

Update on Gibberellin Signaling. A Tale of the Tall and the Short¹

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Bioactive gibberellins (GAs) are plant hormones that control growth and development throughout the life cycle of the plant. Isolation and characterization of mutants that are impaired in GA biosynthesis have been crucial in both understanding the role of GAs in regulating plant development and elucidating the GA biosynthetic pathway (Davies, 1995; Hedden and Phillips, 2000). The broad spectrum of developmental processes controlled by GAs is clearly illustrated by the phenotypes of GA biosynthesis mutants. In general, these GA-deficient mutants exhibit a common phenotype: a dark-green dwarf plant that is defective in leaf expansion and stem elongation (Fig. 1). In many species, GA-deficient mutants also display defects during seed germination, altered floral initiation time, and are impaired in development of flower, fruit, and seed.

In recent years our understanding of GA metabolism has advanced rapidly with the cloning of most biosynthetic and catabolic genes (Hedden and Phillips, 2000; Olszewski et al., 2002). In Arabidopsis and other plant species, GA metabolism is tightly regulated by the GA-response pathway. This homeostatic mechanism involves the feedback and feedforward regulation of the respective biosynthetic and catabolic genes. In contrast to GA metabolism, much less is known about the perception of bioactive GAs, the downstream signaling components, and the changes in gene expression that elicit physiological responses. The cereal aleurone has provided an ideal system to study GA response, with GAs specifically promoting the rapid expression of genes encoding hydrolases, such as α -amylases (Lovegrove and Hooley, 2000; Olszewski et al., 2002). In addition, aleurone studies have provided evidence for a plasma membrane localized GA receptor and have identified *GAMYB* as an early GA-response gene that is important in activating expression of α -amylase. Although the cereal aleurone system has vastly improved our knowledge of GA response, it has helped to identify only a few genes encoding primary signaling components.

The identification of GA-response mutants in many plants has proven instrumental in elucidating the GA-

signaling pathway (Richards et al., 2001; Olszewski et al., 2002). The model plant Arabidopsis initially provided the opportunity to use molecular genetics to isolate genes encoding GA-signaling components (Table I). The studies in Arabidopsis not only provided important insights into the GA signal transduction cascade, but also led to the isolation of orthologous genes in other plant species. In fact, many of the components identified so far are highly conserved and have similar roles in GA signaling in their respective species. Once the novel GA-signaling components have been identified, the rapid, transient assay using the cereal aleurone system will provide a powerful tool to define the functional motifs of GA-signaling components. The aim of this update is to focus on recent advances in our understanding of the Arabidopsis GA-signaling pathway, with a particular emphasis on how these studies have interfaced with investigations in other plant species.

GA SIGNAL TRANSDUCTION IN ARABIDOPSIS

DELLA Proteins, Repressors of GA Signaling in Arabidopsis

Loss-of-function *gai* and *rga* mutants are recessive and partially rescue the GA-deficient phenotype caused by mutations or paclobutrazol treatments that block GA biosynthesis (Peng et al., 1997; Silverstone et al., 1998). Therefore, *GAI* and *RGA* encode negative regulators of GA signaling in Arabidopsis. Interestingly, further studies revealed that *GAI* and *RGA* are homologous to each other with predicted roles as nuclear transcriptional regulators. In Arabidopsis, there are five *GAI/RGA* related genes (*GAI*, *RGA*, *RGL1*, *RGL2*, and *RGL3*; Dill and Sun, 2001; Peng and Harberd, 2002). The *RGA*, *GAI*, and homologous proteins are now referred to as DELLA proteins after a highly conserved N-terminal domain (Fig. 2). In addition to *RGA* and *GAI*, *RGL1* and *RGL2* have also been demonstrated to function as negative regulators of GA signaling (Lee et al., 2002; Wen and Chang, 2002; Cheng et al., 2004; Tyler et al., 2004). It remains to be seen whether *RGL3* has a similar role. The DELLA proteins belong to the larger GRAS family, which consists of more than 30 members in Arabidopsis. The GRAS family is characterized by a highly conserved C-terminal GRAS domain that is potentially

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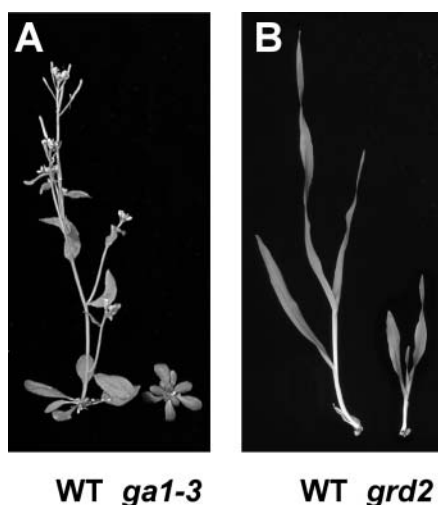


Figure 1. Phenotype of GA-deficient mutants in Arabidopsis and in barley. A, Five-week-old wild-type (WT) Arabidopsis and the GA-deficient *ga1-3* mutant. B, Two-week-old WT Himalaya and a GA-deficient mutant *grd2* (Chandler and Robertson, 1999).

involved in transcriptional regulation (Pysh et al., 1999).

