Gibberellin signal transduction

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Recent studies using biochemical and genetic approaches have identified a number of components, including several negative regulators, of the gibberellin (GA) signal transduction pathway in higher plants. The basal state of GA signaling is likely to be repressive, and the GA signal seems to activate the pathway by de-repression to allow GA-stimulated growth and development.

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Abbreviations

CaM	calmodulin
d1	*dwarf1*
GA	gibberellin
gai-1	*GA-insensitive-1*
GUS	β-glucuronidase
LEC1	*LEAFY COTYLEDON 1*
NLS	nuclear localization signal
***O*-GlcNAc**	*O*-linked N-acetylglucosamine
OGT	*O*-linked N-acetylglucosamine transferase
pkl	*pickle*
Rht1–3	*Reduced height 1–3*
SH2	Src homology 2
shi	*short internodes*
sln	*slender*
sly1	*sleepy 1*
spy	*spindly*
STATs	signal transducers and activators of transcription

Introduction

Bioactive gibberellins (GAs) affect a number of processes during plant development, including seed germination, leaf expansion, stem elongation, flower initiation, and flower and fruit development. Recent molecular and pharmacological studies using cereal aleurone and the molecular cloning of genes that are affected in GA-response mutants have begun to elucidate the mechanism by which GA regulates gene expression in plants. In the past two years, a number of excellent reviews on GA signaling have covered this topic with different emphases [1–4,5•,6•]. This review summarizes the recent findings in the field and focuses mainly on the papers published in the past year.

Perception of GA

Most studies of GA receptors and GA-induced genes have focused on the cereal aleurone system (see [6•] for extensive review). The expression of the genes encoding α-amylase, the most abundant hydrolase induced by GA in the aleurone layer, has been used as a valuable marker to monitor the status of the GA response in this tissue. Studies of aleurone protoplasts treated with either immobilized Sepharose-GA_4 or microinjected GA have provided evidence that GA receptors are located on the external face of the plasma membrane [7,8]. Photoaffinity-labeling experiments identified two GA-binding proteins that are found in the plasma membranes of the shoots and aleurone of several plant species [9]. In contrast, binding studies and protein purification using radiolabeled GAs have detected soluble cytosolic or microsomal GA-binding proteins in cucumber hypocotyls and azuki bean seedlings [10,11]. The function of these GA-binding proteins remains to be elucidated.

Second messengers and G proteins in GA signaling

In addition to studies on GA perception, the cereal aleurone system has also been used to identify protein components and second messengers that are involved in GA signaling. Candidates include heterotrimeric G proteins, Ca^{2+}, calmodulin (CaM), cGMP and protein kinases (see [1,6•] for extensive reviews). Heterotrimeric G proteins, which interact with G-protein-coupled transmembrane receptors, are important components of various signaling pathways [12,13]. Treatment of oat aleurone protoplasts with an agonist of the G proteins was able to mimic the effect of GA on the induction of α-amylase gene expression and secretion of α-amylase [14]. Inhibition of G-protein activity using hydrolysis-resistant guanosine 5′-O-(2-thiodiphosphate) (GDP-β-S) completely abolished the induction by GA of α-amylase promoter-reporter GUS expression. Therefore, the heterotrimeric G proteins are likely to play a role in GA signaling. The concentrations of cytoplasmic Ca^{2+}, CaM and cGMP have been found to increase in barley aleurone protoplasts after GA treatment. Sophisticated microinjection experiments have indicated that cGMP mediates the induction of α-amylase gene expression and enzyme secretion by GA [15], whereas Ca^{2+} and CaM-domain protein kinases only regulate α-amylase secretion [16,17].

