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SPINDLY's role in the gibberellin response pathway

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SUMMARY

The *SPINDLY* (*SPY*) locus of *Arabidopsis thaliana* is believed to be involved in gibberellin (GA) signal transduction. The six known mutations at this locus cause a phenotype that is consistent with constitutive activation of the GA signal transduction pathway. *spy* alleles are epistatic to *gai*, a mutation conferring gibberellin-insensitivity, indicating that SPY acts as a negative regulator of GA signal transduction, downstream of GAI. *SPY* was cloned using a T-DNA insertion in the *spy-4* allele. *SPY* encodes a 914 amino acid protein with an N-terminal TPR region (a likely protein-protein interaction domain) and a novel C-terminal domain. The *spy* mutants show that both the N- and C-terminal domains of SPY are functionally important. *spy-4* is likely to be a null allele and displays some morphological defects not seen in the other alleles. A 35S:*SPY* construct rescues the *spy* mutant phenotype, but does not show any gain-of-function *SPY* phenotypes. Smaller constructs overexpressing different domains of the SPY protein have no effect on plant development.

Key words: Plant hormone, Gibberellin response mutant, *SPINDLY* gene

INTRODUCTION

West and Phinney (1956) first demonstrated that gibberellins (GAs) were endogenous compounds in higher plants. Since this discovery, over 80 naturally occurring GAs have been identified (Pharis et al., 1992), and GAs have been shown to be important for many aspects of plant growth and development (for reviews see Jones, 1973; Pharis and King, 1985; Hooley, 1994).

Much of what we know of the role of GAs in normal plant development comes from the study of GA-deficient mutants. At least 50 mutants have been described in 18 different species, which are deficient in GAs and exhibit decreased plant stature, primarily due to decreased internode elongation (King, 1988). In these mutants GA treatment reverses the phenotype to yield plants with nearly normal stature and internode length. The most extreme GA-deficient mutants, isolated in *Arabidopsis* and tomato, show that GAs are also required for seed germination and for normal floral development (Koornneef and van der Veen, 1980; Koornneef et al., 1981, 1990). Using the *gib1* mutant of tomato it was shown that GA is required for the initiation of premeiotic S phase of the pollen mother cells (Nester and Zeevaart, 1988; Jacobsen and Olszewski, 1991). *gib1* was also used to show that GAs are important in the growth of tomato roots. The elongation rate of excised *gib1* roots was only 40% of wild type, and GA treatment reversed this effect (Butcher et al., 1990).

In *Arabidopsis*, mutations causing GA deficiency have been mapped to five loci *GA1*, *GA2*, *GA3*, *GA4* and *GA5* (Koornneef and van der Veen, 1980). The *GA1* locus encodes the gibberellin biosynthesis enzyme *ent*-kaurene synthase A (Sun et al., 1992). The strong *ga1-2* allele is a chromosomal rearrangement within the *GA1* gene. Relative to wild type, *ga1-2* plants are extremely dwarfed and dark green and the flowering stems fail to elongate. *ga1-2* floral development is abnormal. Petals and stamens fail to elongate properly and stamens fail to open, resulting in complete male sterility (Koornneef and Van der Veen, 1980). GA treatment corrects these mutant defects and yields fertile plants. However, the seeds produced on these plants require exogenous GAs for germination. Another strong allele, *ga1-3*, has been shown to exhibit moderately delayed flower initiation when grown under inductive conditions (long days), and to absolutely require exogenous GA for flowering when grown under non-inductive conditions (short days) (Wilson et al., 1992).

GA is known to stimulate changes in gene expression in a number of systems including the aleurone layer of germinating cereal seeds (for reviews see Jacobsen and Chandler, 1987; Hammerton and Ho, 1986) and shoot tissues (Shi et al., 1992; Weiss et al., 1992; Jacobsen and Olszewski, 1996). The expression of α-amylase, the gene most extensively studied in the aleurone system, is regulated at the level of transcription (Jacobsen and Beach, 1985). Analysis of a barley α-amylase gene promoter has resulted in the isolation of a GA-regulated *myb*-type transcriptional activator (*GAmyb*), which may be a component of the GA response pathway leading to α-amylase gene expression (Gubler et al., 1995). *GAmyb* is induced within 1 hour of GA treatment, preceding the induction of α-amylase, and this induction is not blocked by the protein synthesis inhibitor, cycloheximide.

Very little is known about the mechanisms by which plant cells perceive GAs, or the events that occur between perception and the early GA responses, such as changes in gene expression. We are just beginning to learn about these processes through the study of gibberellin response mutants. Two major classes of these mutants are known from a number

of plant species. The first is the GA-insensitive mutants. These mutants are dominant or semi-dominant and have a phenotype which resembles that of the GA-deficient mutants (reviewed by Hooley, 1994). As the name implies, these mutants show reduced sensitivity to applied GAs. A single semi-dominant mutant of this type called *gibberellin-insensitive* (*gai*) is known from *Arabidopsis* (Koornneef et al., 1985). The *gai* phenotype resembles that of a partial loss-of-function GA biosynthesis mutant. *gai* mutants are shorter in stature and darker green than wild type, but in contrast to the strong GA biosynthesis mutants, *gai* seeds germinate and their flowers are fertile. A number of recessive *gai* alleles were isolated by screening for revertants of the semi-dominant *gai-1* allele (Peng and Harberd, 1993). Plants homozygous for these putative loss-of-function alleles were indistinguishable from wild-type plants, suggesting that the wild-type *GAI* gene function is dispensable. One interpretation of this result is that the GA signal transduction pathway is redundant such that another gene(s) can substitute for the function of *GAI* (Peng and Harberd, 1993).