The importance of DELLA proteins as repressors of GA signaling is illustrated by the observations that DELLA loss-of-function mutations suppress the phenotype of *ga1-3*. In Arabidopsis, the DELLA proteins have overlapping as well as specific roles in controlling plant growth and development. For example, an *rga* null mutation (*rga-24*) partially suppresses many of the defects of *ga1-3*, including those affecting leaf expansion, abaxial trichome initiation, flowering time, stem elongation, and apical dominance (Silverstone et al., 1997). By contrast, the *gai-6* null mutation does not dramatically suppress the *ga1-3* phenotype, but in combination with *rga-24* completely suppresses the above defects; in fact, the triple mutant (*ga1-3/rga-24/gai-6*) has a slender phenotype that resembles GA-overdosed wild type (Dill and Sun, 2001; King et al., 2001). However, the triple mutant seed remains non-germinating and the mutant plants still show GA-deficient floral defects, suggesting a role for the *RGL* genes in controlling these additional developmental processes. This hypothesis was confirmed by recent studies showing that *rgl2* null mutations suppress the germination defect of *ga1-3* (Lee et al., 2002). Furthermore, a combination of *rga*, *rgl1*, and *rgl2* null mutations dramatically rescued the floral development defect and fertility of *ga1-3* (Cheng et al., 2004; Tyler et al., 2004).

GA-Induced Degradation of Arabidopsis DELLA Proteins

Bioactive GAs control plant growth and development, at least in part, by relieving the repression imposed by DELLA proteins. Unlike the loss-of-function

gai-6 allele, the *gai-1* mutant is a GA-insensitive dwarf (Table I). This dominant *gai-1* allele is predicted to encode a mutant *gai-1* protein lacking 17 amino acids within the highly conserved DELLA motif (Peng et al., 1997). Transgenic plants carrying *rga-Δ17* or *rgl1* mutant gene that encodes *rga* or *rgl1* proteins lacking the 17 amino acids of the DELLA motif, as in *gai-1*, also have a GA-insensitive dwarf phenotype (Dill et al., 2001; Wen and Chang, 2002). The DELLA domain deletions appear to produce constitutively active repressors that are not inactivated by GA. It has now been demonstrated that GA induces rapid degradation of the wild-type RGA protein, providing a molecular basis for the GA relief of DELLA repression (Silverstone et al., 2001). In fact, RGA protein levels are significantly reduced 5 to 10 min after GA treatment (S.G. Thomas and T.-p. Sun, unpublished data), indicating that this is a very early event in the GA signaling cascade. Interestingly, the *rga-Δ17* mutant protein is resistant to GA-induced degradation (Dill et al., 2001), suggesting that the GA-insensitive dwarf phenotype of the *rga-Δ17* plants is caused by a constitutively active *rga-Δ17* protein that cannot be targeted for degradation by GA. Other DELLA proteins are also likely to be subject to GA-induced degradation, although recent studies of transgenic Arabidopsis expressing both GAI and RGL1 as green fluorescent protein (GFP) fusion proteins did not observe such an effect (Fleck and Harberd, 2002; Wen and Chang, 2002). By contrast, studies using polyclonal antibodies raised against RGA have demonstrated rapid GA-induced degradation of both endogenous GAI and RGL2 proteins (Dill et al., 2004; Tyler et al., 2004).

SLY1, an F-Box Protein Involved in GA-Induced Degradation of DELLA Proteins

The early events in response to many plant hormones involve the destruction of key regulatory proteins via the ubiquitin-proteasome pathway (Hare et al., 2003; Vierstra, 2003). Proteins destined for degradation by the 26S proteasome are polyubiquitinated by an E3 ubiquitin (Ub) ligase enzyme complex. Pioneering work in the fields of auxin, jasmonate, and ethylene signal transduction have illustrated the importance of Skp1/cullin/F-box (SCF) E3 Ub ligase complexes in these pathways (Gray et al., 1999; Xu et al., 2002; Guo and Ecker, 2003; Potuschack et al., 2003). SCF E3 Ub ligases are complexes consisting of four main protein components: Skp1, cullin/Cdc53, Rbx1/Hrt1/Roc1, and F-box. Studies in yeast (*Saccharomyces cerevisiae*) have defined the F-box subunit as providing substrate specificity of the SCF E3 Ub ligase complex. The F-box proteins are characterized by an N-terminal F-box motif, which has a structural role for mediating binding to the Skp1 component of the complex. The C termini of F-box proteins are often required for binding and targeting the substrate for ubiquitination and in many cases contain a conserved protein-protein interaction domain for this purpose.

Table 1. *Arabidopsis* GA-response mutants

GA-Response Mutants	Phenotype	Dominant/Recessive	Role of Wild-Type Allele in GA Signaling	Predicted Protein	Mutant Screening Strategy
<i>gai-1</i>	GA-insensitive dwarf	Dominant	Negative regulator	Transcriptional regulator	GA-auxotrophic looking dwarfs (Koornneef et al., 1985)
<i>gai-t6</i>	GA-independent growth	Recessive	Negative regulator	Transcriptional regulator	Ds insertion suppressing dwarf phenotype of <i>gai-1</i> (Peng et al., 1997)
<i>gar2-1</i>	GA-independent growth	Dominant	Not clear	?	Growth suppression of <i>gai-1</i> (Wilson and Somerville, 1995)
<i>pk1</i>	Dwarf with reduced GA response	Recessive	Positive regulator?	CHD3 chromatin remodeling factor	GA-auxotrophic looking dwarfs with an embryonic root phenotype (Ogas et al., 1999)
<i>rga</i>	GA-independent growth	Recessive	Negative regulator	Transcriptional regulator	Growth suppression of <i>gai-3</i> (Silverstone et al., 1997)
<i>rga-Δ17</i>	GA-insensitive dwarf	Dominant	Negative regulator	Transcriptional regulator	Transgenic plants expressing <i>rga-Δ17</i> (Dill and Sun, 2001)
<i>shi</i>	GA-insensitive dwarf	Dominant	Negative regulator?	RING finger protein	Activation tagging screen for GA-insensitive dwarfs (Fridborg et al., 1999)
<i>sly1</i>	GA-insensitive dwarf	Recessive	Positive regulator	F-box protein	<i>abi1-1</i> suppressor screen (Steber et al., 1998)
<i>spy</i>	GA-independent growth	Recessive	Negative regulator	OGT	Restoration of growth on paclobutrazol (Jacobsen and Olszewski, 1993), growth suppression of <i>gai-3</i> (Silverstone et al., 1997) or <i>gai-1</i> (Wilson and Somerville, 1995)