GA-response mutants

In addition to the biochemical studies described above, another approach to examining GA perception and response has been to identify mutations affecting these processes (reviewed in [1,2,4,5•,6•,18,19•]). GA-response mutants fall into two phenotypic categories. First, the elongated *slender* (*sln*) mutants have longer petioles, paler green leaves, taller stems, and lower fertility than do wild-type plants. This phenotype resembles wild-type plants that have been treated with an excessive amount of GA (i.e. they have the so-called 'GA-overdose' phenotype). Second, the GA-unresponsive dwarf mutants display a similar phenotype to the GA-biosynthesis mutants, except that they fail to respond to exogenous GA treatment. For example, they are semi- or extreme dwarfs, and have impaired seed germination, dark green and compact leaves, delayed flowering, and abnormal floral development. Several genes

that are affected in these mutants have been cloned from *Arabidopsis* and rice, and their orthologs were subsequently cloned from additional species.

Recessive GA-unresponsive dwarf mutants and positive regulators of GA signaling

Until now, most of the so-called GA-unresponsive dwarf mutants have been identified solely on the basis of their morphology to be phenocopies of GA-deficient mutants. Because GA is not the only endogenous signal that induces plant growth, it is essential to characterize dwarf mutants precisely before classifying them as GA-response mutants (i.e. mutants that are specifically defective in GA signaling). In cereals, the ability of aleurone cells to produce α-amylase in response to GA has been useful in distinguishing between different dwarf mutants. This approach assumes that plants that are defective in the early components of GA signaling should show an overall impaired GA response in all tissues. The GA-response pathway may, however, branch out to control different processes. Additional GA-regulated marker genes are, therefore, needed to identify downstream components of GA signaling.

The *dwarf1* (*d1*) mutants in rice [20], the *gse* (for GA-sensitivity) mutants in barley [21••], and the *sleepy1* (*sly1*) mutant in *Arabidopsis* [22] have a semi-dwarf phenotype, which is similar to that of the leaky GA-biosynthesis mutants. Exogenous GA treatment does not, however, rescue the defective stem elongation of *d1*, *gse* or *sly1* mutants. The *d1* and *gse* mutations also prevented GA-induced α-amylase gene expression in the aleurone cells [20]. The recessive nature of these mutations implies that these genes may encode positive regulators of the GA signal transduction pathway. Recent cloning of the *D1* locus revealed that it encodes the putative α-subunit of the heterotrimeric G protein in rice [23••,24••]. This finding supports the results of earlier pharmacological studies, which showed that the heterotrimeric G proteins are involved in GA signaling [14]. The *sln* mutation (see below) is epistatic to *gse* [21••], indicating that, in barley, SLN may act downstream of the GSE in the GA-response pathway. The *sly1* mutant was isolated as a suppressor of the *abi1-1* (for abcisicic-acid-insensitive-1-1) mutation, but it cannot germinate in the wild-type *ABI1* background [22]. Cloning of *GSE* and *SLY1* will help to reveal their roles in GA response.

Another *Arabidopsis* mutant, *pickle* (*pkl*), has the same shoot phenotype as the GA-unresponsive dwarf mutants [25]. After germination, however, it has a unique embryonic root phenotype, which is absent in other mutants defective in either GA biosynthesis or the GA response. Because the penetrance of this primary root phenotype was reduced by GA application and enhanced by treatment with a GA biosynthesis inhibitor, uniconazole, it has been proposed that PKL mediates the GA-induced differentiation of roots during germination [25]. Positional cloning of *PKL* revealed that PKL contains sequence homology to the CHD3 (for a chromo [chromatin-organization modifier] domain, a helicase/ATPase domain, and a DNA-binding domain) chromatin-remodeling factor, which regulates gene expression by repression of transcription [26•]. Expression of the embryo identity gene *LEAFY COTYLEDON 1* (*LEC1*) was de-repressed in the roots of *pkl* seedlings. Nevertheless, repression of *LEC1* by *PKL* is GA independent.