The second class of mutants is the 'slender' mutants. Slender is a term used to describe the phenotype of mutants in a number of species. The most studied of these are in pea, barley and tomato (reviewed by Hooley, 1994). These mutants are recessive and have a GA-overdose phenotype. This review focuses on the isolation and characterization of *spindly* mutants, slender-like mutants in *Arabidopsis*, and discusses the role of the *SPINDLY* gene in gibberellin signal transduction.

ISOLATION AND GENETIC CHARACTERIZATION OF *spy* MUTANTS

To isolate slender mutants in *Arabidopsis*, a two-stage selection/screen was used. First, EMS mutagenized seed of the Columbia ecotype was selected for mutants that could germinate in the presence of 35 mg/l paclobutrazol (Jacobsen and Olszewski, 1993). Paclobutrazol is a GA biosynthesis inhibitor, which at high concentrations completely inhibits the germination of wild-type seeds. A screen of 440,000 M2 seed yielded 69 true breeding lines, which consistently germinated on paclobutrazol. Many of these mutants phenotypically resembled mutants with defects in ABA biosynthesis or ABA response. Further analysis of these mutants identified two new ABA biosynthesis loci (Leon-Kloosterziel et al., 1996). The second stage of the screen was to analyze these 70 lines for mutants that were resistant to the dwarfing effects of foliar paclobutrazol applications. Initially two mutants, *spy-1* and *spy-2* (Jacobsen and Olszewski, 1993), and later a third mutant *spy-6* (S.E.J., unpublished results), were found to pass both levels of the screen (Table 1). *spy-3* came from a similar screen where the order of the screening steps was reversed. The same two-stage screen was used to test seed from 4900 *Agrobacterium* seed transformant lines (Feldmann, 1991), resulting in the identification of one additional mutant, *spy-4* (Jacobsen et al., 1996). F_1 complementation tests determined that all five of these mutants are allelic. A similar screen was performed by Wilson and Somerville (1995) using EMS mutagenized seeds of the ecotype Landsberg *erecta* (L*er*) resulting in the identification of another allele, *spy-5*.

All of the *spy* mutants resemble wild-type plants that have been repeatedly sprayed with GA. Mutant plants are early flowering, and have yellowish rosette and cauline leaves. Cauline leaves occasionally curl upward and enclose the developing inflorescence. Flowers have a disorganized appearance and are partially male sterile due to a lack of pollen shed. Pistils that are unfertilized often elongate to a much greater extent than unfertilized wild-type pistils (parthenocarpic fruit development). All of these effects have also been observed in wild-type *Arabidopsis* plants sprayed with GA_3 (Jacobsen and Olszewski, 1993).

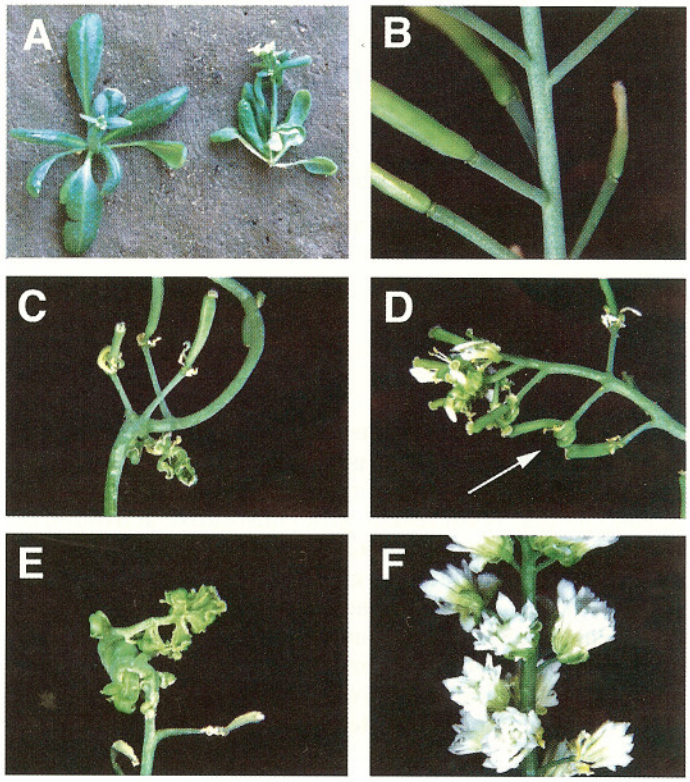

Fig. 1. *spy-4* phenotype. (A) A 20-day-old plant homozygous for *spy-4* (right) and a 20-day-old wild-type L*er* plant (left). *spy-4* was back crossed into the L*er* background five times. (B) A wild-type L*er* inflorescence showing a spiral arrangement of flowers. (C) A *spy-4* inflorescence showing defects in floral phyllotaxy. (D) A *spy-4* inflorescence with abnormal flowers. Arrow shows a carpelloid sepal. (E) A *spy-4* inflorescence that has terminated in carpelloid organs. (F) A *spy-4 ag-3* inflorescence.

