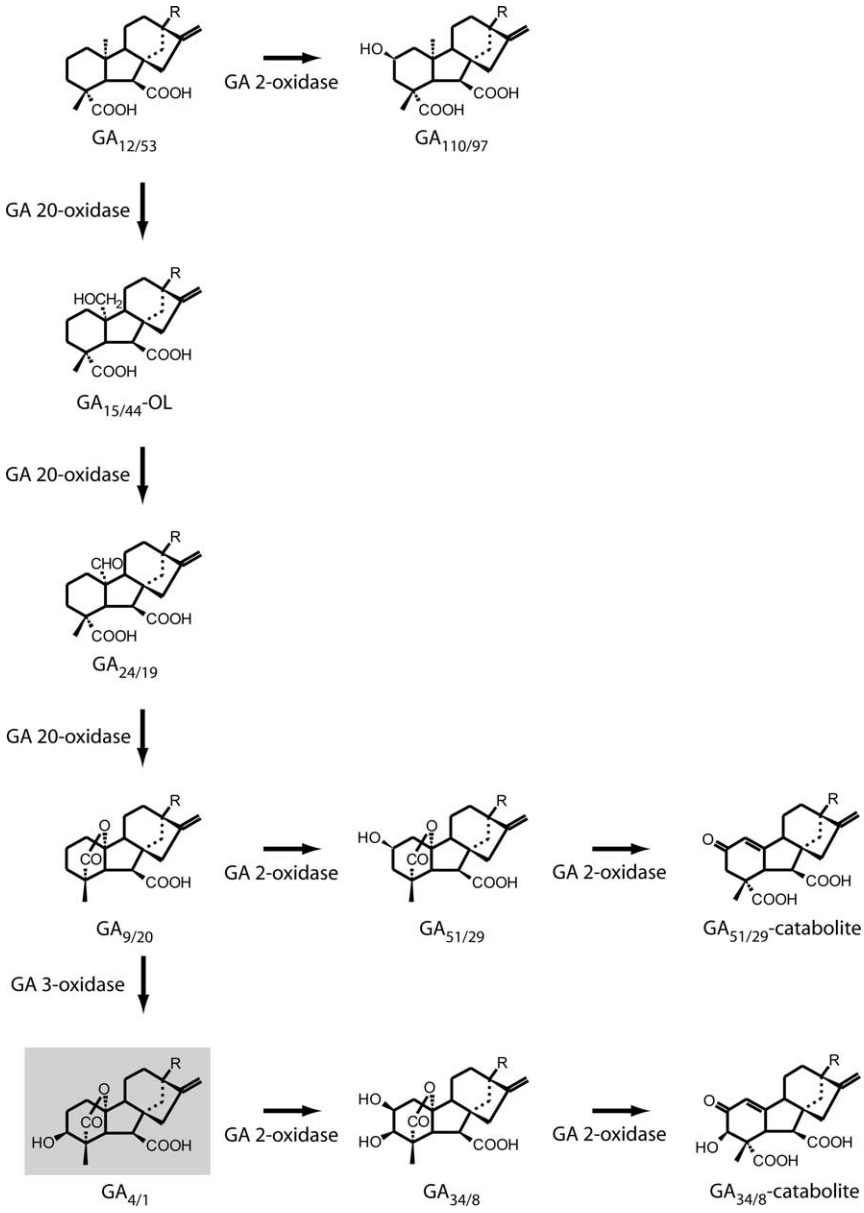


FIGURE 4. Late GA biosynthetic and catabolic pathways. The bioactive GAs, GA_4 and GA_1, are synthesized from GA_{12} and GA_{53}, respectively. Subscripted numbers before the slash indicate the non-13-hydroxylated GA (R=H) and after the slash indicate the 13-hydroxylated equivalent (R=OH).

yeast heterologous expression system developed for testing the functionality of *AtKO*, it was confirmed that the barley and the two *Arabidopsis* CYP88A enzymes catalyzed the three steps of GA biosynthesis from KA to GA_{12} (Helliwell *et al.*, 2001a). They were subsequently designated as *ent*-kaurenoic acid oxidases. It is likely that other CYP88A enzymes, including *D3*, encode KAO enzymes. Interestingly, a novel gene encoding a 2-oxoglutarate–dependent dioxygenase (2-ODD) enzyme (GA 7-oxidase), which catalyzes the single-step conversion of GA_{12}-aldehyde to GA_{12}, was identified from pumpkin (Lange, 1997). The significance of this class of enzymes is unknown as they have not been identified in other plant species.

The presence of two *AtKAO* genes is in contrast to those encoding earlier steps in the GA biosynthetic pathway. It is likely that this functional redundancy explains why no *Arabidopsis* mutants blocked at this step have been identified. Although the AtKAO genes exhibit similar expression patterns (Helliwell *et al.*, 2001a), characterization of knockout mutants is needed to determine whether they have specific roles in regulating plant development. In pea, there are two KAO genes, *PsKAO1* and *PsKAO2*, that do appear to have distinct developmental roles (Davidson *et al.*, 2003). The pea *NA* gene encodes PsKAO1, and *na* mutants exhibit severe GA-deficient phenotypes but normal seed development. These characteristics are potentially explained by the differential expression pattern of the *PsKAO* genes; *PsKAO1* is expressed ubiquitously in the plant whereas *PsKAO2* is only expressed in the developing seeds (Davidson *et al.*, 2003). The role of *PsKAO2* in seed development remains to be resolved.

In some plants, including *Thlaspi arvense*, GAs have an important role in mediating vernalization (or cold)-induced bolting and flowering (Metzger and Dusbabek, 1991). It has been proposed that thermoinduction stimulates GA biosynthesis and the resulting GA accumulation promotes stem elongation. In *Thlaspi*, the site of perception of cold is the shoot apex (Hazebroek and Metzger, 1990) where the levels of KA were dramatically reduced following vernalization (Hazebroek *et al.*, 1993). This suggests that KAO is the primary step in GA metabolism regulated by vernalization in this species. The identification and characterization of GA biosynthetic genes in *T. arvense* should help to determine how vernalization regulates GA metabolism.

G. GIBBERELLIN 13β-HYDROXYLASE

In many plants, including most monocots and pea, GA_1 is the predominant bioactive GA, illustrating the importance of 13-hydroxylation in the biosynthetic pathway. At present, the exact point in GA biosynthesis at which 13-hydroxylation occurs is still not entirely clear. Gibberellin-feeding experiments in pea suggest that this reaction occurs early in the pathway, with both GA_{12} and GA_{12}-aldehyde proving to be good substrates (Kamiya

and Graebe, 1983). Biochemical studies suggest that this class of enzymes is predominantly cytochrome-P450–dependent monooxygenases (Grosselindemann *et al.*, 1992; Hedden *et al.*, 1984; Kamiya and Graebe, 1983), although a soluble enzyme activity was detected in cell-free extracts from spinach leaves (Gilmour *et al.*, 1986). It is tempting to speculate that GA_{12} is the endogenous substrate as this is produced by other microsomal located cytochrome-P450 monooxygenases. There are no characterized GA 13-hydroxylase mutants, and a gene encoding this enzyme has not been identified in plants. A better understanding of the 13-hydroxylase enzymes awaits the cloning of these elusive genes.

H. GIBBERELLIN 20-OXIDASE

The final steps in the metabolism of bioactive GAs are catalyzed by 2-oxoglutarate–dependent dioxygenases (Hedden and Phillips, 2000). These enzymes are believed to be soluble and cytoplasmic. The GA 20-oxidase catalyzes the penultimate step in the biosynthesis of bioactive GAs, a stage that involves the oxidation of C-20 to an aldehyde followed by the removal of this C atom and the formation of a lactone (Hedden and Phillips, 2000). Some plants contain a GA 20-oxidase enzyme with different properties. For example, in spinach a GA_{44}-oxidase activity that converts the lactone, rather than the free alcohol form of this GA, has been identified (Gilmour *et al.*, 1986; Ward *et al.*, 1997).

Over the last decade, our understanding of GA 20-oxidation has improved dramatically since Lange and coworkers identified the first GA 20-oxidase gene (Lange *et al.*, 1994). Their strategy aimed at cloning this gene involved purifying a GA 20-oxidase enzyme from immature pumpkin seeds, a tissue extremely rich in GA metabolic enzymes (Lange, 1994). Antibodies raised against a peptide sequence contained within the purified GA 20-oxidase were subsequently used to isolate a corresponding cDNA clone by expression screening (Lange, 1994). The recombinant pumpkin GA 20-oxidase (CmGA20ox1) expressed from this cDNA clone was confirmed as a multifunctional enzyme capable of converting GA_{12} to GA_9 (Lange *et al.*, 1994). Surprisingly, the predominant reaction catalyzed by Cm20ox1 was the complete oxidation of the carbon-20 to the carboxylic acid, rather than its loss. The C-20 tricarboxylic acid GAs produced by Cm20ox1 are essentially biologically inactive. This raises the question: what functional role does it play in the development of pumpkin seeds?

