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Update on Signal Transduction

Genetic Analysis of Gibberellin Signal Transduction[1]

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In recent years exciting progress has been made in understanding how endogenous plant hormones control plant development. One of the fundamental questions in this area of research is the mechanism by which the presence of a plant hormone molecule (the signal) leads to a change in plant development (the transduction pathway). This *Update* will discuss progress in the genetic analysis of signal transduction for the class of plant hormones collectively known as GAs. More extensive reviews on various aspects of GA research can be found in Hooley (1994), Ross (1994), and Sponsel (1995).

GA PHYSIOLOGY

The best-characterized physiological roles for the GAs are the promotion of shoot extension growth (e.g. internode elongation) and of reserve mobilization immediately after germination of cereal grains. Depending on the plant species, GAs may also be required for seed germination and for complete anther, seed, and fruit development (Takahashi et al., 1991; Hooley, 1994). Other aspects of plant development that may also be modulated by GAs include flower induction and development and anthocyanin biosynthesis. GAs are also used for a variety of commercial purposes, although generally it is not known if their effects in these applications reflect the normal physiological roles of endogenous GAs.

Early experiments involving GA application and the identification of GA-responsive maize dwarves suggested that some GAs promote stem and leaf growth. More recently, research has been aimed at establishing a role for changes in the endogenous levels of biologically active GAs as regulators of plant development, particularly internode elongation (Reid and Howell, 1995). Our present understanding of GA physiology has resulted largely from the manipulation of endogenous GA levels by applying either GAs or chemical inhibitors of GA biosynthesis, or by using single gene mutants. The best examples of the genetic approach are the GA-deficient mutants of Arabidopsis, pea, and maize, which have a reduced ability to carry out step(s) in the GA biosynthetic pathway. Typically, this leads to a 10-fold reduction in the level of active GA(s) compared with WT plants, and a dwarf phenotype attributable to a reduction in internode length. In addition to reduced stature, organs such as leaves and fruits may also be reduced in size. GA-deficient mutants can be identified with relative ease by virtue of their dwarf growth habit, which is corrected by application of an active GA.

Mutants with altered GA signal transduction are usually more difficult to identify and analyze (see below); consequently, much of the research with GA-related mutants has concentrated on GA biosynthesis. Indeed, the relative ease of working with GA biosynthesis mutants has helped determine the nature of GA research. This field has historically focused on establishing (a) a physiological role for endogenous GAs in controlling plant development, and (b) the GA biosynthetic pathways of various organisms and the characteristics of enzymes involved in GA metabolism.

GA BIOSYNTHESIS

Gibberellins are tetracyclic diterpene carboxylic acids. They were identified more than 50 years ago from the fungus *Gibberella fujikuroi*, which infects rice plants and causes Bakanae or "silly rice" disease (Takahashi et al., 1991). Nearly 100 different gibberellins have now been identified from a range of plants and several fungi (Sponsel, 1995) because of the structure of the GAs (19 or 20 carbons) combined with different patterns of hydroxylation and other modifications. Fortunately for those interested in GA physiology and signal transduction, not all GAs are found in any one plant species, and only a handful appear to possess biological activity per se. The GAs thought to be active in their own right are GA_1, GA_3, and possibly GA_4. The remaining GAs are thought to be precursors of active GAs (e.g. GA_9 and GA_{20}), deactivated forms of active GAs (e.g. GA_{34} and GA_{29}), or secondary metabolites that may lack any physiological role. GAs can also occur as conjugates, but, although a role for conjugate formation and breakdown in modifying the amount of active GAs has been suggested, it has not been demonstrated.

GENETIC ANALYSIS OF GA SIGNAL TRANSDUCTION

For the purposes of this review, GA signal transduction is considered to be the series of biochemical events leading

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Abbreviation: WT, wild-type.

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from the perception of the active GA molecule to the final response (Fig. 1, continuous lines). Figure 1 also illustrates some of the difficulties arising from the use of complex responses such as plant growth, which is the primary phenotype employed in identifying mutants affected in GA signal transduction. Because plant growth is affected by multiple signal-transduction pathways in diverse ways, it is often difficult to determine if an alteration in GA signaling is responsible for an observed growth defect.