Morphological and genetic characterization of the *spy* mutant alleles suggests that the wild-type SPY product does not act in GA biosynthesis or catabolism, but more likely acts in GA signal transduction (Jacobsen and Olszewski, 1993).

Table 1. *spindly* alleles

Allele	Ecotype	Mutagen	Strength
spy-1	Col	EMS	Unknown*
spy-2	Col	EMS	Weak
spy-3	Col	EMS	Weak
spy-4	WS	T-DNA	Strong
spy-5	L*er*	EMS	Weak
spy-6	Col	EMS	Weak

*The phenotypic strength of *spy-1* is not known as this line also carries a mutation at the linked *hy2* locus.

spy-1 was found to be largely epistatic to the strong *ga1-2* mutation. *spy-1 ga1-2* seed germinated without added GA, and even germinated when in the presence of paclobutrazol. Since paclobutrazol affects a different step in the GA biosynthesis pathway than does *ga1-2*, the combination of the mutation and the inhibitor treatment probably decrease GA biosynthesis to negligible levels. Since *spy-1* can simultaneously suppress the effects of *ga1-2* and paclobutrazol, it is likely that *spy-1* activates a basal level of gibberellin signal transduction, which is independent of gibberellin.

Later analysis determined that the *spy-1* line probably also contains a linked mutation at the *HY2* locus (Jacobsen et al., 1996). *hy2* mutants have long hypocotyls and flower earlier than wild type. This explains why *spy-1* displays defects not seen in the other alleles in the Columbia background, such as long hypocotyls and extreme early flowering.

Of all of the alleles, *spy-4* clearly has the strongest phenotype (Fig. 1A; Jacobsen et al., 1996). In contrast to the other alleles, the *spy-4* early flowering phenotype is semi-dominant. *spy-4* mutants also display morphological defects not seen in plants homozygous for the other alleles. For phenotypic analysis, *spy-4* was back crossed into the L*er* background five times. In this background, as well as in the WS background, *spy-4* plants exhibit defects in phyllotaxy. Wild-type *Arabidopsis* inflorescence meristems produce flowers in a spiral arrangement (Fig. 1B). *spy-4* plants often show deviations from this spiral pattern. Fig. 1C shows a part of a *spy-4* inflorescence stem where three flowers have emerged from the same region. *spy-4* mutant inflorescence stems also exhibit occasional fasciation and bifurcation (not shown), similar to that of the *clavata* mutants (Leyser and Furner, 1992). *spy-4* flowers are also abnormal. The first whorl sepals are often transformed into carpels or carpelloid organs (Fig. 1D), as is seen in some floral homeotic mutants such as *apetala2* (Bowman et al., 1989). In addition, *spy-4* mutant inflorescences terminate relatively early in carpelloid organs (Fig. 1E). Some of the *spy-4* morphological defects resemble those of plants that are ectopically expressing the floral homeotic gene *AGAMOUS* (Mizukami and Ma, 1992). Furthermore, plants overexpressing *AGAMOUS* are similar to *spy* mutants in that they have curled leaves and flower earlier than the wild type. To test whether some of the *spy-4* morphological defects could be due to inappropriate expression of *AGAMOUS*, the *spy-4 ag-3* double mutant was constructed. The double mutant shows an additive phenotype (Fig. 1F). Flowers and inflorescences of the double mutant are similar to those seen on *ag-3* plants (Bowman et al., 1989). Sepals did not exhibit homeotic conversion to carpels, and the stems did not prematurely terminate in carpel structures. However, the vegetative phenotypes were like those seen in *spy-4* single mutants. Plants still flowered early and showed defects in phyllotaxy. Thus, while AG overexpression may explain aspects of the *spy-4* inflorescence and floral phenotype, it seems not to be responsible for the *spy-4* vegetative defects.

We characterized the genetic interactions of the strong *spy-4* allele with mutations affecting GA biosynthesis or response (Jacobsen et al., 1996). Similar to what was observed for the *spy-1* allele, *spy-4* was largely but not completely epistatic to *ga1-2*. Fig. 2A shows the dramatic difference in overall plant stature and development between the *ga1-2* single mutant and the *spy-4 ga1-2* double mutant. *spy-4 ga1-2* double mutant plants respond to GA treatment to yield plants which closely resemble *spy-4* single mutants (Fig. 2B). These results confirm earlier results obtained with the *spy-1* allele (Jacobsen and Olszewski, 1993), which show that the effects of *spy* and GA biosynthesis are additive. *spy* mutants appear to activate a constitutive level of GA signal transduction, but GA response appears to be unaffected. At least two models can account for this relationship of *spy* and *ga1-2* (Fig. 3). The first is that SPY acts as a negative quantitative modulator of GA signal transduction (Fig. 3A). A second model is that SPY acts to completely shut off one branch of a redundant GA signal transduction pathway (Fig. 3B).