The identity of *Cm20ox1* led directly to the isolation of three GA 20-oxidase genes from *Arabidopsis* and the confirmation that one of these corresponds to the *GA5* locus, *AtGA20ox1* (Phillips *et al.*, 1995; Xu *et al.*, 1995). In *Arabidopsis*, it is now apparent that there are five putative GA 20-oxidase genes (Hedden *et al.*, 2001). Three of these genes, *AtGA20ox1*, *2*, and *3*, have been confirmed to encode functional enzymes that predominantly

metabolize GA_{12} to GA_9 (Phillips *et al.*, 1995; Xu *et al.*, 1995). In rice, the recessive *semidwarf1* (*sd1*) mutations have been instrumental in producing higher yielding dwarf varieties that are more resistant to environmental damage (Hedden, 2003). Studies from three independent labs have demonstrated that the *SD1* locus encodes a GA 20-oxidase, OsGA20ox2 (Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeyer *et al.*, 2002). On the basis of the rice genome sequence, it appears there are four GA 20-oxidase genes in rice (Sakamoto *et al.*, 2004). Further work is necessary to confirm the functional role of the three other putative OsGA20ox genes.

The importance of GA 20-oxidation as a key regulatory step within the biosynthetic pathway of most plants is demonstrated by the finding that the GA 20-oxidase catalyzes a rate-limiting step. This is clearly illustrated in *Arabidopsis*, where GA_{24} and GA_{19} have been shown to accumulate in stems (Coles *et al.*, 1999; Talon *et al.*, 1990a). Furthermore, it was demonstrated that GA 20-oxidase over-expression in transgenic *Arabidopsis* leads to elevated levels of bioactive GAs and a corresponding GA overdose phenotype compared to wild-type plants (Coles *et al.*, 1999; Huang *et al.*, 1998). In contrast, over-expression of enzymes catalyzing earlier steps in the GA biosynthetic pathway do not have this effect (Fleet *et al.*, 2003). It is, therefore, likely that GA 20-oxidase activity provides an important step in the regulation of bioactive GA levels and the subsequent developmental programs these control. This is supported by the observations that transcript levels of GA 20-oxidase genes demonstrate tight spatial and developmental regulation.

The presence of GA 20-oxidase multigene families in higher plants raises the possibility that certain members have roles in regulating specific developmental programs. This hypothesis is supported by studies that show distinct spatial and development expression profiles for individual genes (Carrera *et al.*, 1999; Garcia-Martinez *et al.*, 1997; Phillips *et al.*, 1995; Rebers *et al.*, 1999). For example, in *Arabidopsis*, *AtGA20ox2* is expressed predominantly in flowers and siliques, whereas *AtGA20ox3* expression is exclusively found in siliques (Phillips *et al.*, 1995). In contrast, *AtGA20ox1* is expressed predominantly in the stem, providing a possible explanation for the semidwarf character of the *ga5* mutant (Phillips *et al.*, 1995; Xu *et al.*, 1995). The identification of loss-of-function mutations in other GA 20-oxidase genes should help to uncover specific roles for these family members.

The *KNOX* genes are involved in maintenance of the meristem (Hake *et al.*, 2004). There is evidence to suggest that KNOX proteins achieve this, in part, by controlling GA levels through the regulation of their biosynthesis. Tanaka-Ueguchi and coworkers demonstrated that over-expression of the *NTH15* KNOX gene in tobacco produced a GA-responsive dwarf phenotype caused, in part, by the reduced expression of a GA 20-oxidase gene, *Ntc12* (Tanaka-Ueguchi *et al.*, 1998). They propose that NTH15 directly represses *Ntc12* to maintain the indeterminate state of cells in the SAM.

At the periphery of the meristem, *NTH15* expression is suppressed, allowing GA 20-oxidase expression and subsequent determination of cell fate. Similarly, in *Arabidopsis* the KNOX gene *STM* is involved in repressing *AtGA20ox1* expression in the meristem (Hay *et al.*, 2002).

I. GIBBERELLIN 3-OXIDASE

Growth active GAs are hydroxylated at the C-3β position (Fig. 4). The 2-ODD enzyme responsible for this modification is a GA 3-oxidase (Hedden and Phillips, 2000). The *Arabidopsis ga4* mutants are GA-responsive semidwarf plants (Chiang *et al.*, 1995; Koornneef and van der Veen, 1980) that contain reduced levels of 3β-hydroxy GAs, together with increased levels of GA_{19}, GA_{20}, and GA_9. These observations suggested that *GA4* may encode a 3β-hydroxylase (Talon *et al.*, 1990a). This was subsequently confirmed when the *GA4* locus was identified by T-DNA tagging (Chiang *et al.*, 1995) and the recombinant GA4 enzyme demonstrated to convert GA_9 to GA_4 (Williams *et al.*, 1998). To prevent confusion, the *GA4* gene has been renamed *AtGA3ox1*, following the nomenclature suggested by Coles *et al.* (1999).

Gregor Mendel's pioneering experiments using garden peas to investigate the transmission of hereditary elements are widely accepted as the foundation of genetics. In these studies he followed seven pairs of traits, including stem length (Le) (Mendel, 1865). The *le* mutations are recessive and produce GA-responsive dwarf plants (Brian and Hemming, 1955; Mendel, 1865). Analysis of endogenous GA levels in the *le* mutant (Potts *et al.*, 1982) and the finding that these plants were unable to convert GA_{20} to GA_1 (Ingram *et al.*, 1984) suggested that Le is involved in GA biosynthesis at the step of 3β-hydroxylation. The identity of *AtGA3ox1* directly led to the isolation and characterization of the *Le* gene by two independent groups (Lester *et al.*, 1997; Martin *et al.*, 1997). Both of these groups confirmed that *Le* encodes a functional 3β-hydroxylase, whereas the *le* mutant form exhibited reduced activity, when expressed in *Escherichia coli*. The reduction in activity was associated with an alanine to threonine substitution in the predicted amino acid sequence of the enzyme near its proposed active site.

In addition to *Arabidopsis* and pea, other plant species containing mutations that affect GA biosynthesis at the 3β-hydroxylation step have been identified (Fujioka *et al.*, 1988a; Ross, 1994). In general, all of the GA 3-oxidase loss-of-function mutants have a semidwarf phenotype, in contrast to the severe dwarf phenotype of GA auxotrophs blocked at earlier steps in the pathway. The most likely explanation for this observation is the functional redundancy of GA 3-oxidase genes. For example, there are at least four GA 3-oxidase genes in *Arabidopsis*, whereas rice contains two genes (Phillips and Hedden, 2000, Sakamoto *et al.*, 2004). There is also evidence to indicate that different GA 3-oxidase genes have specific roles regulating plant

development. For example, two of the *Arabidopsis* GA 3-oxidase genes, *AtGA3ox1* and *AtGA3ox2* (formerly *GA4H*), display differential spatial and temporal expression patterns (Yamaguchi *et al.*, 1998b). *AtGA3ox1* was expressed in all growing tissues tested, whereas *AtGA3ox2* was predominantly expressed in germinating seeds and young seedlings but not in other tissues (Yamaguchi *et al.*, 1998b). Similarly, *OsGA3ox2* expression was detected in all aerial portions of the rice plant; in contrast, *OsGA3ox1* was exclusively expressed in floral tissue (Kaneko *et al.*, 2003). A more detailed analysis of *AtGA3ox1* and *AtGA3ox2* mRNA transcripts in germinating seeds found that both were predominantly expressed in the cortex and endodermis of the embryo axes (Yamaguchi *et al.*, 2001). These observations suggest that GA production occurs in GA-responsive cells.

Considering the importance of the GA 3-oxidases in producing bioactive GAs, it is not surprising that expression of the respective genes is tightly regulated, by both developmental and environmental stimuli. Work is underway to uncover the complex environmental regulation of these genes, most notably with respect to germination of *Arabidopsis* seeds (Ogawa *et al.*, 2003; Yamaguchi *et al.*, 1998b, 2001; Yamauchi *et al.*, 2004). These studies have demonstrated that expression of *AtGA3ox1* is regulated by light, bioactive GAs, and temperature (regulation by light and GAs will be discussed later). The treatment of imbibed *Arabidopsis* seeds to low temperatures (stratification) is known to promote germination. Stratification has also been implicated in increasing the GA levels (Derkx *et al.*, 1994). These observations suggest that cold treatment promotes germination of *Arabidopsis* seeds by stimulating GA biosynthesis. Studies have clearly demonstrated that stratification produces an increase in the levels of *AtGA3ox1* transcripts, which is directly responsible for the increase in bioactive GA_4 levels that promote germination (Yamauchi *et al.*, 2004). These elegant studies provide a benchmark for future studies investigating the regulation of GA metabolism. They demonstrate the potential for integration of genomics, genetic analysis, and biochemical studies to improve our understanding of the role of GAs in regulating plant development.

J. GIBBERELLIN 2-OXIDASE AND GIBBERELLIN INACTIVATION

The amount of bioactive GAs is determined by both the rate of GA biosynthesis and inactivation. Inactivation can be achieved by glucosyl conjugation or by 2β-hydroxylation, the relative contributions of the two pathways being unknown (Schneider and Schliemann, 1994). A clear physiological role of GA conjugation, however, has not been shown, whereas the importance of 2β-hydroxylation in regulating bioactive GA content is well established. Gibberellin 2β-hydroxylase activity is abundant in seeds during the later stages of maturation, particularly in legume seeds that accumulate

large amounts of 2β-hydroxylated GAs (Albone *et al.*, 1984; Durley *et al.*, 1971; Frydman *et al.*, 1974). Indeed, GA_8, the first 2β-hydroxy GA to be identified was extracted from seeds of runner bean (*Phaseolus coccineus*) (MacMillan *et al.*, 1962). In certain species, including legumes, further metabolism of 2β-hydroxy GAs occurs to form the so-called catabolites, in which C-2 is oxidized to a ketone and the lactone is opened with the formation of a double bond between C-10 and an adjacent C atom (Albone *et al.*, 1984; Sponsel and MacMillan, 1980). Biochemical characterization of the proteins responsible for 2β-hydroxylation showed they belong to the soluble 2-oxoglutarate–dependent dioxygenases (Griggs *et al.*, 1991).