It is likely that some signal-transduction pathways affect growth independent of GA signaling (Fig. 1, dotted lines), whereas others affect growth by interacting with the GA transduction pathway and modifying its activity (Fig. 1, dashed lines). It is also possible to imagine interaction at a portion of the pathway that is common to all GA responses or at a level that is specific to a subset of the possible GA responses. Finally, complex interactions involving effects on GA signal transduction as well as separate effects on growth can also be envisioned. To understand GA signal transduction, we must be able to distinguish between these different mechanisms and identify effects specific to the GA transduction pathway.

Since GAs are required for normal internode elongation, all mutants affected in stem growth by mechanisms other than altered GA metabolism can be considered as potential GA signal-transduction mutants. They can be collectively referred to as "stature" mutants, and include both specific GA signal-transduction mutants and mutants not directly related to GA signaling. Based on their resemblance to GA-deficient mutants or WT plants treated with GAs, stature mutants can be divided into three groups with regard to GA signal transduction.

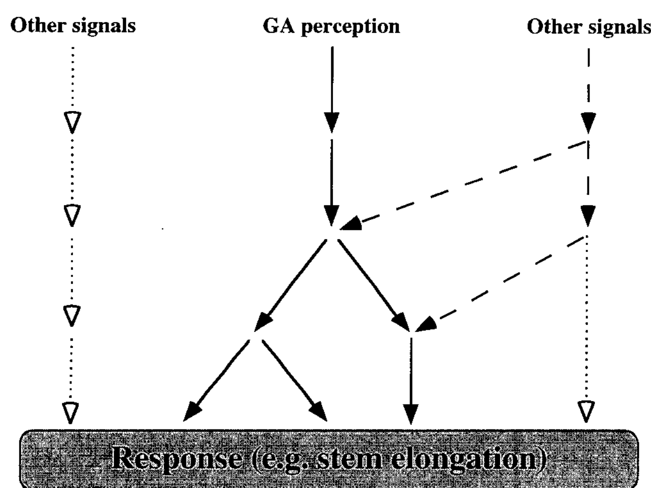

Figure 1. Model of GA signal transduction. Arrows indicate either promoting or inhibiting interactions between components of one signal transduction pathway, and between different pathways. Solid lines represent parts of the GA signal-transduction pathway, and dashed lines indicate other pathways that interact with it. Dotted lines represent signal-transduction pathways that are independent of GA signaling but influence the same response. Mutations affecting any of these signaling pathways may alter plant development and the response to GAs, but only a subset of these mutations will directly involve GA signal transduction.

Decreased GA Signal-Transduction Mutants

The first group of signal-transduction mutants is composed of dwarf mutants that appear to be complete phenocopies of GA-deficient plants but cannot be returned to a WT phenotype by GA application. These properties suggest that the mutants are affected in a portion of the signal-transduction pathway that is common to all GA responses. All of the known mutants in this class are partially or fully dominant and have been studied in greatest detail in maize (*D8–1*), wheat (*Rht3*), and Arabidopsis (*gai*) (Hooley, 1994; Ross, 1994). These mutants are not "GA insensitive," since they are further dwarfed by reducing endogenous GA levels and the original dwarf phenotype can be recovered by GA application (Winkler and Freeling, 1994).

Genetic analysis suggests that *Rht3, D8–1, D8-Mpl* (*Mpl-1* is probably a less severe *d8* allele; Winkler and Freeling, 1994), and *gai* are gain-of-function mutations (Gale et al., 1975; Harberd and Freeling, 1989; Peng and Harberd, 1993). Furthermore, the *D8* mutations are not likely to exert their effects by causing dramatic overproduction of the WT gene product, since plants possessing three or four copies of the WT *d8* gene are not dwarves (Harberd and Freeling, 1989). Putative loss-of-function derivative *gai* alleles have been identified by screening a M_1 population (Peng and Harberd, 1993) and M_2 populations (Wilson and Somerville, 1995) of mutagenized *gai* plants for increased stature. Plants homozygous for these derivative alleles are indistinguishable from WT plants, at least under normal growing conditions.