Studies in yeast and mammalian cells have demonstrated that proteins targeted for ubiquitination are posttranslationally modified, usually by phosphorylation. It is the modified form of the target protein that is recognized by the F-box component of the SCF E3 Ub ligase. The importance of SCF E3 Ub ligases in controlling plant growth and development is illustrated by recent predictions of almost 700 genes encoding F-box proteins in *Arabidopsis* (Gagne et al., 2002).

A role for the ubiquitin-proteasome pathway, and more specifically SCF E3 Ub ligases, in GA signaling comes from work with *SLY1*, which encodes a predicted F-box protein (McGinnis et al., 2003). *SLY1* is a small protein of 151 amino acids, containing an N-terminal F-box motif. Although its C terminus does not have a characteristic protein-protein interaction domain, this region is clearly important for *SLY1* function as both of the loss-of-function *sly1* alleles are predicted to encode *sly1* proteins with C-terminal truncations. The *Arabidopsis* genome encodes a predicted protein, MIF21.6, which is 30% identical to At*SLY1* over a 124-amino acid region. MIF21.6 contains an N-terminal F-box domain but demonstrates higher sequence identity over the C-terminal domain (51% identity over 51 amino acids). Thus, MIF21.6 may also be involved in GA signal transduction.

Several lines of evidence indicate that *SLY1*, presumably as part of the SCF^{*SLY1*} complex, targets

the DELLA proteins for GA-induced degradation by the ubiquitin-proteasome pathway (McGinnis et al., 2003; Dill et al., 2004). In the *sly1* mutants, RGA and GAI protein levels are dramatically elevated and protein levels are not reduced by GA treatment. Furthermore, the dwarf phenotype of *sly1* is completely suppressed by a combination of *rga* and *gai* null alleles (Dill et al., 2004). The triple *sly1/rga/gai* mutant still demonstrates germination and floral defects, suggesting that *SLY1* also plays a role in GA-induced degradation of RGL proteins. A direct interaction of *SLY1* with RGA and GAI was found in a yeast two-hybrid system, providing further support for a role of *SLY1* in recruiting DELLA proteins to the SCF^{*SLY1*} E3 Ub ligase complex. Yeast two-hybrid assays have also been performed to closely map the domains of GAI necessary for the interaction with *SLY1* (Dill et al., 2004). Surprisingly, the DELLA motif of GAI or RGA is not required for their interaction with *SLY1*, whereas the GAI GRAS domain alone is sufficient to interact with *SLY1* in yeast. The DELLA domain may therefore be important for the GA-induced posttranslational modification of the DELLA proteins. Future studies will be needed to verify this hypothesis, as well as to identify additional components in the SCF^{*SLY1*} E3 Ub ligase complex and the regulation of assembly and/or activity of this complex by GA.

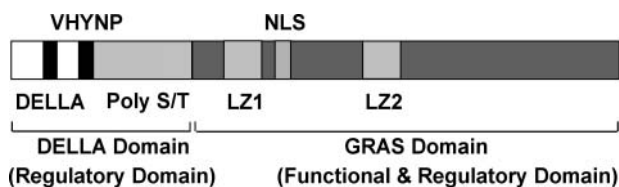


Figure 2. The conserved domains in DELLA proteins. Among the GRAS protein family, the DELLA proteins contain a unique N-terminal DELLA domain with two highly conserved motifs (named DELLA and VHYNP), and a divergent Poly S/T region. The C-terminal region (named GRAS domain) is conserved among all GRAS family members. Poly S/T, polymeric Ser and Thr; LZ, Leu zipper; NLS, nuclear localization signal.

Hormone Cross Talk in Arabidopsis

It has long been known that there is cross talk between different phytohormone signaling pathways (Gazzarrini and McCourt, 2003). A recent microarray study aimed at identifying GA-responsive genes in germinating Arabidopsis seeds demonstrated that GA may affect both ethylene biosynthesis and response, as well as auxin biosynthesis and transport (Ogawa et al., 2003). In addition, both auxin and ethylene can affect GA-regulated root and hypocotyl growth by modifying the stability of RGA (Achard et al., 2003; Fu and Harberd, 2003). The molecular mechanism(s) involved in the interactions among these hormones remain to be determined. Considering the central role of SCF E3 Ub ligase-mediated protein degradation in each of the auxin, ethylene, and GA signaling cascades, it will be intriguing to see whether these SCF complexes provide the molecular link to this phytohormone cross talk. Biochemical and proteomics approaches should help to provide answers to these questions.