As first demonstrated by Koornneef et al. (1985), *gai* mutants show a reduced sensitivity to applied GAs. Whereas 50 μM GA_3 completely reverses the phenotypic effects of a strong GA-deficient mutant (Fig. 2C), it has little effect in the *gai* back-

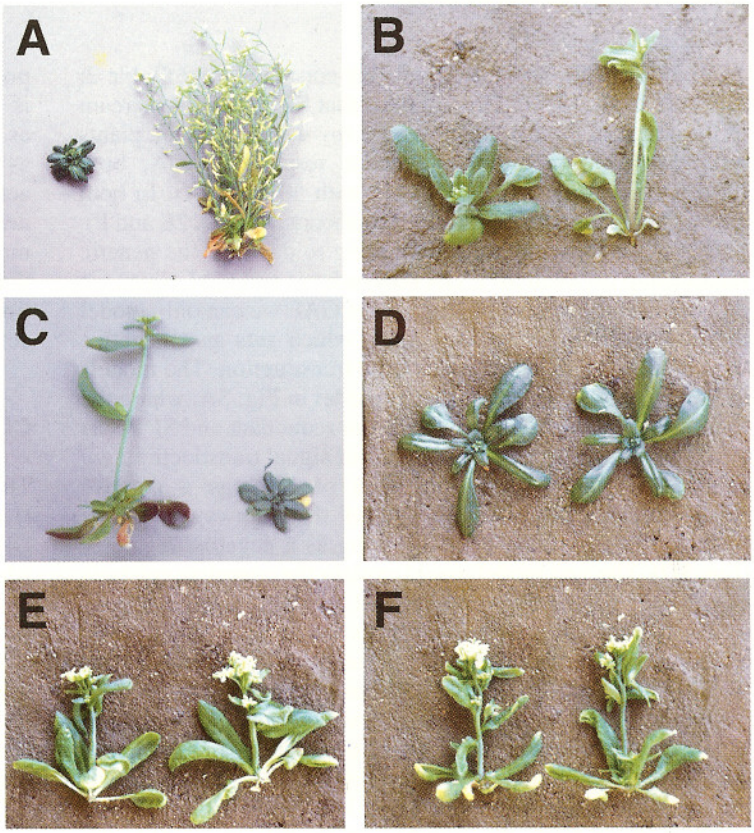

Fig. 2. *spy-4 ga1-2* double mutants and the response of different genotypes to GA treatment. (A) *ga1-2* plant (left) and a *spy-4 ga1-2* plant (right). Both plants are the same age. (B) A *spy-4 ga1-2* plant that has been sprayed with water (left) and a *spy-4 ga1-2* plant that has been sprayed with 50 μM GA_3 (right). (C) A *ga1-2* plant sprayed with water (right) and a *ga1-2* plant sprayed with 50 μM GA_3 (left). Both plants are the same age. (D) A *gai* plant sprayed with water (left) and a *gai* plant sprayed with 50 μM GA_3 (right). (E) A *spy-4* plant sprayed with water (left) and a *spy-4* plant sprayed with 50 μM GA_3 (right). (F) A *spy-4 gai* plant sprayed with water (left) and a *spy-4 gai* plant sprayed with 50 μM GA_3 (right). All of the plants in B, D-F were 20 days old.