These results suggest two mechanisms by which the WT RHT3, D8, and GAI proteins may act (for clarity, all proteins are written in uppercase in this article, although convention varies between the different species). The WT genes may either encode a component of the signal-transduction pathway or encode a protein that is not normally involved in GA signaling. In the former case, the mutant *Rht3, D8,* and *gai* alleles are dominant-negative mutations that encode altered signal-transduction proteins that prevent normal functioning of the GA signal-transduction pathway. One example of this type of mutation is the "poison subunit" model, in which the WT protein is part of a multisubunit protein complex involved in GA signaling. However, this model predicts that the mutant phenotype should be sensitive to the WT gene dosage, which is not observed for *Rht3, D8–1, D8-Mpl* (Gale et al., 1975; Harberd and Freeling, 1989), and possibly *gai* (Peng and Harberd, 1993; Wilson and Somerville, 1995). In the latter case, the mutant protein has acquired a new function or a new pattern of expression that allows it to interfere with GA signaling. Although it is not possible to distinguish between these models at present, the *GAI* locus has recently been cloned (N.P. Harberd, personal communication). Detailed analysis of the molecular and biochemical nature of *gai* and of the derivative alleles should help to resolve these questions.

Increased GA Signal-Transduction Mutants

The second group of GA signal-transduction mutants includes those that resemble WT plants treated with

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active GAs. They have been best characterized in pea (*la* and *cry*^{s}), tomato (*pro*), barley (*sln*), and Arabidopsis (*spy*) (Hooley, 1994; Reid, 1994). Common phenotypes include elongated internodes, parthenocarpic fruit growth (among the dicotyledonous species), and poor pollen production. All of these mutants potentially have increased GA signal transduction and are genetically recessive, and it appears that they represent loss-of-function mutations affecting a portion of the signal-transduction pathway that suppresses GA action. Furthermore, since these mutants closely resemble WT plants treated with GA, this negatively acting portion of the signal-transduction pathway appears to influence all aspects of plant development that can be controlled by changing GA levels. Two models of the signal-transduction pathway are consistent with this observation. The simplest model is that all GA-regulated processes share a common positively acting component of the signal-transduction pathway that is negatively regulated by the protein products of the WT genes (Fig. 2A). More complex models envisage several distinct positively acting signal-transduction components, possibly specific for different aspects of GA-regulated development, all of which are regulated by the same negatively acting portion of the signal-transduction pathway (Fig. 2B).

The barley *sln* mutation results in a plant that appears to be completely saturated for GA-induced growth (Chandler, 1988; Lanahan and Ho, 1988). In addition, *sln* plants have both increased and GA-independent α-amylase production in germinating seeds and are male-sterile. Since homozygous *sln* plants are unaffected by changes in endogenous GA levels, it appears that SLN is critical for normal GA signaling in barley and that other proteins are unable to compensate, even partially, for loss of SLN activity.

In contrast to *sln*, the *spy* (Arabidopsis) and *pro* (tomato) mutants both possess a phenotype that mimics WT plants treated with a nonsaturating GA dose, which increases signal transduction (Jones, 1987; Jacobsen and Olszewski, 1993). Both *spy* and *pro* plants retain the ability to respond to changing GA levels: growth is promoted when exogenous GA is applied, and both mutants can only partially suppress the dwarf phenotype caused by severely reduced GA biosynthesis. In contrast to their effects on stem growth, *spy* mutations can completely overcome the inability of GA-deficient seeds to germinate (Jacobsen and Olszewski, 1993). It is not known if any of the *spy* or *pro* mutations are null, so it is not clear if SPY or PRO are each partially redundant or whether sufficiently severe *spy* or *pro* mutations would result in constitutive signal transduction (as in *sln*). Genetic analysis reveals that the relatively severe *spy-4* allele is epistatic to the *gai* mutation (Jacobsen et al., 1996), suggesting that the mutant GAI protein and SPY both act in the same pathway to suppress GA signaling. The epistasis also suggests that SPY acts downstream of the mutant GAI protein (Fig. 3).

The *SPY* locus has recently been cloned and found to encode a member of the tetratricopeptide-repeat-containing class of proteins (Jacobsen et al., 1996). These proteins form a diverse group but share the common feature that they are thought, and in some cases have been shown, to function via tetratricopeptide-repeat-domain-mediated protein-protein interactions (Lamb et al., 1995). Although it is not known if SPY interacts with other proteins involved in signal transduction, such a model is clearly consistent with the genetic model of SPY action.