Recessive elongated (*slender*) mutants identify negative regulators in GA signaling

The *sln* mutants [18] exhibit complete (e.g. *la* ; *cry*^{s} in pea and *sln* in barley) or partial (*spindly* [*spy*] and *rga* (for repressor of *ga1-3*) in *Arabidopsis*, and *procera* in tomato [27•]) GA-independent growth response. These mutations are recessive, and therefore are likely to affect negative regulators of GA signaling. The *Arabidopsis* genes *SPY* and *RGA* have been isolated [28,29].

Germination of *Arabidopsis* seeds has an absolute requirement for GAs. *SPY* was identified because of the ability of the mutant *spy* alleles to rescue seed germination in the presence of a GA biosynthetic inhibitor, paclobutrazol [30]. Cloning of *SPY* [28] and its homolog in barley (*HvSPY*, [31]) revealed that SPY is highly similar to the Ser/Thr *O*-linked N-acetylglucosamine (*O*-GlcNAc) transferases (OGT) in rat and humans [32]. OGTs modify target proteins by glycosylation of Ser/Thr residues, which either interfere or compete with kinases for phosphorylation sites. Like OGTs in animals, SPY contains tetratricopeptide repeats that are likely to be important for protein–protein interaction [5•,33]. Transient expression of *HvSPY* in barley aleurone protoplasts is able to inhibit GA-induced α-amylase gene expression [31]. This result further supports the role of SPY and HvSPY as repressors of GA signaling. Although SPY has not been confirmed to be OGT *in planta*, the insect-cell-produced SPY protein did show OGT activity *in vitro* [5•].

RGA was identified because of the ability of recessive *rga* alleles to partially suppress the phenotype of the GA-biosynthesis mutant *ga1–3* [34]. Cloning and characterization of the *RGA* gene revealed that the RGA protein is most probably a transcriptional regulator, which represses GA signaling in *Arabidopsis* ([29], see below). The mutant *early flowering 1* (*eaf1*) has recently been characterized [35•]. This recessive mutant has pale green leaves and elongated petioles, and shows an increased resistance to paclobutrazol during seed germination. It has been proposed that EAF1 is a repressor that regulates GA-induced germination and flowering by affecting either GA concentrations or GA response.

Semi-dominant GA-unresponsive dwarf mutants identify negative regulators in GA signaling

The *GA-insensitive-1* (*gai-1*) mutant in *Arabidopsis* [36], *Reduced height 1–3* (*Rht1–3*) mutants in wheat [37], and *D8* and *D9* in maize [38] are semi-dominant GA-response mutants. These mutants also accumulate high levels of

bioactive GA_1 [39–41], suggesting that their impaired GA response upregulates GA biosynthesis via a feedback mechanism (reviewed in [1,42•]). Nevertheless, because of the semi-dominant (gain-of-function) nature of the mutations, the role of *GAI*, *Rht* and *D8* and *D9* in GA signaling were unclear until the intragenic suppressors were isolated and these genes were cloned.

The intragenic suppressors of *gai-1* were isolated from γ-ray- or fast-neutron-mutagenized and Ds-insertion lines [43–45]. The loss-of-function (null) alleles created in these ways restored the plant phenotype to wild-type. These plants were, however, able to grow on media containing paclobutrazol, indicating that the GA-response pathway was partially de-repressed [45]. Thus, GAI is probably a negative regulator of the GA response.

Cloning of *GAI* and *RGA* revealed that GAI and RGA share 82% sequence identity [29,45] and that they belong to the plant-specific GRAS (for GAI, RGA, SCARECROW) family of regulatory proteins [46•]. To date, more than 33 GRAS family members have been identified in *Arabidopsis*. All GRAS family members contain highly conserved central (VHIID) and carboxy-terminal (RVER) regions, named after the conserved amino-acid motifs (using the single letter code for amino acids) that are found in each region [29,47]. The specific function of different members may reside in the amino-terminal region, which is divergent among the GRAS family members.