SPINDLY Encodes an O-Linked N-Acetylglucosamine Transferase

The importance of SPY as a negative regulator of GA signaling is clearly illustrated by the finding that *spy* mutations partially suppress all of the GA-related growth defects of the *ga1* mutants (Jacobsen and Olszewski, 1993; Silverstone et al., 1997). SPY encodes a novel Arabidopsis protein that shows sequence similarity to animal O-linked N-acetylglucosamine (O-GlcNAc) transferases (OGTs) over its C terminus (Thornton et al., 1999). Support for SPY being a functional OGT is provided by the findings that SPY has *in vitro* OGT activity and that *spy* mutants exhibit a reduction in O-GlcNAcylated proteins. The SECRET AGENT (*SEC*) gene encodes a second Arabidopsis OGT, which also demonstrates OGT activity when expressed in an *Escherichia coli* system (Hartwek et al., 2002). Although the *sec* mutant has a wild-type phenotype, the *spy/sec* double mutant is synthetic lethal in gamete and seed development, indicating that OGT is essential in these developmental stages and that SPY regulates other cellular pathways in addition to GA signaling. It is unclear whether SEC plays a minor role

in GA signaling. The N termini of both SPY and SEC have tetratricopeptide repeat (TPR) domains that appear necessary for mediating protein-protein interactions. Overexpression of the SPY TPR domain in Arabidopsis confers a *spy*-like phenotype (Tseng et al., 2001), suggesting that the overexpressed TPR domain may block SPY function by forming inactive protein complexes and/or by interacting with SPY substrates and preventing modification. O-GlcNAcylation of target proteins in animal systems is specific to Ser or Thr residues and is a dynamic modification analogous to phosphorylation that can affect the activity of target proteins (Wells et al., 2001). In addition, O-GlcNAcylation may compete with phosphorylation for identical or close-by target residues. Target proteins of SPY have not been identified, but potential candidates include the DELLA proteins (Olszewski et al., 2002).

GA-Responsive Genes in Arabidopsis

To improve our understanding of the roles of GAs in regulating Arabidopsis growth and development, it is essential to study the GA-mediated changes in spatial and temporal patterns of gene expression. DNA microarray analysis provides a powerful tool to identify GA-responsive genes. A recent study identified 138 up-regulated and 120 down-regulated genes after a 12-h GA₄ treatment of imbibed *ga1-3* seeds, using the oligo-based microarrays representing about 8,200 Arabidopsis genes (Ogawa et al., 2003). A subset of the GA-regulated genes in *ga1-3* was identified in germinating wild-type seeds, the expression of which demonstrated a direct correlation with increases in endogenous levels of the bioactive GA₄. Previous studies in Arabidopsis have identified potential promoter elements that may have a role in GA responsiveness (Blazquez and Weigel, 2000). An 8-bp cis-element was identified in the promoter of the floral meristem identity gene *LEAFY* as being necessary for GA-responsive expression. This element is a potential MYB transcription factor binding site. Furthermore, the expression of *AtMYB33*, a *GAMYB*-like gene in Arabidopsis, was detected in the shoot apex prior to *LEAFY* expression (Gocal et al., 2001). Only a single GA-responsive gene identified in the study by Ogawa et al. (2003) contained the GA-responsive *LEAFY* cis-element. This indicates that this element does not serve as a common cis-element for GA-responsive genes. Interestingly, a previously identified abscisic acid-responsive element was found in many of the promoters of the GA down-regulated genes.

Because DELLA proteins are thought to be transcriptional regulators, it is critical to identify the genes they control. The use of DNA microarray studies to establish global gene expression profiles in DELLA loss-of function and gain-of-function mutants should help to identify candidate target genes of DELLA proteins.

GA SIGNALING COMPONENTS ARE CONSERVED IN OTHER PLANTS

DELTA Proteins Repress GA Signaling in Other Plant Species

In the search for improved crops, geneticists and plant breeders have generated and characterized many mutants. These collections include GA-deficient and -response mutants that have proven immensely important in both furthering our understanding of GA action as well as increasing agronomic yields. In particular, many GA-response mutants have been identified in cereals. These GA-insensitive mutants fall into the same categories as those seen in *Arabidopsis*, showing either a GA-dwarf or -overdose (slender) phenotype. In contrast to *Arabidopsis* GA-overdose mutants, the corresponding cereal slender mutants display a more dramatic, easily observed phenotype (Olszewski et al., 2002). The importance of GA-response mutations in producing commercially important crops is clearly illustrated by the *Rht* semi-dominant dwarfing alleles of wheat (*Triticum aestivum*; Perkins, 1997). These dwarfing mutations have been essential in producing higher yielding lines that are also more resistant to wind damage; these dwarfing alleles have been a crucial component of the development of Green Revolution varieties and are critical for the success of the Green Revolution. In addition to *Rht* in wheat, dominant mutations producing GA-insensitive dwarf plants have also been identified in other species, including maize (*Zea mays*; *D8*), rice (*Oryza sativa*; *slr1*), barley (*Hordeum vulgare*; *sln1-d*), *Brassica rapa* (*dwf2*), and grape (*Vitis vinifera*; *Vvgai1*; Peng et al., 1999; Ikeda et al., 2001; Boss and Thomas, 2002; Chandler et al., 2002; Muangprom and Osborn, 2004). Identification of DELLA protein genes in *Arabidopsis* has facilitated the cloning of orthologs in other plant species. Interestingly, the dwarfing cultivars described above all contain gain-of-function mutations in genes encoding DELLA protein orthologs (Peng et al., 1999; Boss and Thomas, 2002; Gubler et al., 2002). Moreover, these dwarfing alleles are predicted to encode mutant DELLA proteins that are altered in the N-terminal region encompassing the highly conserved DELLA domain. Similar to *Arabidopsis*, GA opposes the action of SLR1 and SLN1 in rice and in barley, respectively, by targeting their rapid degradation. The DELLA motif-mutated *slr1* and *sln1-d* proteins are resistant to GA-induced degradation (Gubler et al., 2002; Itoh et al., 2002), consistent with property of the *rga-Δ17* protein in *Arabidopsis*. These observations illustrate that the role of the DELLA proteins in controlling GA signaling is conserved between dicots and monocots.