The *la* and *cry*^{s} mutations of pea are of particular interest because plants of genotype *lala CRY-* or *LA-cry*^{s}*cry*^{s} have only a mildly elongated phenotype compared with WT plants, whereas the *la cry*^{s} double mutant appears, like *sln* in barley, to possess saturated signal transduction regardless of endogenous GA levels (Potts et al., 1985). The *LA* and *CRY* loci are therefore thought to be duplicate genes, since a WT allele at either locus prevents the mutant phenotype. Nevertheless, it is not clear if both the LA and CRY proteins are similar to SPY and PRO. If all of these proteins do possess similar functions, then greater functional redundancy must exist in pea compared with the other two species. An alternative explanation for the phenotype of the *la cry*^{s} double mutant is that CRY is functionally distinct from LA, and loss of both LA and CRY function has a strongly synergistic effect on GA signaling. The *la cry*^{s} phenotype cannot be completely mimicked at the cellular level by exogenous GA application (Murfet, 1990), suggesting that LA or CRY may also be involved in other pathways controlling plant development in addition to GA signal transduction. Since the *cry*^{s} allele is thought to alter

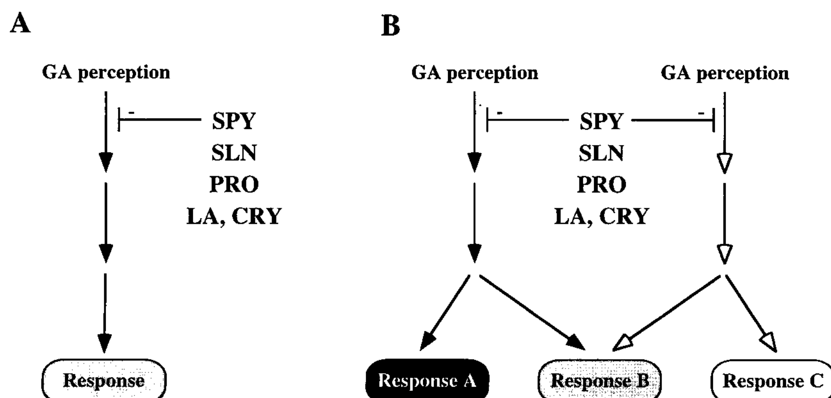

Figure 2. Models explaining the similar phenotypes of WT plants treated with GAs and plants homozygous for the *spy* (Arabidopsis), *sln* (barley), *pro* (tomato), or *la* and *cry*^{s} (pea) mutations. A, The WT proteins inhibit a positively acting branch of the GA signal-transduction pathway that is shared by all known GA responses. B, A more complex model in which the WT proteins inhibit two distinct positively acting pathways that together control GA responses.

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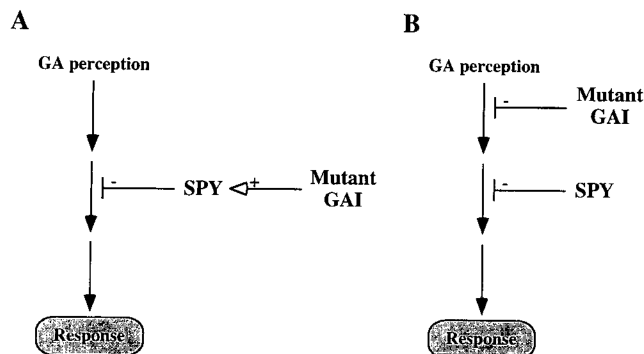

Figure 3. Models of the role of SPY and the mutant GAI protein in the GA signal-transduction pathway that can explain the epistasis of *spy-4* over *gai*. A, The mutant GAI protein positively regulates (directly or indirectly) SPY activity, which in turn negatively regulates GA signal transduction. B, The mutant GAI protein and SPY independently negatively regulate GA signal transduction, and SPY acts downstream. Arrows may represent more than one biochemical event in the transduction pathway.

phytochrome-mediated control of internode elongation (Murfet, 1990), an exciting possibility is that CRY might be involved in integrating GA and phytochrome signal transduction.