Both RGA and GAI have hallmarks of transcriptional regulators [29,45]. These include homopolymeric Ser and Thr, Leu heptad repeats, and putative nuclear localization signals (NLSs). In support of an NLS function, the green fluorescent protein (GFP)::RGA fusion protein was shown to be in the nucleus both in onion epidermal cells in a transient expression assay [29] and in stably transformed *Arabidopsis* plants (AL Silverstone, H-S Jung, T-p Sun, unpublished data). RGA and GAI may act as co-activators or repressors by interacting with other transcription factors because neither proteins contains a well-defined DNA-binding domain.

Sequence analysis indicates that *RGA* and *GAI* have a unique conserved region near the amino terminus called DELLA, after a set of conserved amino acids [29]. This region is likely to be involved in modulating GA response because the gain-of-function *gai-1* allele contains a 51-base-pair in-frame deletion within the DELLA region [45]. Peng *et al.* [45] hypothesized that this deletion in the gai-1 protein makes it a constitutive repressor of GA response, whose activity cannot be inhibited by the GA signal. More recently, two new GRAS family members in *Arabidopsis*, RGA-like (RGL) [48] and RGA1-like (GenBank accession number AC009895), have been identified and shown to contain the DELLA region. Future reverse-genetics studies would help to determine whether these proteins play a similar role in GA signaling.

Another GA-unresponsive dwarf *Arabidopsis* mutant, *short internodes* (*shi*) was identified from a mutant population generated by transposon insertion [49••]. This mutation is semi-dominant, and its phenotype is caused by the over-expression of the *SHI* gene that is adjacent to the CaMV-35S-promoter-containing transposon. On the basis of the *shi* mutant phenotype, SHI was proposed to be a negative regulator of GA signaling [49••]. Cloning of *SHI* revealed that SHI is a putative zinc-finger transcription factor. Isolation of loss-of-function *shi* alleles is essential to rule out the possibility that over-expression and ectopic expression of *SHI* may cause the dwarf phenotype by interfering with components of the GA response.

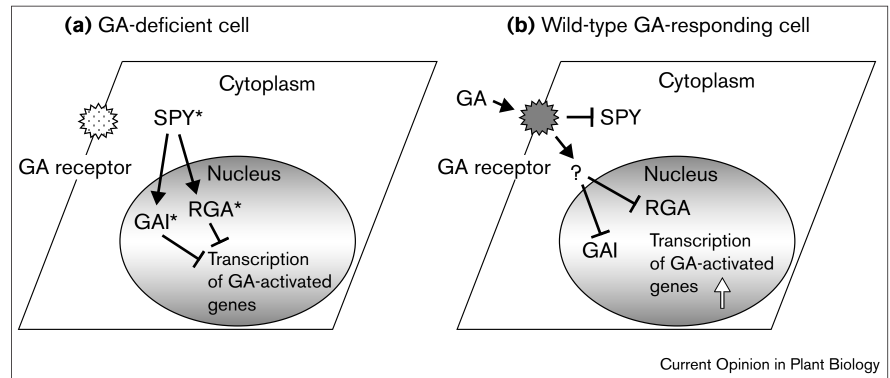
Extragenic suppressors of *gai-1*

The dominant *gar2-1* (for GA-responder) allele and leaky *spy* alleles (e.g. *spy-5* and *spy-7*) partially suppress all defects of *gai-1* mutants [44,50•]. In addition, the effects of *gar2-1* and *spy-7* are additive, and in combination these mutations restore the phenotype and GA levels of *gai-1* to those of the wild-type [50•]. Because *gar2-1* is dominant, it is difficult to predict the function of *GAR2* in the GA signaling pathway at present. SPY was initially thought to function downstream of GAI in the GA-response pathway because the severe *spy-4* allele was completely epistatic to *gai-1* [28]. Once *SPY*, *GAI* and *RGA* were cloned, an alternative model has been suggested ([5•,29,45], see below). After taking account of the predicted biochemical functions of these genes, it was proposed that SPY may activate RGA and GAI by GlcNAc modification.