A gene encoding for GA 2-oxidase was first identified in runner bean by screening an embryo-cDNA expression library for 2β-hydroxylase activity (Thomas *et al.*, 1999) and studies using a similar approach with seed-cDNA libraries led to the identification of two GA 2-oxidase genes from pea (*P. sativum* L.; Lester *et al.*, 1999; Martin *et al.*, 1999). Five *Arabidopsis* GA 2-oxidase genes have since been identified based on sequence homology and their identity has been confirmed by activity assays (Hedden and Phillips, 2000; Thomas *et al.*, 1999; Wang *et al.*, 2004). Two more *Arabidopsis* proteins capable of GA 2β-hydroxylation were identified using an activation tagging screen for dwarf mutants (Schomburg *et al.*, 2003). Interestingly, these two proteins, AtGA2ox7 and AtGA2ox8, are more related to GA 20-oxidases than to the other GA 2-oxidases. Evidence that all these proteins function in GA inactivation *in vivo* comes from experiments in which over-expression in *Arabidopsis* resulted in dwarfed plants (Schomburg *et al.*, 2003; Thomas, Phillips, and Hedden, 2000, unpublished data; Wang *et al.*, 2004). Similar results have been obtained with GA 2-oxidases from poplar and rice (Busov *et al.*, 2003; Sakamoto *et al.*, 2001).

Detailed characterization of the enzymatic activities of GA 2-oxidases from various plants has shown that they can convert a range of GAs. Most of the enzymes tested show activity towards the bioactive $GA_{1/4}$ and their non-3-hydroxylated precursors $GA_{20/9}$, although there are differences in the preferred substrate (e.g., Lester *et al.*, 1999; Thomas *et al.*, 1999). A subset of the enzymes is capable of further oxidation to a ketone at C-2. AtGA2ox7 and AtGA2ox8 are somewhat exceptional, in that they are specific for C-20 GAs (Schomburg *et al.*, 2003).

Because of the highly similar activities of the various GA 2-oxidases, any functional diversity between the family members may be expected to lie in differential expression patterns. Support for this comes from studies in pea, where *PsGA2ox1* is highly expressed in maturing seed and *PsGA2ox2* preferentially in the shoot (Lester *et al.*, 1999; Martin *et al.*, 1999). This differentiation may partly explain the strong block in the conversion of GA_{20} to GA_{29} observed in seed of the *sln* mutant that carries a point mutation in *PsGA2ox1* (Lester *et al.*, 1999; Martin *et al.*, 1999; Ross *et al.*, 1995). The elongated shoot phenotype of this mutant is due to enhanced elongation

of the first internodes only and appears to arise from transport of GA_{20} from the seed into the young shoot after germination (Reid *et al.*, 1992; Ross *et al.*, 1993).

A very specific expression pattern has been reported for *OsGA2ox1* in rice (Sakamoto *et al.*, 2001). mRNA from this gene was observed in a ring around the vegetative shoot apical meristem, at the bases of the youngest leaf primordia. After phase transition to the inflorescence stage, however, expression was drastically reduced. This prompted the authors to speculate on a role of GA 2-oxidases in floral transition, a hypothesis further elaborated by King and Evans (2003) to account for the effects of various applied GAs on floral transition in *L. temulentum*. However, this interesting hypothesis still awaits testing using knockout mutants.

Due to their rather recent discovery, little is known about the regulation of the GA 2-oxidase genes. Using a chromatin immunoprecipitation approach, Wang *et al.* (2002) isolated a portion of the *AtGA2ox6* promoter. They convincingly showed that *AtGA2ox6* is a direct target of AGL15 and is transcriptionally activated during embryogenesis (Wang *et al.*, 2004). The function of *AtGA2ox6* expression during embryogenesis is not yet fully clear, but it seems to contribute to seed dormancy.

K. FEEDBACK AND FEEDFORWARD REGULATION OF GIBBERELLIN METABOLISM

In plants, a homeostatic regulatory mechanism exists whereby biologically active GAs control their own levels through the processes of feedback and feedforward regulation of GA metabolism. Evidence for this level of regulation originally came from studies in which GA levels were compared between GA-response mutants and the respective wild-type controls (Hedden and Croker, 1992). The GA-insensitive dwarf *rht3* and *d8* mutants in wheat and maize, respectively, were found to contain highly elevated levels of the bioactive GA_1, whereas the levels of GA_{19} were lower, compared to wild-type seedlings (Appleford and Lenton, 1991; Fujioka *et al.*, 1988b). Similar observations were made in the GA-insensitive *gai-1* mutant in *Arabidopsis* (Talon *et al.*, 1990b). These studies suggested that GA 20-oxidation was increased in these GA-response mutants and hence under feedback control. Hedden and Croker subsequently demonstrated that the maize *d1* mutant, which is defective in 3-oxidation, has high levels of GA_{20} but reduced levels of GA_{53} and GA_{19} compared to wild-type plants (Hedden and Croker, 1992). The subsequent application of bioactive GA to *d1* restored the levels of these GAs close to those of wild-type plants, providing strong support for feedback regulation of GA 20-oxidation in maize.

The identity of genes encoding GA biosynthetic enzymes has provided further clues to the control of GA metabolism by feedback and feedforward regulation. It was found that the *Arabidopsis ga4-1* mutant accumulated high

levels of the *ga4* transcripts compared to wild-type plants (Chiang *et al.*, 1995). Treating the *ga4-1* plants with GA dramatically reduced the *ga4* transcript levels, indicating that the expression of *AtGA3ox1* is under feedback control by bioactive GAs. Further evidence for the GA 20-oxidation step being under feedback control was also provided by the demonstration that the expression of *AtGA20ox1*, *AtGA20ox2*, and *AtGA20ox3* genes were reduced by exogenous applications of GA (Phillips *et al.*, 1995; Xu *et al.*, 1995). It is now apparent that feedback regulation of most GA 20-oxidase and GA 3-oxidase genes is conserved in higher plants. Although, it is interesting to note that the expression of *AtGA3ox2* is apparently not under feedback control (Yamaguchi *et al.*, 1998b). Currently, there is no evidence to suggest that earlier steps in the GA biosynthetic pathway are controlled by feedback regulation. In contrast to the GA-induced downregulation of GA biosynthetic 2-ODD genes, the expression of the inactivating *Arabidopsis* GA 2-oxidase genes, *AtGA2ox1* and *AtGA2ox2*, is upregulated by GA treatment of *ga1-2* plants (Thomas *et al.*, 1999). A similar effect on the expression of *PsGA2ox1* and *PsGA2ox2* genes was observed in pea (Elliott *et al.*, 2001). In this case, levels of the *PsGA2ox1/2* transcripts were elevated in the WT background compared to the *ls* and *na* mutants. Interestingly, in this study there was no evidence of feedforward regulation based on the endogenous levels of 2β-hydroxy GAs. The authors suggest that other uncharacterized 2-oxidase activities could account for this anomaly. These two studies suggest that bioactive GAs regulate their own levels by adjusting inactivation through a feedforward controlling mechanism. It will be necessary to confirm the biological significance of feedforward regulation. The isolation of GA 2-oxidase loss-of-function mutants in *Arabidopsis* should provide help in these studies.

The studies showing that feedback regulation is perturbed in GA-insensitive response mutants support a direct role for the GA-response pathway in controlling this process. In *Arabidopsis*, the GA-induced decrease in expression of *AtGA3ox1* is currently one of the earliest markers of GA-responsive gene expression (Ogawa *et al.*, 2003; Thomas and Sun, unpublished). Changes in *AtGA3ox1* and *AtGA20ox1* expression levels are observed only 30 min after treating the *ga1-3* mutant with bioactive GAs (Thomas and Sun, unpublished). It is not clear whether these are primary responses to GA signaling because studies using cycloheximide demonstrate that protein synthesis is necessary for GA-mediated feedback regulation of *AtGA20ox1* expression (Bouquin *et al.*, 2001).

L. REGULATION OF GA METABOLISM BY LIGHT

The intrinsic ability of plants to respond to their environmental conditions is clearly essential for them to survive and reproduce. Light quantity, quality, and photoperiod are certainly the most important of these factors,

and it is therefore not surprising that they regulate all aspects of plant growth and development. The role of light in controlling plant developmental processes has been studied in great detail. It has emerged that in some of these cases, light exerts its effect by causing changes in the concentration and/or sensitivity to GAs (Kamiya and Garcia-Martinez, 1999; Olszewski *et al.*, 2002; Yamaguchi and Kamiya, 2000). The most well-characterized examples that we will discuss further include seed germination, de-etiolation, photoperiodic control of flowering, and tuberization in potato.