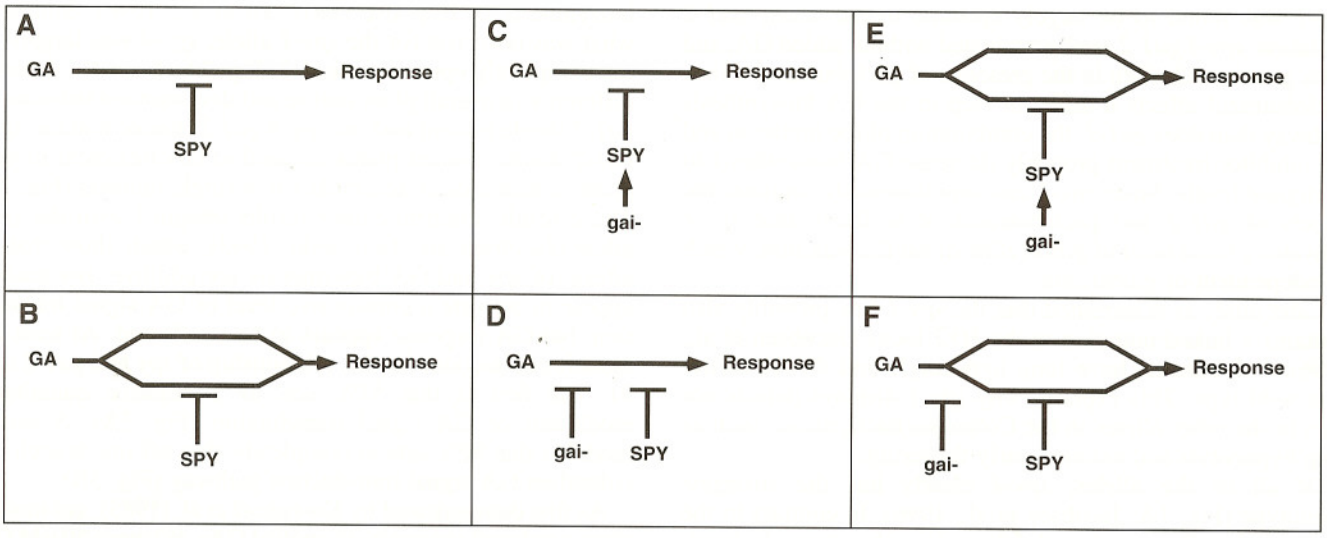

Fig. 3. Alternative models to explain the relationship between gibberellin biosynthesis, the action of wild-type SPY protein and the action of the mutant *gai* gene product. (A and B) Two different models to explain the relationship between SPY and gibberellin biosynthesis. (C-F) Four different models to explain the relationship between gibberellin biosynthesis, the action of wild-type SPY protein and the action of the mutant *gai* gene product. See text for details.

ground (Fig. 2D). *spy-4* is completely epistatic to *gai* (Table 2; Jacobsen et al., 1996). This suggests that SPY acts downstream of the action of GAI. To test whether *spy-4* single mutant plants or *spy-4 gai* double mutant plants respond to GA, both genotypes were repeatedly sprayed with 50 μM GA_3. In both cases, GA treatment did not alter the phenotype (Fig. 2E and F).

Fig. 3C-F shows models attempting to describe the genetic relationship between SPY, GA biosynthesis and GAI. Since we do not know the wild-type function of GAI, we can only model the action of the *gai* mutant form, which acts as a negative regulator of GA perception or signal transduction. The models in Fig. 3C and D are based on the model in Fig. 3A, where the GA signal transduction pathway is not redundant and SPY acts as a quantitative negative modulator of signal transduction. *gai* could either act directly upstream of SPY as a positive regulator (Fig. 3C), or at a part of the pathway which is upstream of the action of SPY, acting as a negative regulator (Fig. 3D). The first model would explain the complete epistasis of *spy* over *gai*. In the second model, *spy*'s complete epistasis over *gai* but incomplete epistasis over *ga1-2* could be explained by the fact that *gai* is phenotypically weaker than *ga1-2*. A second set of models is based on the model in Fig. 3B, where we assume that the pathway is redundant and that SPY keeps one part of the pathway off. Here again, *gai* could either act directly upstream of SPY (Fig. 3E) or on a part of the pathway upstream of SPY (Fig. 3F).

As is clear from the number of models presented, the precise relationship between GA biosynthesis, SPY and GAI is still unclear. This relationship should become more clear as we identify additional genes that act on this pathway, and as we learn more about the exact molecular mechanism of action of SPY and GAI. It will also be useful to know the GA dose-response relations of all of the single, double and triple mutant genotypes. What is consistent in all of the models is that SPY acts as a negative regulator of GA signal transduction and acts downstream of both GA biosynthesis and the action of *gai*.

Table 2. *spy-4* is epistatic to *gai*

Genotype	Number of rosette leaves
spy-4	4.6±0.88*
spy-4 gai	3.6±0.92
gai	10.6±1.42

*Numbers represent the mean ± the standard deviation. Measurements were taken 18 days post-germination.

CLONING OF *SPINDLY*

The cloning of *SPINDLY* has recently been described (Jacobsen et al., 1996). Briefly, genomic DNA flanking the T-DNA insertion in *spy-4* was used to isolate a 3.5 kb cDNA whose 5′ end is 13 bp downstream of the insertion. This cDNA has a complete ORF composed of 18 exons, which is predicted to encode a 914 amino acid protein. All of the *spy* alleles contain mutations in the *SPY* gene (Table 3) except for *spy-6*, which has not been analyzed.

The N terminus of SPY contains ten tetratricopeptide repeats (TPRs). TPRs are 34 amino acid repeated sequence motifs found in many eukaryotic and prokaryotic proteins. TPRs are

Table 3. Mutations in the *SPY* gene

Allele	Nucleic acid change	Alteration
spy-1	G to A at 3051	Alters splice site, exon 8 is skipped
spy-2	G to A at 2942	Alters splice site, exon 8 is skipped
spy-3	G to A at 3582	Gly 593 to Ser
spy-4	T-DNA insertion at 236	Reduced RNA abundance
spy-5	G to A at 5439	Cys 845 to Tyr

6479 bp of the *SPY* gene sequence was determined. All positions noted above are relative to the first nucleotide of this sequence. The start of the *SPY* cDNA sequence is at nucleotide 276 and the start methionine is at 946.