Opposite to the dwarf phenotype caused by the dominant *slr1* and *sln1* alleles, recessive mutations in these loci confer a GA-insensitive slender phenotype in both rice and barley (Ikeda et al., 2001; Chandler et al., 2002). Rice appears to contain only a single

DELLA protein gene (Ikeda et al., 2001). Removing this gene function, therefore, results in a constitutively active GA response and a dramatically taller phenotype. By contrast, a single DELLA protein gene knockout in *Arabidopsis* only produces subtle effects on GA responses, due to functional redundancy among the five DELLA family members (Dill and Sun, 2001; King et al., 2001). The nonredundancy of GA-signaling components, coupled with the availability of the aleurone system, makes cereals an attractive system to elucidate GA signal transduction. The study of DELLA proteins in cereals has highlighted the similarities between GA signaling in dicots and monocots, in addition to improving our understanding of the role of DELLA proteins.

Functional Analysis of DELLA Protein SLR1 in Rice

To further investigate the role of SLR1 in GA signaling, functional domain analysis was performed by overexpressing truncated forms of SLR1 as GFP fusions in transgenic rice (Itoh et al., 2002). Deletions in the highly conserved N-terminal DELLA and VHYNP motifs (Fig. 2) produced severe GA-insensitive dwarf plants in which the GFP fusion proteins were not degraded in response to GA. This supports other studies described above that show the N terminus of DELLA proteins is essential for perceiving the GA signal. Interestingly, deletion of an N-terminal Ser/Thr rich region (Poly S/T motif; Fig. 2), adjacent to the GRAS domain, produces a dwarf that has reduced sensitivity to GA. However, deleting the Poly S/T motif does not confer resistance to GA-induced degradation or cause an elevated mutant protein accumulation. It was proposed that this domain may be modified by phosphorylation or O-GlcNAcylation to regulate the repressive activity of SLR1. Deletions in the C-terminal GRAS domain of SLR1 appear to abolish functionality, which supports the findings that null alleles of DELLA proteins often contain substitutions or deletions in the GRAS coding region (Silverstone et al., 1998; Ikeda et al., 2001; Gubler et al., 2002). In addition, the mutant proteins with the C-terminal truncations are not degraded in response to GA. These findings support a dual role for the GRAS domain as having both functional and regulatory activity. The C terminus also contains a Leu zipper motif, which may play a role in formation of SLR1 homodimers. Expression of a C-terminal truncated form containing the Leu zipper motif had a dominant negative effect, producing a slender phenotype in the wild-type background. It was proposed that the truncated form produced this effect by forming inactive dimers with wild-type SLR1 (Itoh et al., 2002).

SCF E3 Ub Ligase-Mediated Degradation of SLR1

Studies described above have clearly illustrated that DELLA proteins have a highly conserved role in GA signaling in different species. The mechanisms

controlling GA-mediated degradation of DELLA protein also appear to be highly conserved, at least in Arabidopsis and rice. An important study in rice by Sasaki et al. (2003) identified the recessive *gid2* alleles that produce GA-insensitive dwarf plants. The *GID2* gene encodes an F-box protein that is highly homologous to SLY1. Genetic and biochemical analyses strongly support a role for GID2, as part of a SCF^{GID2} E3 Ub ligase complex, in GA-induced degradation of SLR1 in rice. The SLR1 protein level is elevated in the *gid2* mutant, and ubiquitinated forms of SLR1 are observed after GA treatment. Furthermore, GA promotes phosphorylation of SLR1 in the *gid2* mutant (Sasaki et al., 2003), and phosphorylated SLR1 protein specifically binds to GID2 in an in vitro pull-down assay (Gomi et al., 2004). These results suggest that phosphorylation of SLR1 is necessary for its degradation.

Role of D1 and PHOR1 in GA Signaling

Studies of plants other than Arabidopsis have uncovered several novel potential GA-signaling components. In rice, the recessive *dwarf1* (*d1*) alleles have a GA-insensitive dwarf phenotype, suggesting that D1 is a positive regulator of GA signaling (Mitsunaga et al., 1994). The *D1* gene encodes a putative α -subunit ($G\alpha$) of the heterotrimeric G protein (Ashikari et al., 1999; Fujisawa et al., 1999). This finding supports pharmacological data suggesting a role for heterotrimeric G proteins in GA response (Jones et al., 1998). However, it is important to note that D1 also has additional roles in other signaling pathways, including disease resistance (Suharsono et al., 2002). In Arabidopsis, $G\alpha$ is encoded by a single gene, GPA1. In contrast to *d1*, the *gpa1* loss-of-function mutants do

not display a dwarf phenotype, although they are less responsive to GA during germination (Ullah et al., 2002). Characterization of the *gpa1* mutant supports a role for GPA1 in regulating multiple signaling pathways.

In potato (*Solanum tuberosum*), *PHOTOPERIOD REGULATED 1* (*PHOR1*) was identified as an up-regulated gene in the potato leaves grown under short days (Amador et al., 2001). Transgenic potato plants expressing an antisense *PHOR1* construct had a semi-dwarf phenotype. These plants displayed reduced response to GA application and had higher levels of endogenous GAs compared to control plants. These findings support *PHOR1* having a role as a positive regulator of GA signaling. Furthermore, 3 to 4 h of GA treatment induces translocation of the *PHOR1*-GFP fusion protein to the nucleus in tobacco (*Nicotiana tabacum*) BY-2 cells. The predicted *PHOR1* protein has recently been shown to contain a U-box (UFD2 homology) arm repeat, which is present in a class of Ub E3 ligase proteins (Monte et al., 2003). It will be interesting to determine whether *PHOR1* plays a role in GA-mediated degradation of DELLA proteins.