The germination of *Arabidopsis* seeds has an absolute requirement for both GAs and red light. There is strong evidence to suggest that the response of seeds to light is mediated by an increase in GA biosynthesis (Yamaguchi and Kamiya, 2000). In addition, red light was shown to increase the sensitivity of the seed to the concentration of GA required for germination (Hilhorst and Karssen, 1988). Studies of germination in *Arabidopsis* (Yamaguchi *et al.*, 1998b) and lettuce (Toyomasu *et al.*, 1993, 1998) have provided strong evidence that phytochrome upregulates GA biosynthesis by promoting GA 3-oxidation. In *Arabidopsis*, red light was demonstrated to upregulate the expression of both *AtGA3ox1* and *AtGA3ox2* within 1 h of treatment (Yamaguchi *et al.*, 1998b). Interestingly, the red light induction of these two genes appears to be mediated by different phytochrome (PHY) light receptors.

Bioactive GAs are required for establishing etiolated growth and repressing photomorphogenesis. This was illustrated in a study demonstrating that reductions in *Arabidopsis* GA levels partially derepress photomorphogenesis in the dark (Alabadi *et al.*, 2004). Similarly, in pea, the GA-deficient mutant *na* exhibited a dramatic photomorphogenic phenotype when grown in the dark.

Upon exposure to light, dark-grown seedlings demonstrate a dramatic change in phenotype that is known as de-etiolation. These changes include a significant reduction in stem elongation, which coincides with decreased levels of bioactive GAs in peas (Ait-Ali *et al.*, 1999; O'Neill *et al.*, 2000; Reid *et al.*, 2002). A study by Reid and coworkers demonstrated that this reduction mediated by red and blue light is most likely caused by a rapid (within 30 min) downregulation in the expression levels of *PsGA3ox1* and by an upregulation of *PsGA2ox1* (Reid *et al.*, 2002). They went on to confirm that the light-induced reduction in GA levels is mediated through phytochrome A and a blue light receptor. After the initial decline in GA_1 levels following 8-h exposure of light, there is a subsequent increase over the next 16 h, which results in plants that have similar GA_1 concentrations to those grown exclusively in the dark (O'Neill *et al.*, 2000; Reid *et al.*, 2002). There is a direct correlation between the recovery in GA_1 levels and increases in the expression levels of *PsGA20ox1* and *PsGA3ox1*, presumably due to feedback regulation of GA biosynthesis (Reid *et al.*, 2002). The continued inhibition of stem elongation by light appears to be attributed to reduced

responsiveness to GA in the light-grown plants compared to those grown in the dark (O'Neill *et al.*, 2000; Reid, 1988).

Tuberization of *Solanum tuberosum* (potato) occurs when the plants are exposed to a SD photoperiod (Jackson, 1999). There is strong evidence to suggest that the photoperiodic control of tuberization is mediated, in part, by GAs. More specifically, GAs appear to inhibit tuberization in long days (LD). This is illustrated by the observation that exogenous applications of GAs can inhibit or delay tuberization under inductive SD photoperiods (Jackson and Prat, 1996). In contrast, reducing the levels of GAs promotes tuberization under noninducing LD (Jackson and Prat, 1996; Vandenberg *et al.*, 1995). Furthermore, endogenous levels of GA_1 were reduced in stolons and leaves of plants induced to tuberize compared to those grown under noninductive conditions (Xu *et al.*, 1998). It has been demonstrated that the potato leaves are the principal site of photoperiod perception (Ewing and Wareing, 1978). A role for PHYB in inhibiting tuberization in LD has been suggested by the findings that transgenic potato plants with reduced PHYB levels will tuberize in both SD and LD (Jackson and Prat, 1996). The identification of potato genes encoding GA biosynthetic enzymes has provided important tools to help understand the role of GAs in the photoperiodic control of tuberization. Using a degenerate PCR-based approach, Carrera and coworkers cloned three GA 20-oxidase genes from potato that displayed differential tissue-specific expression profiles (Carrera *et al.*, 1999). One of these genes, *StGA20ox1*, was expressed at relatively high levels in leaves and exhibited photoperiodic regulation of transcript levels (Carrera *et al.*, 1999, 2000; Jackson *et al.*, 2000). The photoperiodic transcriptional regulation of *StGA20ox1* appears to be controlled by PHYB, along with an unidentified blue light receptor (Jackson *et al.*, 2000). The role of *StGA20ox1* in tuberization was investigated by producing transgenic potato plants expressing sense or antisense copies of this gene (Carrera *et al.*, 2000). Although the over-expression of *StGA20ox1* did not prevent tuberization under SD, it did result in plants that required a longer duration of SD photoperiod to tuberize compared to control plants. Conversely, the *StGA20ox1* antisense lines tuberized earlier than the controls and showed increased tuber yields. This study supports a role for StGA20ox1 in tuberization, although it indicates that other factors are also necessary for LD inhibition of this process.

In *Arabidopsis* and spinach (*Spinacia oleracea*), *GA20ox* genes are also subject to transcriptional regulation by LD photoperiods (Wu *et al.*, 1996; Xu *et al.*, 1997). Expression of *AtGA20ox1* is enhanced by exposure to LDs that promotes rapid stem elongation and flowering. Spinach has an absolute requirement for LD photoperiods to initiate bolting and flowering. The increase in GA levels, which is a necessary requirement for LD-induced bolting in spinach, is directly attributable to increased transcription of the *SoGA20ox1* gene (Lee and Zeevaart, 2002; Wu *et al.*, 1996). It was also found

that expression of *SoGA2ox1* was repressed by LDs (Lee and Zeevaart, 2002). This suggests that the LD-induced increases in bioactive GA levels may also be maintained by a reduction in the rate of their inactivation.

III. GIBBERELLIN SIGNAL TRANSDUCTION

Much has been learned about GA-signal transduction using a combination of genetic, physiological, and biochemical analyses. Regulatory elements of the GA-signal transduction pathway have been identified using: (1) screens for mutants with altered GA sensitivity, (2) identification of transcriptional regulators of the GA-responsive genes, and (3) methods for identifying differentially expressed genes. Such approaches have recovered both positive and negative regulators of GA response that have been the subject of several reviews (Jacobsen *et al.*, 1995; Olszewski *et al.*, 2002; Sun and Gubler, 2004).

Mutant analysis is often used to determine the role of a gene in a signaling pathway. The hallmark of a GA-insensitive mutant is that it shares all or a subset of the phenotypes of a GA biosynthesis mutant, but cannot be rescued by hormone application. This failure to be rescued by GA indicates that plants are unable to perceive the GA signal. Gibberellin-insensitive mutants may show poor germination or increased seed dormancy, growth as a dark green dwarf, delayed flowering, and reduced fertility. Conversely, mutants with a constitutive GA response have phenotypes expected in plants subject to a GA overdose, such as increased plant height and internode length, slender stems, parthenocarpy, and a reduced requirement for GA in germination. Table I summarizes the GA-response genes identified to date.

A. DELLA PROTEINS IN GIBBERELLIN SIGNALING

The current model of GA signaling is centered on the control of DELLA protein accumulation (see model in Fig. 5A). DELLA proteins are negative regulators of GA response subject to GA-stimulated disappearance (Itoh *et al.*, 2003). Loss of DELLA gene function results in a recessive constitutive GA-response phenotype. Such mutants can be tall and slender with a reduced requirement for GA in stem elongation and transition to flowering. Gain-of-function mutations in DELLA genes have the opposite effect resulting in a semidominant GA-insensitive semidwarf phenotype and increased sensitivity to GA biosynthesis inhibitors (Dill *et al.*, 2001; Peng *et al.*, 1997). The DELLA proteins in a number of species have been shown to disappear following GA application including: (1) *Oryza sativa SLENDER RICE1* (rice *OsSLR1*; Itoh *et al.*, 2002); (2) *Hordeum vulgare SLENDER1* (barley *HvSLN1*; Gubler *et al.*, 2002); and (3) *Arabidopsis thaliana REPRESSOR OF ga1-3* (*RGA*; Silverstone

TABLE I. GA Signaling Genes

Gene	Isolated in	Phenotypes	Encodes
Positive regulators			
D1	Rice	GA-insensitive dwarf	α-Subunit of heterotrimeric G-protein
GAMYB	Barley, rice	Activator of α-amylase	Myb transcription factor
GID1	Rice	Recessive GA-insensitive dwarf	Serine hydrolase
GID2	Rice	GA-insensitive dwarf, poor fertility, overproduces SLR1 protein	F-box protein, homologous to SLY1
GSE1	Barley	Recessive GA-insensitive dwarf, SLN1 protein overproduced	Unknown
PHOR1	Potato	Antisense gives a GA-insensitve dwarf, over-expression gives increased internode length	U-box protein with Armadillo repeats, a potential component of an E3 Ub ligase
PKL	*Arabidopsis*	Recessive dark green semidwarf, GA overproduction, embryonic root in mature plant	Chromatin remodeling factor
SLY1	*Arabidopsis*	GA-insensitive dwarf, increased seed dormancy, poor fertility, overproduces RGA protein	F-box protein, homologous to GID2
SNE	*Arabidopsis*	Over-expression suppresses sly1 dwarf	F-box protein, homologous to SLY1
Negative regulators			
GAI	*Arabidopsis*	Semidominant semidwarf, also recessive increased internode length, partly redundant with RGA	DELLA subfamily of GRAS family of putative transcription factors
RGA	*Arabidopsis*	Recessive increased internode length, reduced requirement for GA in germination	DELLA
RGL1, *RGL2*, *RGL3*	*Arabidopsis*	RGL1 is involved in germination and stature, RGL2 is specific to germination	DELLA
RSG	Tobacco	Dominant-negative dwarf, reduced GA_1	bZIP transcription factor
SHI	*Arabidopsis*	Over-expression leads to dwarf stature	Ring finger protein
SLN1	Barley	Recessive increased internode length	DELLA
SLR1	Rice	Recessive increased internode length	DELLA
SPY	*Arabidopsis*, barley	Recessive increased internode length, parthenocarpy, reduced requirement for GA in germination	O-Glc-NAc transferase