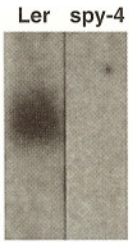

Fig. 4. RNA blot analysis of *SPY* RNA. The left lane contains RNA from wild-type L*er* plants. The right lane contains an equivalent amount of RNA from *spy-4* plants. The blot was probed with ^{32}P-labeled *SPY* cDNA.

thought to form amphipathic alpha-helices, which act as protein-protein interaction domains (Lamb et al., 1995). The *spy-1* and *spy-2* splicing mutations both delete the eighth exon, which encodes part of the eighth and ninth TPRs. These mutations show that the TPRs are important for SPY function, and suggest that SPY acts in part by interacting with other proteins through one or more of these domains. The 485 amino acid non-TPR C-terminal region does not contain any sequence motifs of known function. However, the *spy-3* and *spy-5* missense mutations in this domain show that it is also important for SPY function. The closest known SPY homologue is a *Caenorhabditis elegans* gene of unknown function, K04G7.3, which contains homology to both the TPR and the non-TPR domains. Database searches of the recently completed *Saccharomyces cerevisiae* and *Methanococcus jannaschii* genomes did not reveal any significant SPY homologues.

RNA blot analysis indicates that the size of the wild-type *SPY* RNA is approximately 3.5 kb (Fig. 4), a size similar to that of the *SPY* cDNA clone (Jacobsen et al., 1996). Sequences hybridizing to this cDNA clone are not detected in the T-DNA insertion allele *spy-4*, suggesting that this allele is an RNA null.

Preliminary in situ localization experiments suggest that *SPY* RNA is expressed in most plant tissues and in all of the layers (L1, 2 and 3) of each tissue (not shown). *SPY* expression was detected in apical inflorescence meristems, young flowers, stems, developing rosette and cauline leaves, shoot apical meristems and root tips. This pattern is consistent with the pleiotropic *spy* mutant phenotype. We have not yet examined older tissues such as developed rosette leaves, older stems or roots.

INAPPROPRIATE *SPINDLY* EXPRESSION

To help understand the wild-type function of SPY we made a series of constructs that overexpress either the full length SPY protein or specific portions of SPY. Each construct is driven by the constitutive 35S promoter and terminated by NOS 3′ sequences. Fig. 5A shows each construct and the number of primary transformants generated for each. SCO1 expresses the full length SPY coding region, SCO2 expresses only the SPY TPR region, SCO3 expresses only the SPY non-TPR C-term region, and SCO4 expresses only the eighth and ninth TPRs, which are missing in the *spy-1* and *spy-2* mutants. These lines all overexpressed transgenic *SPY* RNA of the appropriate size (Fig. 5B and data not shown). Amongst 112 primary SCO1 transformants, two displayed a *spy*-like phenotype, which was inherited in a dominant fashion. We also observed two *spy*-like plants amongst the 82 SC03 primary transformants. Because of the low frequency of these *spy*-like phenotypes, we presume that they are caused by transgene-induced silencing of the *SPY* locus. Besides these *spy*-like plants, no other phenotypes were observed for any of the four constructs. The fact that we did not observe any consistent dominant negative or dominant positive phenotypes from SCO 2, 3, or 4 may suggest that the smaller SPY domains are not functional when not in the context of the whole SPY protein. Another possibility is that these shorter proteins are not stable.

To characterize the full length SCO1 transformants in more detail, ten T1 lines were selfed and the T2 plants were analyzed for any phenotypic abnormalities. Again, these plants showed no difference from the wild type. To show that the SCO1 transformants produce excess *SPY* RNA, we used RNA blot analysis to compare plants homozygous for the transgene with wild-type plants. Fig. 5B shows that one line, SCO1 line#5, contained approximately ten times more SPY RNA than the wild type.

To test whether the SCO1 construct is sufficient for SPY function, we crossed SCO1 line#5 to the *spy-5* mutant. Analysis of the F_2 and F_3 progeny from this cross indicate the SCO1 construct completely rescues the *spy-5* mutant phenotype (data not shown). Preliminary evidence suggests that SCO1 also complements the stronger *spy-4* mutant. These results indicate that the 35S:*SPY* construct can substitute for the wild-type *SPY* gene.

Since the *spy* mutations result in an increase in GA signal transduction, one might expect that overexpression of the *SPY* protein could act to decrease gibberellin signal transduction, resulting in dwarfed plants. To more carefully analyze any possible phenotypes of *SPY* overexpression, we compared plants homozygous for SCO1 with wild-type plants. Fig. 5C shows that there was no difference from wild type in overall morphology or flowering time. We also analyzed the phenotype of SCO1 line#5 in the presence of 50 μM GA_3, in the presence of 35 mg/l paclobutrazol, or in double mutant combinations with the *gai* mutant (not shown). Again, the transgenic plants always had the same phenotype as the wild type.