Two additional mutants, *gar2* and *gas1–1*, that appear to possess increased GA signal transduction, have recently been described in Arabidopsis (Carol et al., 1995; Wilson and Somerville, 1995). Both mutants were identified as extragenic suppressors of the *gai* mutant. The *gas1–1* mutation is recessive and a weak suppressor of *gai*; the *gar2* mutant is fully dominant and is also a partial *gai* suppressor. It is not yet clear if *gar2* represents a gain-of-function mutation or if both WT *GAR2* genes are absolutely required for normal GAR2 function.

Potential GA Signal-Transduction Mutants

The third and by far the largest group of stature mutants includes those that possess altered vegetative growth and response to GAs, but are not complete phenocopies of WT plants with increased or decreased endogenous GA levels. Examples include mutants deficient in type-B phytochromes (increased GA response) and numerous mutants with defects that prevent normal elongation growth (decreased GA response) (see Reid, 1994). Since growth is a complex phenomenon, this group is probably composed mostly of mutants unaffected in GA signal transduction, but is also likely to contain mutants affected in some, but not all, aspects of GA signaling. The inability of GA signal-transduction mutants in this class to fully mimic GA-deficient or GA-treated plants would be consistent with their being affected at a point in the GA signal-transduction pathway that is specific to only a subset of GA responses (Fig. 1). In addition, if multiple independent GA signal-transduction pathways exist (Fig. 2B), it is possible that all loss-of-function mutants in positively acting components of one signal-transduction pathway will not fully mimic GA-deficient plants. The major problem with dealing with this group, and with a genetic analysis of GA signal transduction in general, is distinguishing which mutants are in fact specifically affected in GA signaling.

One method of characterizing potential GA signal-transduction mutants that has not been used to its full potential is to construct a GA dose-response curve. Since GA signal transduction appears to regulate GA biosynthesis (see below), it is essential that these experiments be performed with the endogenous concentration of the active hormone as low as is practicable. This can easily be achieved by using either well-characterized biosynthesis mutants or chemical inhibitors of GA biosynthesis (Takahashi et al., 1991; Ross, 1994). It is also important that doses ranging from a concentration insufficient to elicit a measurable response to a concentration that saturates the response are used, since mutations may alter only part of the dose-response curve. Analysis of these curves is also aided by the use of a consistent terminology, such as that described by Firn (1986). In particular, the term "sensitivity" is confusing because it is used in a variety of conflicting ways to describe changes in the response to applied GAs.

Dose-response curves allow a detailed description of the change of a particular organ in response to GA but do not necessarily define mutants specifically involved in GA signaling. This limitation stems from the complex nature of virtually all whole-plant GA responses, particularly those involving growth. One solution is to measure a simpler GA response that is affected by no other, or few other, regulators of plant development, such as molecular markers specifically regulated by GAs. The best examples of this type of molecular marker for GA signal transduction are the α-amylase genes expressed in the aleurone cells of germinating cereal grains in response to GA. The expression of the α-amylase genes not only provides an excellent marker for signal transduction in this system, but analysis of the promoter region has identified elements involved in the transcriptional regulation of these genes (Jacobsen et al., 1995). This has allowed the identification of a GA-regulated gene encoding a trans-acting factor that regulates the expression of α-amylase, which may itself be a component and also an excellent marker of signal transduction (Gubler et al., 1995).

Since essentially all of the potential GA signal-transduction mutants are stature mutants, vegetative molecular markers highly specific for GA signal transduction are required. Candidate genes include *GA4*, *GA5*, *GASA*, and γ-*TIP* (Arabidopsis), *GAST1* and *GAD* (tomato), chalcone synthase (petunia), a cell-wall invertase (pea), α and β tubulin (oat), and a *cdc2* homolog and two cyclins (rice) (Huttly and Phillips, 1995; Jacobsen and Olszewski, 1995). The problem with these markers is that none has yet been shown to be specifically regulated by GA signal transduction rather than by growth or other signals regulating plant development.