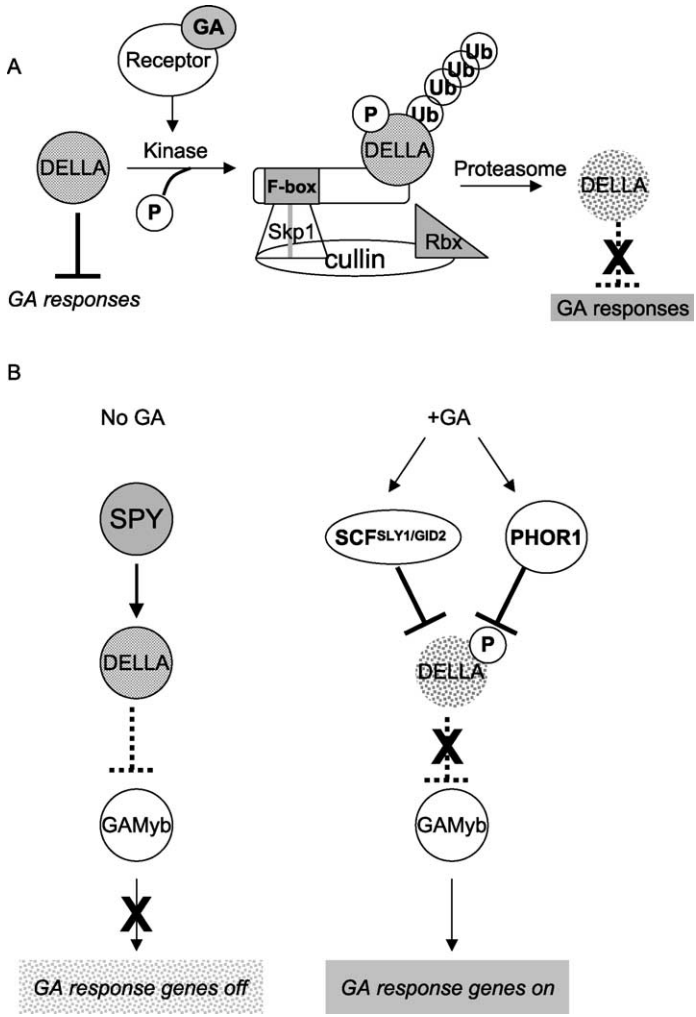


FIGURE 5. Gibberellin signaling in plants. (A) Regulation of DELLA proteins by the ubiquitin-proteasome pathway is mediated by GA-dependent phosphorylation. In the absence of GA, DELLA inhibits GA responses. Gibberellin-binding by the GA receptor stimulates a kinase to phosphorylate the DELLA protein. The phosphorylated DELLA is recognized by the $SCF^{SLY1/GID2}$ E3 ubiquitin ligase complex (F-box protein, Skp1 homologue, cullin, and ring finger protein Rbx). The SCF complex catalyzes the transfer of ubiquitin from Rbx to the target protein. Formation of a polyubiquitin chain targets the DELLA for degradation by the 26S proteasome. (B) Genetic model for GA signaling. In the absence of GA, DELLA proteins inhibit expression of GA-responsive genes either directly or indirectly through inhibition of transcription factors like GAMYB. SPINDLY may negatively regulate GA response by stabilizing the DELLA protein by O-Glc-NAc modification. In the presence of GA, DELLA is negatively regulated by the $SCF^{SLY1/GID2}$ and possibly by the U-box protein PHOR1. DELLA destruction allows activation of GA-responsive gene expression possibly via GAMYB or other transcription factor.

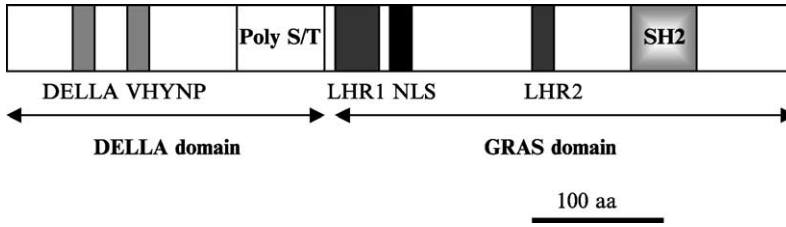


FIGURE 6. DELLA protein structure. The DELLA protein family consists of a number of conserved domains. This schematic is drawn approximately to scale based on an alignment of *Arabidopsis* and rice DELLA proteins. The DELLA protein domain consists of two conserved elements, DELLA and VHYNP. Deletions within this domain lead to loss of GA regulation. The GRAS superfamily domain contains two LHR and one SH2-like domain. These domains are found in STAT transcription factors of metazoans.

et al., 2001), *GA-INSENSITIVE* (*GAI*; Dill *et al.*, 2004; Fu *et al.*, 2004), and *RGA-LIKE2* (*RGL2*; Tyler *et al.*, 2004). Thus, the model is that GA induces GA responses like stem elongation by triggering the destruction of the DELLA protein inhibiting stem elongation (Dill and Sun, 2001; King *et al.*, 2001).

DELLA proteins consist of a DELLA domain required for GA regulation and a GRAS domain required for function (Fig. 6). The DELLA genes are members of the GRAS (GAI-RGA and Scarecrow) family of putative transcription factors (Pysh *et al.*, 1999). The C-terminal GRAS domain contains sequences similar to those found in metazoan signal transducers and activators of transcription (STAT) factors including two leucine heptad repeats (LHR) and an SH2-like domain (Peng *et al.*, 1999). GAI-RGA and Scarecrow proteins contain a variable N-terminal domain. The N-terminal domain of the DELLA subfamily is defined by the consensus "DELLA" and "VHYNP" amino acid sequences. Deletions, N-terminal truncations, and amino acid substitutions within the DELLA domain have been shown to result in a semidominant GA-insensitive dwarf phenotype (Boss and Thomas, 2002; Dill *et al.*, 2001; Peng *et al.*, 1999). Thus, the DELLA domain is required for GA regulation. The DELLA proteins are nuclear localized. A consensus nuclear localization sequence is located within the GRAS domain (Fig. 6). Domain analysis of the rice DELLA protein OsSLR1 was performed by over-expressing *SLR1* constructs containing domain deletions. In spite of the fact that LHR1 domain deletion (termed LZ; Itoh *et al.*, 2002) does not disappear when treated with GA, it results in no phenotype. In contrast, deletion of sequences on the C-terminal side of the NLS results in a dominant-negative tall/slender phenotype. Itoh and coworkers suggest the C-terminal domain is required for function while the LHR1 domain is required for homodimerization. Failure to form homodimer makes the LHR1 deletion both inactive and unregulated, whereas the dominant-negative phenotype of the C-terminal deletion results from dimerization of

the truncated protein with wild-type protein via the LHR1 domain. Leucine heptad repeat1-dependent homodimerization was detected by 2-hybrid analysis. Further studies are needed to establish whether SLR1 forms a homodimer in plants.

DELLA gene function is conserved in a wide range of plant species. A single DELLA gene has been functionally defined in monocot species barley (*SLN1*; Gubler *et al.*, 2002), rice (*SLR1*; Ikeda *et al.*, 2001), and maize (*dwarf8* or *d8*; Peng *et al.*, 1999). There are three known DELLA genes in hexaploid wheat, *Rht-A1*, *Rht-B1*, and *Rht-D1* (Peng *et al.*, 1999). It has been demonstrated that mutations in the DELLA domain of *Rht-B1* and *Rht-D1* resulted in the semidominant GA-insensitive semidwarf varieties that were the basis of the 20% increase in yield called the "Green Revolution" in the 1960s and 1970s (Allan, 1986; Peng *et al.*, 1999). These semidwarf mutations appear to increase yield by: (1) making plants with shorter and stronger stems that are resistant to falling over, and (2) causing the plant to put more energy into producing grain than into biomass. Two DELLA genes have been identified in Hawaiian Silversword and a single DELLA gene has been characterized in wine grape (Boss and Thomas, 2002; Remington and Purugganan, 2002). There are five DELLA genes in the dicot species *A. thaliana* (Itoh *et al.*, 2003). It is not yet known why this dicot species has evolved so many copies of this gene family. However, it is known that the five *Arabidopsis* genes serve partly overlapping functions. *RGA* and *GAI* have been shown to act redundantly in repressing stem elongation, transition to flowering, and the juvenile-to-adult phase transition (Dill and Sun, 2001; King *et al.*, 2001). *RGA* and *RGL1* have the strongest role in the transition to flowering (Cheng *et al.*, 2004; Tyler *et al.*, 2004; Yu *et al.*, 2004). *RGL2* is the main DELLA regulating seed germination, but also appears to act in the regulation of flower development (Lee *et al.*, 2002; Tyler *et al.*, 2004; Yu *et al.*, 2004). The combination of knockouts in *RGA* and *RGL2* is sufficient to restore normal flower development in *ga1-3*. While the function of *RGL3* is not yet known, its transcript appears mainly in young plant tissues (Tyler *et al.*, 2004).