GA Signaling in Cereal Aleurone Cells

It is beyond the scope of this update to give a thorough review of GA signaling and response in cereal aleurone cells. However, there are excellent recent reviews on this topic (Lovegrove and Hooley, 2000; Olszewski et al., 2002). The aleurone cell has a number of advantages that make it an ideal system to study GA signaling and response using biochemical approaches. Although aleurone cells do not produce GA, transcription of a number of genes encoding hydrolytic enzymes is induced by GA treatment.

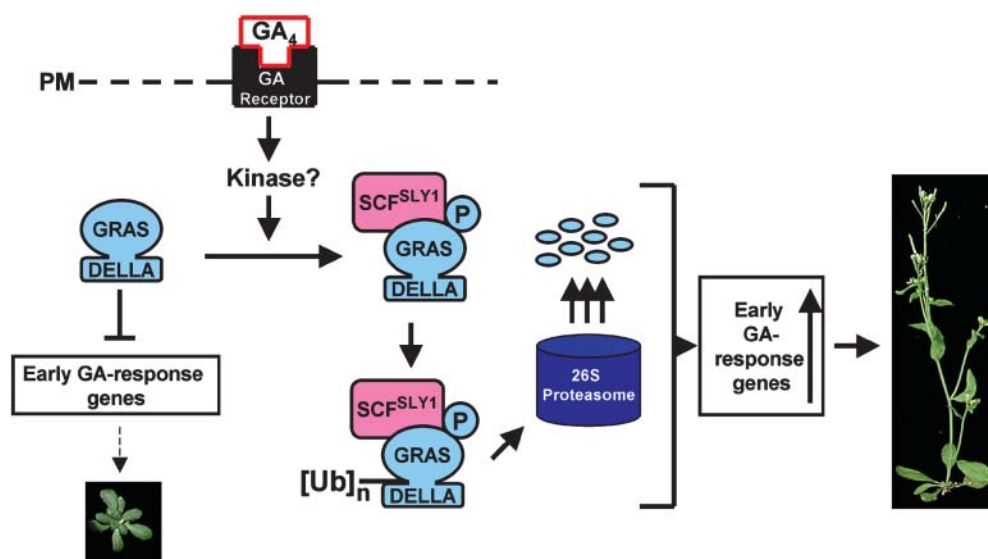


Figure 3. A model of GA-signaling pathway in Arabidopsis. Arrows and the T-bar indicate positive and inhibitory effects, respectively. Weak versus stronger effects are represented by dotted and solid lines, respectively. PM, plasma membrane; [Ub]_n, polyubiquitination.

Therefore, promoters of these genes, when fused to reporter genes, are convenient indicators of GA response in the aleurone system. In addition, GA-responsive protoplasts can be easily isolated from aleurone cells for transient expression studies.

GAMYB is a transcription factor that positively regulates expression of α -amylase genes in cereal aleurone cells (Gubler et al., 1999). In barley, GAMYB has been shown to specifically bind to the GA-response element of α -amylase genes and transactivate their expression. The identity of primary GA-signaling components in cereals has provided the opportunity to test their roles in controlling *GAMYB* induction of α -amylase expression (Robertson et al., 1998; Gubler et al., 2002; Zentalla et al., 2002). For example, *GID2* and *D1* are positive regulators, whereas *DELLA* proteins and *SPY* are negative regulators of the GA response in aleurone cells. Studies aimed at understanding the kinetics of GA response have shown that *GAMYB* expression is induced 1 h after treating aleurone cells with GA. By contrast, *SLN1* degradation occurs within 10 min (Gubler et al., 2002). It will be important to understand the molecular events that occur immediately after *SLN1*-induced degradation. The use of aleurone cells in DNA microarray experiments provides an ideal opportunity to elucidate these early transcriptional changes caused by GA.

MODEL OF GA SIGNALING IN ARABIDOPSIS

Our model of the GA signaling cascade in Arabidopsis is shown in (Fig. 3). This model centers on the five *DELLA* proteins (*RGA*, *GAI*, *RGL1*, *RGL2*, and *RGL3*) that are predicted to be the key repressors of downstream GA-response genes. The *DELLA* proteins appear to have both individual and redundant roles in regulating various aspects of GA-mediated growth and development in Arabidopsis. Bioactive GAs produced by the plant are perceived by a putative plasma membrane receptor, triggering a signaling cascade of events. Based on studies in rice, an early step in this signaling cascade is the activation of a protein kinase that phosphorylates *DELLA* proteins. Of course, an alternative form of posttranslational modification may occur. The *DELLA* domain is predicted to play a role in perceiving the GA signal. Subsequently, the modified form of the *DELLA* protein is recognized and bound by the *SLY1* subunit of a SCF^{*SLY1*} E3 Ub ligase complex through the GRAS domain. This results in polyubiquitination of the *DELLA* protein, which in turn promotes recognition and subsequent degradation by the 26S proteasome. The reduction in *DELLA* protein levels allows changes in downstream gene expression that result in GA-responsive growth and development. The role of *SPY* as a negative regulator in the GA signaling cascade is not clearly defined. One possibility is that O-GlcNAcylation of *DELLA* proteins may stimulate their activity leading to increased repression of GA

responses. Further studies will be necessary to confirm this model as well to incorporate other potential signaling components into it.