Thus it seems that a tenfold overexpression of *SPY* has no phenotypic consequences. Two possible explanations for this are that: (1) when SPY is made in excess, other components of the GA signal transduction machinery become limiting; or (2) post-transcriptional regulation limits the amount of active SPY protein produced. In the future the second possibility can be tested with SPY antibodies.

CONCLUSIONS

The genetic analysis of *spy* mutants suggest that the wild-type SPY protein acts as a negative regulator of gibberellin signal transduction. It is curious that the putative null *spy-4* allele, is completely epistatic to the gibberellin-response mutant *gai*, but not completely epistatic to a mutation causing a strong decrease in gibberellin biosynthesis. This may suggest that the gibberellin response pathway is more than a simple linear flow of signal from the perception of GA to the ultimate GA responses. The cloning of *SPINDLY* gives us almost no clue as to its specific biochemical function, except that it probably interacts with other proteins through its N-terminal TPR domains. Thus, the discovery of the biochemical nature of the

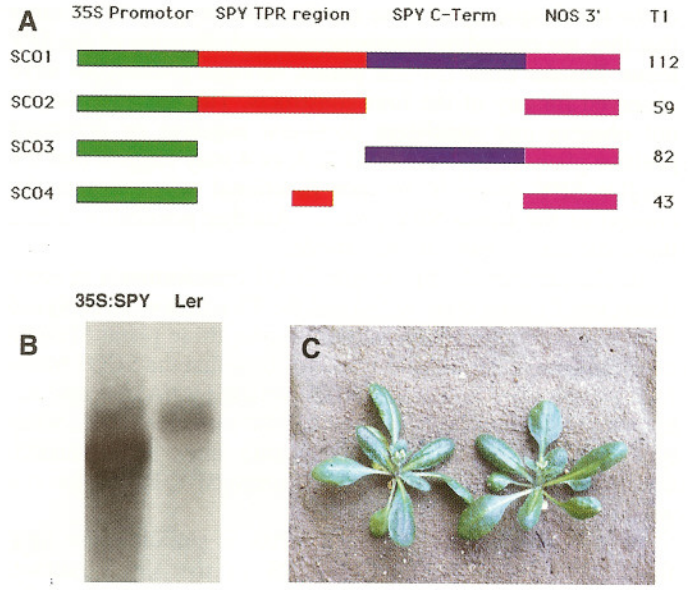

Fig. 5. *SPY* RNA overexpression. (A) Schematic diagram of the constructs used to overexpress the entire *SPY* RNA (SCO1) or only parts of the *SPY* RNA (SCO2-4). The column on the right (T1) shows the number of primary transformants generated for each of the SCO constructs. (B) RNA blot analysis of *SPY* RNA. The left lane contains RNA from plants homozygous for the SCO1 construct. The right lane contains RNA from wild-type L*er* plants. The blot was probed with ^{32}P-labeled *SPY* cDNA. The transgenic RNA is smaller than the wild-type RNA because the construct does not include the 5′ and 3′ untranslated RNA sequences. (C) A plant homozygous for the SCO1 construct (left) and a wild-type L*er* plant (right).

GA response pathway still awaits us. One step in this direction will be the cloning of SPY partner proteins and their molecular analysis.

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REFERENCES

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in Arabidopsis. *Plant Cell* **1**, 37-52.

Butcher, D. N., Clark, J. A. and Lenton, J. R. (1990). Gibberellins and the growth of excised tomato roots: Comparison of the *gib-1* mutant and wild type and responses to applied GA_3 and 2*S*, 3*S* paclobutrazol. *J. Exp. Bot.* **41**, 715-722.

Feldmann, K. A. (1991). T-DNA insertion mutagenesis in *Arabidopsis* - Mutational spectrum. *Plant J.* **1**, 71-82.

Gubler, F., Kalla, R., Roberts, J. K. and Jacobsen, J. V. (1995). Gibberellin-regulated expression of a *myb* gene in barley aleurone cells: evidence for Myb transactivation of a high-pI α-amylase gene promoter. *The Plant Cell* **7**, 1879-1891.

Hammerton, R. W. and Ho, T. H. D. (1986). Hormonal regulation of the development of protease and carboxypeptidase activities in barley aleurone layers. *Plant Physiol.* **80**, 692-697.

Hooley, R. (1994). Gibberellins-perception, transduction, and responses. *Plant Mol. Biol.* **26**, 1529-1555.

Jacobsen, J. V. and Beach, L. R. (1985). Control of transcription of α-amylase and rRNA genes in barley aleurone protoplasts by gibberellin and abscisic acid. *Nature* **316**, 275-278.

Jacobsen, J. V. and Chandler, P. M. (1987). Gibberellin and abscisic acid in germinating cereals. In *Plant Hormones and their Role in Plant Growth and Development* (ed. P. J. Davies), pp. 164-193. Martinus Nijhoff Publishers, Dordrecht.

Jacobsen, S. E. and Olszewski, N. E. (1991). Characterization of the arrest in anther development associated with gibberellin deficiency of the *gib-1* mutant of tomato. *Plant Physiol.* **97**, 409-414.