B. CONTROL OF DELLA PROTEIN ACCUMULATION BY E3 UBIQUITIN LIGASES

Growing evidence suggests that GA targets the DELLA proteins for destruction via the ubiquitin-26S proteasome pathway. Supporting evidence comes from the study of a conserved F-box protein of a *S*kp1, *C*ullin or Cdc53, F-box (SCF) E3 ubiquitin ligase in rice and in *Arabidopsis* GA signaling. *S*kp1, *C*ullin or Cdc53, F-box complexes are one form of E3 ubiquitin ligase previously defined in yeast and animals (Itoh et al., 2003). The crystal structure of SCF^{Skp2} has been solved and was used as a basis for the model structure in Fig. 5A (Zheng *et al.*, 2002). The F-box protein binds

to a specific substrate at its C-terminus that typically contains a consensus protein–protein interaction domain such as leucine rich repeats (LRR), WD repeats, or kelch repeats. The N-terminus contains an F-box domain for Skp1 binding. Skp1 tethers the F-box protein to the N-terminus of cullin, the backbone of the complex. Cullin binds a RING-H2 motif subunit (Rbx1/Hrt1/Roc1) like Rbx1 at the C-terminus. The RING-H2 motif protein binds to the E2-conjugating enzyme. The E3 catalyzes the transfer of ubiquitin from the cysteine of E2 to a lysine residue on the substrate. Addition of four or more ubiquitin moieties to the substrate protein targets it for destruction by the 26S proteasome. The presence of 694 F-box proteins in the *A. thaliana* genome points to their important role in plant signal transduction. The ubiquitin-proteasome has become a recurrent theme in plant hormone signaling as E3 ubiquitin ligases act in auxin, jasmonic acid, ethylene, abscisic acid (ABA), and gibberellin signaling.

The F-box genes rice *GA-INSENSITIVE DWARF2* (*OsGID2*) and *Arabidopsis SLEEPY1* (*AtSLY1*) appear to be positive regulators of GA response because they are negative regulators of the DELLA negative regulators of GA response (Fig. 5B). This model is supported both by genetic and biochemical evidence. Recessive mutations in *sly1* and *gid2* result in a recessive GA-insensitive phenotype. Double mutant analysis showed that the *sly1-10* and *gid2-1* dwarf phenotype was suppressed by knockout mutations in DELLA genes, indicating that the DELLA genes act downstream of GID2/SLY1 in GA signaling (Fig. 5B). Moreover, recessive mutations in *OsGID2* and in *AtSLY1* result in high-level accumulation of DELLA proteins even in the presence of GA (Dill *et al.*, 2004; Fu *et al.*, 2004; McGinnis *et al.*, 2003; Sasaki *et al.*, 2003; Tyler *et al.*, 2004). These results suggested that the GA signal causes $SCF^{GID2/SLY1}$ to target the DELLA proteins for destruction by ubiquitylation. Further evidence for this model include: (1) these F-box proteins have been shown to interact with DELLA proteins using yeast two-hybrid, GST pull down assay, and co-immunoprecipitation (Dill *et al.*, 2004; Fu *et al.*, 2004; Gomi *et al.*, 2004); (2) DELLA protein accumulates in a ubiquitylated form in wild-type plants, but not in *gid2* mutants (Sasaki *et al.*, 2003); and (3) 26S proteasome inhibitors cause the DELLA protein HvSLN1 to accumulate at elevated levels (Fu *et al.*, 2002).

How does GA signal to $SCF^{SLY1/GID2}$ to ubiquitylate the DELLA proteins and target them for destruction? In yeast and in mammals, SCF complexes often ubiquitylate their substrate when the substrate is phosphorylated. It appears that phosphorylation of the DELLA protein is at least one signal that stimulates their ubiquitylation by the $SCF^{SLY1/GID2}$ complex (Fig. 5A). The DELLA OsSLR1 accumulates in a phosphorylated form in the *gid2* mutant. In addition, only the phosphorylated form of OsSLR1 interacts with the OsGID2 protein (Gomi *et al.*, 2004). Similarly, AtSLY1 interacts more strongly with the phosphorylated form of the gai-1 protein, the form of GAI that contains the 17 amino acid deletion of the

DELLA domain (Fu *et al.*, 2004). Thus, it will be important to define the DELLA phosphorylation sites and to identify the kinase responsible for DELLA protein phosphorylation.

Is *AtSLY1*/*OsGID2* the only F-box protein acting in GA signaling? Evidence suggests that the homologue of *SLY1* in *Arabidopsis*, *SNEEZY* (*SNE*) may act redundantly with *SLY1* in GA signaling. Over-expression of *SNE* suppresses the *sly1–10* phenotype (Fu *et al.*, 2004; Strader *et al.*, 2004). In addition, it appears that the C-terminal truncations encoded by *sly1-2* and *sly1-10* mutant alleles can interfere with wild-type *SNE* function (Strader *et al.*, 2004). Thus, it is possible that SLY1 and SNE interact in GA signaling. The *SNE* gene is conserved in plant species ranging from grape to rice (Strader *et al.*, 2004).

C. NEGATIVE REGULATION OF GIBBERELLIN RESPONSE

This section summarizes additional genes that have been identified as negative regulators of GA response.

1. *SHI*

Over-expression of the *SHORT INTERNODES* (*SHI*) gene leads to a semidwarf GA-insensitive phenotype in *Arabidopsis*. The *SHI* gene is a member of a multigene family whose predicted protein sequence has homology to RING fingers that mediate protein–protein interactions in ubiquitylation and in transcription (Fridborg *et al.*, 1999, 2001). Epistasis studies may shed light on the position of *SHI* in the GA-signaling pathway.

2. *SPY* and *SEC*

Recessive mutations in *SPINDLY* (*SPY*) were isolated in *Arabidopsis* based on resistance to the inhibitory effect of the GA biosynthesis inhibitor paclobutrazol on germination (Jacobsen and Olszewski, 1993) and on the ability to suppress the GA biosynthesis mutant *ga1-3* (Silverstone *et al.*, 1997a). Loss of *SPY* function results in a GA-overdose phenotype including increased internode length, parthenocarpy, and increased resistance to the GA biosynthetic inhibitor paclobutrazol both vegetatively and in germination. The SPY homologue of barley has also been shown to negatively regulate GA response in the aleurone (Robertson *et al.*, 1998). *SPINDLY* encodes an O-linked-β-*N*-acetylglucosamine transferase (OGT; Thornton *et al.*, 1999). O-linked-β-*N*-acetylglucosamine transferases catalyze post-translational modification of Ser/Thr residues by addition of a single O-linked β-*N*-acetylglucosamine. Evidence from animal systems suggests that OGTs can regulate transcription factors by multiple mechanisms, including competition with kinases for modification of protein phosphorylation sites (Vosseller *et al.*, 2002). *SPINDLY* has a single homologue in

Arabidopsis, *SECRET AGENT* (*SEC*). Genetic data indicate that *SPY* and *SEC* agent are required for plant viability as the double mutant is defective in gametogenesis and embryogenesis (Hartweck *et al.*, 2002). Since DELLA protein destruction is apparently induced by DELLA phosphorylation, it will be important to determine whether *SPY* negatively regulates GA signaling by stabilizing DELLA proteins through competition for phosphorylation sites (Fig. 5B). It is known that the dwarf phenotype of the gain-of-function mutation *gai-1* requires *SPY* function (Swain *et al.*, 2001; Tseng *et al.*, 2001). The possibility that SPY may directly affect DELLA protein activity needs to be investigated.

D. POSITIVE REGULATION OF GIBBERELLIN RESPONSE

This section reviews additional genes that have been identified as positive regulators of GA response in plants.

1. *D1*

The *d1* mutant of rice has a recessive GA-insensitive dwarf phenotype. The *DWARF1* (*D1*) gene encodes the α-subunit of a heterotrimeric G-protein (Ueguchi-Tanaka *et al.*, 2000). Epistasis analysis suggests that *D1* acts upstream of the DELLA gene *OsSLR1* to positively regulate GA signaling. Heterotrimeric G-proteins in yeast and other systems can act in conjunction with a G-protein–coupled receptor. More research is needed to determine if D1 may play a similar role in rice GA signaling. The notion that the heteromeric G-protein plays a role in GA signaling is supported by pharmacological studies in oat aleurone (Jones *et al.*, 1998). G-protein α-subunit (*GPA1*) is the single α-subunit of heterotrimeric G-proteins found in the *Arabidopsis* genome (Jones and Assman, 2004). While T-DNA disruption of *gpa1* does cause reduced response to GA in germination, it does not cause reduced plant height. Thus, the heterotrimeric GA protein may have different roles in GA signaling in different plant species.

2. *GAMYB*

GAMYB is a GA-regulated transcription factor first isolated as a positive regulator of α-amylase in the barley aleurone system (Cercos *et al.*, 1999; Gubler *et al.*, 1995, 1999) and subsequently found to regulate anther development (Murray *et al.*, 2003). GAMYB has been shown to act by directly binding to the GA-response element (GARE) promoter element (Sun and Gubler, 2004). Three transposon insertions have been identified in *GAMYB* of rice (Kaneko *et al.*, 2004). As expected, these mutants produced no α-amylase in the endosperm. These mutants show no change in vegetative growth or in the timing of floral induction. However, upon induction of flowering they show reduced internode length, reduced number of spiklets

per panicle, and varying degrees of floral defects, including pale shrunken sterile anthers, whitened lemma, malformed palea, and malformed pistils.