FUTURE PERSPECTIVES

Although our understanding of the GA signal transduction cascade has greatly improved in recent years, there are still huge gaps in our knowledge. Molecular genetic approaches have proven highly successful in identifying GA-signaling components in a number of plant species. Potential GA signaling components identified in other plants can now be studied in Arabidopsis. For example, Arabidopsis has three genes encoding closely related proteins to *PHOR1* (Monte et al., 2003). Combining the power of Arabidopsis genetics and the transient cereal aleurone system will continue to allow us to elucidate the functions of known GA-signaling components and to discover novel players in the GA-response pathway. The *DELLA* proteins are highly conserved in higher plants and are central modulators of GA-mediated growth and developmental events. These findings provide an important opportunity to dissect the GA-signaling pathway, both downstream and upstream of the *DELLA* proteins. Targeted DNA microarray studies should help in deciphering the transcriptional events that follow *DELLA* protein degradation and may also identify potential *DELLA* target genes. In addition, biochemical and proteomics approaches have the potential to elucidate further the events promoting *DELLA* protein degradation. It is also hoped that these approaches may finally unearth the elusive GA receptor.

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LITERATURE CITED

- Achard P, Vriezen WH, van der Straeten D, Harberd NP (2003) Ethylene regulates Arabidopsis development via the modulation of *DELLA* protein growth repressor function. *Plant Cell* **15**: 2816–2825
- Amador V, Monte E, García-Martínez JL, Prat S (2001) Gibberellins signal nuclear import of *PHOR1*, a photoperiod-responsive protein with homology to *Drosophila* armadillo. *Cell* **106**: 343–354
- Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A (1999) Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the α -subunit of GTP-binding protein. *Proc Natl Acad Sci USA* **96**: 10284–10289
- Blazquez MA, Weigel D (2000) Integration of floral inductive signals in Arabidopsis. *Nature* **404**: 889–892
- Boss PK, Thomas MR (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* **416**: 847–850

- Chandler PM, Marion-Poll A, Ellis M, Gubler F (2002) Mutants at the *Slender1* locus of barley cv Himalaya: molecular and physiological characterization. *Plant Physiol* **129**: 181–190
- Chandler PM, Robertson M (1999) Gibberellin dose-response curves and the characterization of dwarf mutants of barley. *Plant Physiol* **120**: 623–632
- Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. *Development* **131**: 1055–1064
- Davies PJ, ed (1995) *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Kluwer Academic Publishers, Dordrecht, The Netherlands
- Dill A, Jung H-S, Sun T-p (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. *Proc Natl Acad Sci USA* **98**: 14162–14167
- Dill A, Sun T-p (2001) Synergistic de-repression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. *Genetics* **159**: 777–785
- Dill A, Thomas SG, Hu J, Steber CM, Sun T-p (2004) The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* **16**: 1392–1405
- Fleck B, Harberd NP (2002) Evidence that the Arabidopsis nuclear gibberellin signalling protein GAI is not destabilized by gibberellin. *Plant J* **32**: 935–947
- Fridborg I, Kuusk S, Moritz T, Sundberg E (1999) The Arabidopsis dwarf mutant *shii* exhibits reduced gibberellin responses conferred by over-expression of a new putative zinc finger protein. *Plant Cell* **11**: 1019–1031
- Fu X, Harberd NP (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. *Nature* **421**: 740–743
- Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, Sasaki T, Asahi T, Iwasaki Y (1999) Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice. *Proc Natl Acad Sci USA* **96**: 7575–7580
- Gagne JM, Downes BP, Shin-Han S, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in *Arabidopsis*. *Proc Natl Acad Sci USA* **99**: 11519–11524
- Gazzarrini S, McCourt P (2003) Cross-talk in plant hormone signalling: what Arabidopsis mutants are telling us. *Ann Bot* **91**: 605–612
- Gocal GF, Sheldon CC, Gubler F, Moritz T, Bagnall DB, MacMillan CP, Li SF, Parish RW, Dennis ES, Weigel D, et al (2001) *GAMYB-like* genes, flowering and gibberellin signaling in Arabidopsis. *Plant Physiol* **127**: 1682–1693
- Gomi K, Sasaki A, Itoh H, Ueguchi-Tanaka M, Ashikari M, Kitano H, Matsuoka M (2004) GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J* **37**: 626–634
- Gray WM, del Pozo JC, Walker L, Hobbie L, Risseuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev* **13**: 1678–1691
- Gubler F, Chandler P, White R, Llewellyn D, Jacobsen J (2002) GA signaling in barley aleurone cells: control of SLN1 and GAMYB expression. *Plant Physiol* **129**: 191–200
- Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen JV (1999) Target genes and regulatory domains of the GAMYB transcriptional activator in cereal aleurone. *Plant J* **17**: 1–9
- Guo H, Ecker RD (2003) Plant responses to ethylene gas are mediated by SCF^{EBF1/EBF2}-dependent proteolysis of EIN3 transcription factor. *Cell* **115**: 667–677
- Hare PD, Seo HS, Yang J-Y, Chua N-H (2003) Modulation of sensitivity and selectivity in plant signaling by proteasomal destabilization. *Curr Opin Plant Biol* **6**: 453–462
- Hartwek LM, Scott CL, Olszewski NE (2002) Two O-linked N-acetylglucosamine transferase genes of *Arabidopsis thaliana* L. Heynh. have overlapping functions necessary for gamete and seed development. *Genetics* **161**: 1279–1291
- Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci* **5**: 523–530
- Ikedo A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. *Plant Cell* **13**: 999–1010
- Itoh H, Ueguchi-Tanaka M, Sato Y, Ashikari M, Matsuoka M (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell* **14**: 57–70
- Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of Arabidopsis alter gibberellin signal transduction. *Plant Cell* **5**: 887–896
- Jones HD, Smith SJ, Desikan R, Plakidou-Dymock S, Lovegrove A, Hooley R (1998) Heterotrimeric G proteins are implicated in gibberellin induction of α -amylase gene expression in wild oat aleurone. *Plant Cell* **10**: 245–253
- King K, Moritz T, Harberd N (2001) Gibberellins are not required for normal stem growth in Arabidopsis thaliana in the absence of GAI and RGA. *Genetics* **159**: 767–776
- Koornneef M, Elgersma A, Hanhart CJ, van Loenen MEP, van Rijn L, Zeevaert JAD (1985) A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol Plant* **65**: 33–39
- Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J (2002) Gibberellin regulates Arabidopsis seed germination via *RGL2*, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes Dev* **16**: 646–658
- Lovegrove A, Hooley R (2000) Gibberellin and abscisic acid signalling in aleurone. *Trends Plant Sci* **5**: 102–110
- McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun T-p, Steber CM (2003) The Arabidopsis *SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Plant Cell* **15**: 1120–1130
- Mitsunaga S, Tashiro T, Yamaguchi J (1994) Identification and characterization of gibberellin-insensitive mutants selected from among dwarf mutants of rice. *Theor Appl Genet* **87**: 705–712
- Monte E, Amador V, Russo E, Martínez-García J, Prat S (2003) PHOR1: A U-Box GA signaling component with a role in proteasome degradation? *J Plant Growth Regul* **22**: 152–162
- Muangprom A, Osborn TC (2004) Characterization of a dwarf gene in *Brassica rapa*, including the identification of a candidate gene. *Theor Appl Genet* **108**: 1378–1384
- Ogas J, Kaufmann S, Henderson J, Somerville C (1999) PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proc Natl Acad Sci USA* **96**: 13839–13844
- Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell* **15**: 1591–1604
- Olszewski N, Sun T-p, Gubler F (2002) Gibberellin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell* **14** (Suppl.): S61–S80
- Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis *GAI* gene defines a signalling pathway that negatively regulates gibberellin responses. *Genes Dev* **11**: 3194–3205
- Peng J, Harberd NP (2002) The role of GA-mediated signalling in the control of seed germination. *Curr Opin Plant Biol* **5**: 376–381
- Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintam JE, Beales J, Fish LJ, Worland AJ, Pelica F, et al (1999) ‘Green Revolution’ genes encode mutant gibberellin response modulators. *Nature* **400**: 256–261
- Perkins JH (1997) *Geopolitics and the Green Revolution*. Oxford University Press, New York
- Potuschack T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. *Cell* **115**: 679–689
- Pysch LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN (1999) The GRAS gene family in Arabidopsis: sequence characterization and basic expression analysis of the SCARECROW-LIKE genes. *Plant J* **18**: 111–119
- Richards DE, King KE, Ait-ali T, Harberd NP (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. *Annu Rev Plant Physiol Plant Mol Biol* **52**: 67–88
- Robertson M, Swain SM, Chandler PM, Olszewski NE (1998) Identification of a negative regulator of gibberellin action, HvSPY, in barley. *Plant Cell* **10**: 995–1007
- Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong D-H, An G, Kitano J, Ashikari M, et al (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. *Science* **299**: 1896–1898