At present, the best candidate molecular marker is the *GA5* gene of Arabidopsis, which is thought to encode one of at least three GA biosynthesis enzymes with GA 20-oxidase activity (Phillips et al., 1995; Xu et al., 1995). Previous studies suggest that in addition to promoting shoot growth, GA signal transduction might negatively regulate

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the activity of the GA 20-oxidase(s), which would lead to reduced biosynthesis of active GAs and a corresponding reduction in the activity of the signal-transduction pathway in a type of negative feedback control (Hedden and Croker, 1992). The endogenous GA levels in several known GA signal-transduction mutants are consistent with this hypothesis: those with increased signal transduction possess reduced levels of active GAs, whereas the mutants with decreased signal transduction possess higher levels of active GAs compared with WT plants (Ross, 1994). Quantification of mRNA in GA-deficient mutants with or without applied GAs suggests that the negative feedback control of GA biosynthesis results, at least in part, from changing mRNA levels of the *GA5* gene (Phillips et al., 1995; Xu et al., 1995). Whether the regulation of 20-oxidase activity is regulated by changes in seedling growth itself, or more directly by GA signal transduction, has been addressed by determining GA levels in seedlings of the *lk*, *lka*, and *lkb* mutants of pea. These dwarf mutants are not phenocopies of GA-deficient pea plants, and GA levels are not altered compared with WT plants (Lawrence et al., 1992). Therefore, it appears that at least in these mutants altered stem growth is not in itself sufficient to regulate 20-oxidase activity. Thus, this feedback mechanism may be entirely mediated by GA signal transduction. One potential complication with using *GA5* as a molecular marker is that expression may also be slightly increased when long days are used to promote bolting and flowering (Xu et al., 1995). It is possible that the regulation of *GA5* in response to photoperiod and the GA signal-transduction pathway involves the same mechanism, so that all mutants with altered *GA5* expression will be involved in GA signaling.

Finally, an additional strategy that can be used to identify new GA signal-transduction mutants from this class may be to examine their interaction with established GA signaling mutants. This approach may be most successful with Arabidopsis, since it is the only species in which both increased and decreased signal-transduction mutants have been identified. For example, the *spy-4* mutation is epistatic to the *gai* mutation in Arabidopsis, suggesting that SPY acts downstream of the mutant GAI protein in the signal-transduction pathway. The interaction of *spy* with other potential GA signal-transduction mutants might also be informative. As mentioned above, one possibility is that the SPY protein acts to negatively regulate several, distinct, positively acting components of the GA signal-transduction pathway (Fig. 2B). If this hypothesis is correct, it may be possible to identify novel GA signaling mutants based on their interaction with *spy* or other GA signal-transduction mutants. For example, new mutants that affect only one component of the GA signal-transduction pathway and do not mimic GA-deficient WT plants could be identified if, like *gai*, they are hypostatic to *spy-4*.

ARE SOME GA SIGNAL-TRANSDUCTION MUTANTS EMBRYO-LETHAL?

One potential problem with a genetic analysis of GA signal transduction is the assumption that all GA signal-transduction mutants can be identified at the seedling stage. Although this is clearly true for the mutants isolated to date, it may not be possible to identify all GA signaling mutants in this manner. If the model of GA signal transduction presented in Figure 1 is correct, it is surprising that no loss-of-function mutations reducing GA signaling have been identified. This deficiency could be explained if the phenotype of mutants with severely reduced GA signal transduction is different from that assumed previously. It has generally been thought that although they are present at relatively high endogenous levels, GAs are not essential for normal embryo and seed development. However, recent analysis of GA biosynthesis mutants of pea suggests that GAs are required for embryo growth and seed survival: reduced endogenous GA levels in the embryo and endosperm of young seeds increases the probability of seed abortion and reduces the final size of surviving seeds (Swain et al., 1995).

Based on the results from pea, a physiological role for GAs in developing seeds predicts that mutants with reduced GA signal transduction (caused by reduced levels of active GAs or by impaired GA signaling) will be more likely to abort during seed development and may be found predominantly as embryo-lethal mutants in genetic screens. This suggestion is supported by the apparent absence of any viable GA biosynthesis mutants that completely lack endogenous active GAs. The weakly penetrant alteration in cotyledon position and number in *spy-2* seedlings (Jacobsen and Olszewski, 1993) also suggests that GAs may be involved in seed development. Furthermore, since *spy* mutants possess increased GA signal transduction, this observation indicates that both increased and decreased signal transduction may have deleterious effects on embryo and seed development. A large number of embryo-lethal mutants have already been identified in maize and Arabidopsis (e.g. Clark and Sheridan, 1991; Franzmann et al., 1995), but none have yet been shown to be involved in GA biosynthesis or signal transduction.