The dicot *Arabidopsis* contains three homologues of barley and rice *GAMYB*, AtMYB33, AtMYB65, and AtMYB101. Each of these three homologues is able to induce α-amylase expression when expressed in barley (Gocal *et al.*, 2001). Expression of the AtMYB33 transcript, the closest homologue to *GAMYB* of barley and rice, is induced by GA and LD in the shoot apex. AtMYB33 appears to mediate GA induction of flowering because it is able to bind the GARE of the *LEAFY* gene promoter (Gocal *et al.*, 2001). Based on microarray analysis, 20% of the GA-inducible genes of *Arabidopsis* contain a consensus GARE element in the promoter region, suggesting that *GAMYB* may regulate additional GA-response genes (Ogawa *et al.*, 2003). AtMYB33 transcript is negatively regulated by a microRNA, miR159 (Achard *et al.*, 2004). Accumulation of miR159 is positively regulated by GA and negatively regulated by DELLA proteins.

3. *GID1*

Recessive mutations in *GA-INSENSITIVE DWARF1* (*GID1*) result in a GA-insensitive dwarf phenotype. The predicted GID1 protein is a member of the serine hydrolase family that includes esterases, lipases, and proteases. Epistasis analysis indicates that *GID1* acts upstream of the DELLA protein *OsSLR1*. The SLR1 protein accumulates at high levels in *gid1* mutants suggesting that *GID1* is involved in control of SLR1 protein degradation (Gomi and Matsuoka, 2003).

4. *GSE1*

Recessive mutations in *GA-SENSITIVITY1* (*GSE1*) of barley result in a GA-insensitive dwarf phenotype (Chandler and Robertson, 1999). While the gene remains uncloned, studies indicate that *GSE1* is required for the GA-stimulated disappearance of the DELLA protein SLN1 (Gubler *et al.*, 2002). It will be interesting to learn whether *GSE1* is a unique gene or whether it encodes the barley homologue of rice genes *GID1* or *GID2*.

5. *PHOR1*

PHOTOPERIOD-RESPONSIVE1 (*PHOR1*) is a GA-signaling gene identified in potato based on its role in promoting tuberization (Amador *et al.*, 2001). Tuberization of wild potato plants is induced under SDs (8 h of light) and not under LDs (16 h of light). The tuberization process under SD appears to be due, in part, to inhibition of GA signaling (Garcia-Martinez and Gil, 2001). *PHOTOPERIOD-RESPONSIVE1* was recovered using RT-PCR differential display to identify genes expressed during SD-induced tuberization. Antisense expression of *PHOR1* results in a GA-insensitive semi-dwarf phenotype, whereas over-expression of PHOR1 results in enhanced

GA response. In addition, the observation that a PHOR1-GFP translation fusion protein shows GA-dependent nuclear localization supports the view that *PHOR1* is involved in GA signaling. The predicted PHOR1 protein encodes a U-box protein with armadillo repeats (Amador *et al.*, 2001). Evidence suggests that U-box proteins may act independently as E3 ubiquitin ligases (Hatakeyama and Nakayama, 2003). It will be interesting to see whether future research supports a role for *PHOR1* in negatively regulating DELLA proteins via the ubiquitin-proteasome pathway (Monte *et al.*, 2003).

6. *PKL*

The possible role for GA in the transition from embryo to adult development is highlighted by studies of the recessive *pickle* (*pkl*) mutant of *Arabidopsis*. Originally identified based on its tendency to retain embryonic characteristics upon germination, it was subsequently suggested that *PICKLE* (*PKL*) is a positive regulator of GA response (Ogas *et al.*, 1997). This recessive mutation imparts a partially GA-insensitive semidwarf phenotype, reduced response to GA in hypocotyl elongation assays, and enhancement of its embryo-like phenotype when treated with GA biosynthesis inhibitor uniconazole-P (Henderson *et al.*, 2004). While the *pkl* mutant results in overaccumulation of bioactive GAs, a hallmark of GA-insensitive mutants, it does not result in overproduction of *GA3ox1* or of *GA20ox1*. Thus, unlike GA-insensitive mutants *gai-1* and *sly1-10*, the *pkl* mutant does not stimulate positive feedback control of these GA biosynthetic genes. It will be interesting to learn if *pkl* alters expression of the GA inactivating enzyme *GA2ox*. The *PKL* gene encodes a CHD3 protein, a chromatin remodeling factor found throughout eukaryotes that acts as a developmentally regulated repressor of transcription (Dean Rider *et al.*, 2003; Ogas *et al.*, 1999). The model proposed is that *PKL* is a hormone-responsive negative regulator of embryo-specific gene transcription (Henderson *et al.*, 2004). In this case, GA stimulates the transition from embryo to adult developmental state both via a *PKL*-dependent and *PKL*-independent pathway. This raises the intriguing possibility that GA is needed in germination, in part, to signal for the transition to adult development. If this is true, one expects GA biosynthesis mutants to retain some embryonic characteristics after germination. Evidence supporting this model includes: (1) *PKL* appears to be a negative regulator of master regulators of embryonic identity genes *FUSCA3* (*FUS3*), and *LEAFY COTYLEDONS1* and *LEAFY COTYLEDONS2* (*LEC1* and *LEC2*) (Dean Rider *et al.*, 2003; Ogas *et al.*, 1999); (2) *pkl* mutants accumulate seed storage compounds in roots including triacylglycerol, seed storage proteins, and phytate (Rider *et al.*, 2004); (3) GA is able to suppress embryonic characteristics in the *pkl* mutant (Henderson *et al.*, 2004); and (4) GA appears to destabilize a FUS3-GFP fusion protein (Gazzarrini *et al.*, 2004). Mutations in *PKL* have also been

identified as enhancers of *crabsclaw* (*crc*) based on ectopic production of ovules on carples, suggesting that *PKL* may be a general inhibitor of indeterminacy (Hay *et al.*, 2004).

E. GIBBERELLIN-RESPONSE GENES

The final targets of GA signaling are the GA-response genes responsible for the effects of the hormone. Known GA-response genes include: (1) hydrolytic genes acting in germination such as genes encoding α-amylase, endo-[beta]-mannase, and β-1,3-glucanase (Jacobsen *et al.*, 1995; Ni and Bradford, 1993; Wu *et al.*, 2001); (2) cell cycle and cell wall loosening enzymes involved in stem elongation such as cyclins, CDKs, XETs, and expansins (Cosgrove, 2000; Ogawa *et al.*, 2003; Sauter, 1997); and (3) genes involved in induction of flowering and floral development such as *LEAFY*, *APETELA3*, *PISTILLATA*, and *AGAMOUS* (Gocal *et al.*, 2001; Yu *et al.*, 2004).

The precise mechanism by which DELLA genes control expression of GA-response genes is still unknown. However, it is known that the DELLA gene *HvSLN1* of barley is required for repression of *GAMYB* transcription (Fig. 5B, Gubler *et al.*, 2002). *GAMYB* family members positively control expression of α-amylase and of the flowering gene *LEAFY* by direct binding to a GARE promoter element (Gocal *et al.*, 1999, 2001; Gubler *et al.*, 1999; Rogers *et al.*, 1992). GAMYB is known to regulate both α-amylase in germination and LEAFY expression in flowering. Future research will need to determine if *GAMYB* or related genes participate in regulation of other GA-response genes, including those involved in stem elongation or feedback regulation of GA biosynthesis (Fig. 5B).

F. MODEL FOR GIBBERELLIN SIGNALING

Figure 5B shows a current model for control of GA-responsive gene expression in plants. In the absence of GA, DELLA proteins inhibit expression of GA-responsive genes. *GAMYB* is known to induce expression of GA-responsive genes such as those encoding α-amylase and *AtLEAFY*. It will be important to determine whether DELLAs inhibit GA-response gene expression directly or indirectly through inhibition of *GAMYB* or other transcription factors. *SPINDLY* negatively regulates GA response, possibly by stabilizing the DELLA protein by O-Glc-NAc modification. In the presence of GA, $SCF^{SLY1/GID2}$ and possibly also the U-box protein PHOR1 target the DELLA protein for destruction via the ubiquitin-proteasome pathway. This relieves DELLA repression, allowing GAMYB or other transcription factors to induce expression of GA-response genes. It appears that GA targets the DELLA protein for destruction by phosphorylation (Fig. 5A). In this case, the unidentified GA receptor causes activation of a kinase that phosphorylates the DELLA protein. The phosphorylated

DELLA is recognized by the $SCF^{SLY1/GID2}$ E3 ubiquitin ligase. Polyubiquitylation of DELLA by $SCF^{SLY1/GID2}$ targets the DELLA protein for destruction by the 26S proteasome. Degradation of DELLA allows activation of GA-responsive gene expression possibly via GAMYB.

IV. CROSS-TALK WITH OTHER HORMONE-SIGNALING PATHWAYS

The regulation of specific developmental processes is controlled by multiple plant hormones. It is therefore not surprising to find the existence of multiple levels of cross-talk between these phytohormone-signaling pathways. Cross-talk between hormone-signaling pathways is seen both in the control of hormone accumulation and in control of hormone sensitivity.