Jacobsen, S. E. and Olszewski, N. E. (1993). Mutations at the *SPINDLY* locus of Arabidopsis alter gibberellin signal transduction. *The Plant Cell* **5**, 887-896.

Jacobsen, S. E., Binkowski, K. A. and Olszewski, N. E. (1996). SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in Arabidopsis. *Proc. Nat. Acad. Sci. USA* **93**, 9292-9296.

Jacobsen, S. E. and Olszewski, N. E. (1996). Gibberellins regulate the abundance of RNAs with sequence similarity to proteinase inhibitors, dioxygenases, and dehydrogenases. *Planta* **198**, 78-86.

Jones R. L. (1973). Gibberellins: their physiological role. *Annu. Rev. Plant Physiol.* **24**, 571-598.

King, P. J. (1988). Plant hormone mutants. *Trends Genet.* **4**, 157-162.

Koornneef, M. and van der Veen, J. H. (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theor. Appl. Genet.* **58**, 257-263.

Koornneef, M., van der Veen, J. H., Spruit, C. J. P. and Karssen, C. M. (1981). Isolation and use of mutants with an altered germination behaviour in *Arabidopsis thaliana* and tomato. In *Induced Mutation: A Tool in Plant Research* (ed. P. H. Kitto), pp. 227-232. IAEA-SM-251/7, Vienna.

Koornneef, M., Elgersma, A., Hanhart, C. J., van Loenen-Martinet, E. P., Rijn, L. and Zeevaart, J. A. D. (1985). A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol. Plant.* **65**, 33-39.

Koornneef, M., Bosma, T. D. G., Hanhart, C. J., van der Veen, J. H. and Zeevaart, J. A. D. (1990). The isolation and characterization of gibberellin-deficient mutants in tomato. *Theor. Appl. Genet.* **80**, 852-857.

Lamb, J. R., Tugendreich, S. and Hieter, P. A. (1995). Tetratricopeptide repeat interactions - To TPR or not to TPR. *Trends Biochem. Sci.* **20**, 257-259.

Leon-Kloosterziel, K. M., Gil, M. A., Ruijs, G. J., Jacobsen, S. E., Olszewski, N. E., Schwartz, S. H., Zeevaart, J. A. D. and Koornneef, M. (1996). Isolation and characterisation of abscisic acid-deficient Arabidopsis mutants at two new loci. *Plant J.* **10**, 655-661.

Leyser, H. M. O. and Furner, I. J. (1992). Characterization of three shoot apical meristem mutants of *Arabidopsis thaliana*. *Development* **116**, 397-403.

Mizukami, T. and Ma, H. (1992). Ectopic expression of the floral homeotic gene *AGAMOUS* in transgenic *Arabidopsis* plants alters floral organ identity. *Cell* **71**, 119-131.

Nester, J. E. and Zeevaart, J. A. D. (1988). Flower development in normal tomato and a gibberellin-deficient (*ga-2*) mutant. *Am. J. Bot.* **75**, 45-55.

Peng, P. and Harberd, N. P. (1993). Derivative alleles of the *Arabidopsis* gibberellin-insensitive (*gai*) mutation confer a wild-type phenotype. *The Plant Cell* **5**, 351-360.

Pharis, R. P. and King, R. W. (1985). Gibberellins and reproductive development in seed plants. *Annu. Rev. Plant Physiol.* **36**, 517-568.

Pharis, R. P., Ruichuan, Z., Jiang, I. B., Dancik, B. P. and Yeh, R. C. (1992). Differential efficacy of gibberellins in flowering and vegetative shoot growth, including heterosis and inherently rapid growth. In *Progress in Plant Growth Regulation* (ed. C. M. Karssen, L. C. van Loon and D. Vreugdenhil), pp. 13-27. Kluwer Academic Publishers, Dordrecht.

Shi, L., Gast, R. T., Gopalraj, M. and Olszewski, N. E. (1992). Characterization of a shoot-specific, GA_3- and ABA-regulated gene from tomato. *Plant J.* **2**, 153-159.

Sun, T., Goodman, H. M. and Ausubel, F. M. (1992). Cloning the *Arabidopsis* GA1 locus by genomic subtraction. *Plant Cell* **4**, 119-128.

Weiss, D., van Blokland, R., Kooter, J. M., Mol, J. N. M. and van Tunen, A. J. (1992). Gibberellic acid regulates chalcone synthase gene transcription in the corolla of *Petunia hybrida*. *Plant Physiol.* **98**, 191-197.

West, C. A. and Phinney, B. O. (1956). Properties of gibberellin-like factors from extracts of higher plants. *Plant Physiol.* **S31**, 20.

Wilson, R. N. and Somerville, C. R. (1995). Phenotypic suppression of the *gibberellin-insensitive* mutant (*gai*) of *Arabidopsis*. *Plant Physiol.* **108**, 495-502.

Wilson, R. N., Heckman, J. W. and Somerville, C. R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiol.* **100**, 403-408.