- Silverstone AL, Ciampaglio CN, Sun T-p** (1998) The Arabidopsis *RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10**: 155–169
- Silverstone AL, Jung H-S, Dill A, Kawaide H, Kamiya Y, Sun T-p** (2001) Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *Plant Cell* **13**: 1555–1566
- Silverstone AL, Mak PYA, Casamitjana Martínez E, Sun T-p** (1997) The new *RGA* locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*. *Genetics* **146**: 1087–1099
- Steber CM, Cooney S, McCourt P** (1998) Isolation of the GA-response mutant *sly1* as a suppressor of *AB11-1* in *Arabidopsis thaliana*. *Genetics* **149**: 509–521
- Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K** (2002) The heterotrimeric G protein α subunit acts upstream of the small GTPase Rac in disease resistance of rice. *Proc Natl Acad Sci USA* **99**: 13307–13312
- Thornton TM, Swain SM, Olszewski NE** (1999) Gibberellin signal transduction presents...the SPY who O-GlcNAc'd me. *Trends Plant Sci* **4**: 424–428
- Tseng T-s, Swain SM, Olszewski NE** (2001) Ectopic expression of the tetratricopeptide repeat domain of SPINDLY causes defects in gibberellin response. *Plant Physiol* **126**: 1250–1258
- Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun T-p** (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. *Plant Physiol* **135**: 1008–1019
- Ullah H, Chen J-G, Wang S, Jones AM** (2002) Role of a heterotrimeric G protein in regulation of Arabidopsis seed germination. *Plant Physiol* **129**: 897–907
- Vierstra RD** (2003) The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends Plant Sci* **8**: 135–142
- Wells L, Vosseller K, Hart GW** (2001) Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. *Science* **291**: 2376–2378
- Wen C-K, Chang C** (2002) Arabidopsis *RGL1* encodes a negative regulator of gibberellin responses. *Plant Cell* **14**: 87–100
- Wilson RN, Somerville CR** (1995) Phenotypic suppression of the gibberellin-insensitive mutant (*gai*) of Arabidopsis. *Plant Physiol* **108**: 495–502
- Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D** (2002) The SCF^{COI1} ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. *Plant Cell* **14**: 1919–1935
- Zentalla R, Yamauchi D, Ho T-hD** (2002) Molecular dissection of the gibberellin/abscisic acid signaling pathways by transiently expressed RNA interference in barley aleurone cells. *Plant Cell* **14**: 2289–2301