A. GIBBERELLIN AND ABSCISIC ACID SIGNALING

The antagonism between GA and ABA in the control of seed germination is a well-characterized interaction between two plant hormone-signaling pathways (Koornneef *et al.*, 2002). Studies in *Arabidopsis* show that ABA biosynthesis is transiently induced during embryo maturation and is needed for the embryo to achieve dormancy and dessication tolerance (Karssen *et al.*, 1983). Gibberellin is needed to break seed dormancy and induce germination. Many studies have shown that mutations in ABA and GA biosynthesis and signaling pathways alter response to the other hormone in germination. One can think of this as a tug-of-war over germination with the ABA players pulling for seed dormancy and the GA players pulling for germination. For example, mutations that reduce ABA biosynthesis or sensitivity suppress the requirement for GA in germination (Karssen and Lacka, 1986; Léon-Kloosterziel *et al.*, 1996; Nambara *et al.*, 1991; Steber *et al.*, 1998). This failure to respond or synthesize ABA alleviates the requirement for GA in germination because the seeds never become dormant in the first place. Conversely, the GA-insensitive mutants in *SLY1* result in increased seed dormancy and increased sensitivity to ABA in germination while GA-hypersensitive mutations in *SPY* cause slight ABA-insensitivity in germination (Steber *et al.*, 1998; Strader *et al.*, 2004; Swain *et al.*, 2001).

Abscisic acid and GA may negatively regulate the other hormone-signaling pathway at multiple levels including: (1) hormone biosynthesis, (2) hormone signaling, and (3) transcriptional control. Gibberellin treatment has been shown to reduce accumulation of ABA in dark-germinating lettuce seeds after a pulse of far-red light (Toyomasu *et al.*, 1994). Further research is needed to examine the effects of GA and ABA on one another's biosynthesis. Gibberellin and ABA have been shown to differentially regulate the transcription of genes in a number of plant systems. The cereal aleurone system

has eloquently demonstrated the ability of ABA to block GA induction of α-amylase at the level of transcription (Jacobsen *et al.*, 1995). This may occur, in part, via the ABA-induced protein kinase *PKABA1*, as transient over-expression of *PKABA1* represses the GA-induced genes *GAMYB* and α-amylase (Gomez-Cadenas *et al.*, 2001; Zentella *et al.*, 2002). (B) In tomato, ABA induces and GA represses expression of the sugar-sensing gene *LeSNF4* (Bradford *et al.*, 2003). Finally, microarray analysis in *Arabidopsis* has shown that many GA-downregulated genes have ABA response elements (ABRE) in their promoters (Ogawa *et al.*, 2003). The downregulation of these genes in GA-treated *ga1-3* did not appear to correlate with reduced endogenous ABA suggesting that GA is downregulating ABA signaling. Further research is needed to precisely determine how these hormones negatively regulate one another's signaling cascades.

B. GIBBERELLIN AND BRASSINOSTEROID SIGNALING

Cross-talk has been seen between GA and brassinosteroid (BR) signaling during seed germination and hypocotyl elongation. Brassinosteroid partially rescues seed germination and elongation of dark-grown hypocotyls in the *Arabidopsis* GA biosynthesis mutant *ga1-3* and in GA-insensitive mutant *sly1-2* (Steber and McCourt, 2001). Gibberellin does not, however, rescue hypocotyl elongation of dark-grown BR biosynthesis mutant *det2-1*. Thus, BR appears to be able to bypass GA signaling in these processes, but GA cannot bypass BR in hypocotyl elongation. Work on tobacco indicates that GA and BR promote germination by distinct mechanisms (Leubner-Metzger, 2001). Gibberellin and light appear to act in a common pathway to release photodormancy and to induce expression of the hydrolytic enzyme β-1,3-glucanase in the endosperm. In contrast, BR could not overcome photodormancy or induce β-1,3-glucanase. However, both BR and GA could stimulate germination of ABA-inhibited seeds and accelerate the germination of non-photodormant seeds. Leubner-Metzger proposes that BR stimulates germination solely through stimulation of hypocotyl elongation. This would suggest that BR acts in parallel with, rather than downstream of GA signaling to stimulate germination and hypocotyl elongation of *ga1-3* in *Arabidopsis*.

Further research is needed to understand the interaction between GA-, BR-, and ABA-signaling pathways in germination. One possibility is that GA and BR may regulate one another's biosynthesis. Interestingly, Bouquin and coworkers found that whereas GA negatively regulates the GA biosynthesis gene *AtGA20ox1*, BR positively regulates *AtGA20ox1* (Bouquin *et al.*, 2001). Thus, BR may act, in part, by stimulating GA biosynthesis. This does not fully explain the interaction between GA and BR because BR is able to rescue the germination of *ga1-3*, a mutant blocked upstream of *AtGA-20ox1* in GA biosynthesis (Steber and McCourt, 2001). However, the fact

that a mutation in the BR receptor *AtBRI1* resulted in increased expression of *AtGA20ox1* suggest that plants may induce GA biosynthesis in response to reduced flux in the BR-signaling pathway. Future research will need to determine whether the converse is true.

Research in *Arabidopsis* suggests that the heterotrimeric GTP-binding protein (G-protein) and putative G-protein–coupled receptor may be involved in GA and BR signaling in germination (Chen *et al.*, 2004; Ullah *et al.*, 2002). The *Arabidopsis* genome contains one prototypical *GPA1*, one G-protein β-subunit (*AGB1*), and two G-protein γ-subunits (*AGG1* and *AGG2*) (Jones and Assmann, 2004). One putative G-protein–coupled receptor (*GCR1*) containing a predicted seven-transmembrane domain has been identified in *Arabidopsis*. T-DNA insertional mutations in *GPA1* and in *GCR1* result in reduced response to GA and BR in germination (Chen *et al.*, 2004; Ullah *et al.*, 2002). Ullah *et al.* (2002) proposed that BR may potentiate GA signaling in *Arabidopsis* via *GPA1*.

C. GIBBERELLIN AND AUXIN SIGNALING

In pea, elegant experiments studying both the shoot apex regulation of stem elongation and seed regulation of pericarp growth have provided many insights into the interaction of GA and auxin (O'Neill and Ross, 2002; Ozga *et al.*, 2003; Ross *et al.*, 2001; van Huizen *et al.*, 1997).

Removal of the pea shoot apex inhibits stem elongation because the growth promoting IAA source has been removed. Ross *et al.* (2000) have demonstrated that auxin exerts this effect on stem growth by increasing bioactive GA. This is achieved by promoting expression of the *PsGA3ox1* gene, whereas the levels of *PsGA2ox1/2* transcripts were suppressed (O'Neill and Ross, 2002). Similarly, in the case of seed-stimulated pericarp growth, it has been demonstrated that auxin (4-Cl-IAA) and the presence of seeds promotes pericarp growth by upregulating expression of *PsGA3ox1* (Ozga *et al.*, 2003). The effect of auxin on GA metabolism and promotion of stem growth appears conserved in monocots. Wolbang and coworkers confirmed that auxin from the developing inflorescence of barley plants is required for bioactive GA production and subsequent growth in the stem (Wolbang *et al.*, 2004). A barley GA 3-oxidase gene, *HvGA3ox2*, is implicated in this response to auxin. Interestingly, a study in tobacco indicates that auxin promotes a different GA biosynthetic step, GA 20-oxidation (Wolbang and Ross, 2001). These studies have demonstrated that auxin is likely transported to its site of action where it stimulates the biosynthesis of GAs, which in turn promote growth. Further work is necessary to understand the molecular basis of this cross-talk.

Considering the role of GA signaling in regulating the expression levels of 2-ODD genes, one possible explanation for the auxin-mediated regulation of GA metabolism is that this hormone directly modulates the GA-signal

transduction pathway. It is this effect on GA signaling that leads to changes in expression of GA metabolic genes. This model is supported by a study of root growth in *Arabidopsis*. Fu and Harberd (2003) demonstrated that the shoot apex-derived auxin controls root elongation by modulating the GA-response pathway. More specifically, auxin was shown to affect GA-regulated root growth by modifying the stability of the DELLA protein, RGA. The same group has also demonstrated that ethylene can affect GA-regulated root and hypocotyl growth by a similar process (Achard *et al.*, 2003). In view of the role of SCF E3 ubiquitin ligases in these three hormone-signaling pathways, it is tempting to speculate that these complexes may provide the molecular link to this hormone cross-talk. Biochemical and proteomics approaches should help to provide answers to these questions.

V. PERSPECTIVES

We have seen that mutations affecting GA biosynthesis and response have been essential for improving yields in many agronomically important crops. Although the molecular basis of several of the mutations has been revealed, in most cases, we still have little understanding of how they confer these beneficial traits. In contrast to GA metabolism, our knowledge of GA signaling and the downstream processes that promote GA-responsive growth is rather limited. To further our understanding, it is crucial that we identify the respective components of these processes. It will then be possible to fully understand the developmental and environmental factors that regulate GA metabolism, signaling, and responsive components. Furthermore, the precise spatial and temporal localization patterns can be determined, leading to an understanding of the relationships between these components and their roles in mediating GA-responsive growth. We believe that this understanding will, in part, lead to a second "Green Revolution" in the not too distant future.

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