NEW SCREENS FOR GA SIGNAL-TRANSDUCTION MUTANTS

In addition to identifying GA signal-transduction mutants from the existing collection of GA stature mutants, it is probable that mutations in some loci related to GA signal transduction have not yet been described. One approach to identify GA signal-transduction mutants involves the use of genes that are specifically regulated by GA signaling. Assuming that a GA signal-transduction-specific gene can be identified, screens could be designed using the expression of a reporter gene fused to the appropriate promoter. For example, it may be possible to use changes in the expression of a reporter gene fused to the *GA5* promoter to identify new GA signal-transduction mutants. Removing the requirement for a change in stature from the screen might also allow the identification of mutants with unexpected phenotypes (e.g. with no clear growth abnormalities under standard conditions) that are involved in GA signaling. This type of screen has recently enabled the isolation of phytochrome signal-transduction mutants affecting a subset of phytochrome responses (Li et al., 1995).

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Another strategy is to identify mutants that suppress or enhance the phenotypes of known GA signaling mutants. The *gas1–1* and *gar2* mutants have already been identified by screening for suppressors of *gai*. This approach should also allow identification of signal-transduction mutants that cannot otherwise be obtained. In particular, functional genetic redundancy may prevent the isolation of some loss-of-function GA signal-transduction mutations when WT plants are mutagenized. For example, GA signal-transduction genes in both maize (*d8* and *d9*) and pea (*LA* and *CRY*) may be duplicate genes with redundant functions (Potts et al., 1985; Winkler and Freeling, 1994). Mutagenizing plants already homozygous for a loss-of-function GA signaling mutation may therefore allow additional signal-transduction genes to be identified by their effect on plant development.

INTERACTIONS BETWEEN GA AND PHYTOCHROME SIGNAL TRANSDUCTION

The possible interaction between phytochromes and GAs has been the subject of recent debate. This is largely due to the broad similarity between the phenotypes of GA-treated plants and plants deficient in type B (light-stable) phytochromes, and because ectopic expression of various *phy* genes results in a dwarf phenotype (Whitelam and Harberd, 1994). Although there is still debate on the possible control of GA biosynthesis by phytochrome, most studies suggest that type-B phytochromes modify the response to GAs (e.g. Weller et al., 1994; Lopez-Juez et al., 1995). Since GAs and phytochromes both influence plant growth, it is likely that the signal-transduction pathways of various phytochromes (at least five are present in Arabidopsis) and GAs interact in some way. Very little has been done to investigate this question using a genetic approach. Further use of GA dose-response curves and a detailed analysis of the interaction between mutants possibly involved in GA and phytochrome signal transduction may provide information about both pathways.

CONCLUSIONS

The genetic analysis of GA signal transduction will continue to provide insights into the nature of the GA signaling pathway and the control of plant development by GAs. Although difficulties with the identification of new GA signaling mutants remain, further analysis of GA-regulated genes may help to resolve these problems. It is clear that altered plant growth is not by itself sufficient to identify all GA signal-transduction mutants. One alternative is to identify GA signal-transduction-specific molecular markers that can be used to measure the activity of this pathway. Although several candidates have been identified, the regulation of these genes by GAs, growth, and other factors needs to be completely characterized before this approach can be used to its full potential.

More detailed analyses of existing GA signaling mutants, including the cloning of the *GAI* and *SPY* loci, should also provide new solutions. For example, it may be possible to identify proteins involved in GA signal transduction that directly interact with GAI and SPY. The existing mutants also reveal that GA signal transduction is composed of both positively and negatively acting components that interact with each other, and perhaps with other signal-transduction pathways, to determine the final GA response. Hence, GA signal transduction is unlikely to be a simple linear pathway connecting GA perception to the final response. Finally, a more complete understanding of the genetics of GA signal transduction may reveal aspects of GA physiology that have not been identified previously by using GA biosynthesis mutants.

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