Working model of GA signaling in *Arabidopsis*

Our model of GA signaling in *Arabidopsis* focuses on *SPY*, *RGA* and *GAI* because the genetic interaction among these three genes has been tested. OGT target sites are typically rich in Ser/Thr and are found near Pro, Val or acidic residues [51]. Both RGA and GAI contain such sequences at their amino termini; hence, SPY may modify and activate RGA and GAI in GA-deficient conditions (Figure 1a) [5•,29,45]. In animal systems, GlcNAc modification of proteins could facilitate their nuclear localization, increase their stability, alter their protein–protein interaction and/or compete with their phosphorylation [52,53]. The GA signal could inactivate RGA and GAI by inhibiting SPY, and/or by increasing the phosphorylation of RGA and GAI, or by activating an *O*-GlcNAc specific N-acetylglucosaminidase that removes GlcNAc residues (Figure 1b). Previously, the *rga* and *spy* mutations were shown to have additive effects in suppressing the defects of the *ga1–3* mutants [34]. This observation would be consistent with this model if RGA and GAI in the GA-deficient background still maintain a basal repressor activity without activation by SPY. Future biochemical analyses are crucial to verify this model. Because *spy* suppresses all defects in *ga1–3*, but *rga* does not rescue the *ga1–3* defects in seed germination and flower development [30,34], GAI and/or additional RGA/GAI homologs (e.g. RGL, RGA1-like) are likely to be involved in these two GA-regulated processes.

Figure 1



Proposed roles of SPY, GAI and RGA in the GA signaling pathway. **(a)** A GA-deficient cell in a GA-biosynthesis mutant or a wild-type cell without the GA signal. The hypothetical transmembrane GA receptor is inactive in the absence of GA signal. In this situation, SPY* is an active OGT. RGA and GAI become activated RGA* and GAI* proteins upon GlcNAc modification by SPY*. Active RGA* and GAI* function as activators or repressors of transcription, and indirectly or directly inhibit the expression of GA-induced genes. **(b)** A GA-responding cell in a wild-type plant. The GA receptor (dark gray) is activated by binding of bioactive GA. The GA signal inhibits RGA and GAI not only by deactivating SPY, but also by an unidentified factor (?), which may deactivate RGA and GAI through interaction with the DELLA region. The intensity of signal coming from the GA–receptor complex will determine the level of SPY, RGA and GAI activities in a given cell. Arrows and T-bars indicate positive and inhibitory effects, respectively. *Active forms of the proteins.

Isolation of the functional orthologs of RGA and GAI in crops

Yields of cereal crops around the world have increased dramatically since the 1950s, and this 'Green Revolution' was greatly facilitated by the introduction of semi-dwarf wheat varieties carrying the *Rht* mutations [37,54]. Today, almost all of the commercial wheat varieties employ one of the *Rht* mutant alleles. Interestingly, the wheat *Rht-B1* and *Rht-D1* genes and the maize *d8* gene are, in fact, orthologs of *RGA* and *GAI* [55••,56•]. Internal deletions or amino-terminal truncations of the DELLA domain in the Rht and D8 proteins resulted in the semi-dominant dwarf phenotype in wheat and maize, respectively. These results strongly support the hypothesis that the DELLA domain in the GAI/RGA/Rht-B1/Rht-D1/d8 protein family is required to modulate the activity of these proteins in response to the GA signal. Presumably, deleting the DELLA region locks the protein into a conformation that can no longer, or only very weakly, respond to the GA signal. Sequence analysis revealed a putative Src homology 2 (SH2) phosphotyrosine binding domain in Rht/d8/GAI/RGA [55••]. This SH2 domain is known to mediate the binding of a family of transcription factors in animals, called STATs (signal transducers and activators of transcription), to various receptor tyrosine kinases [57]. Future biochemical studies are necessary to determine whether this SH2-like domain in GAI/RGA/Rht/d8 has a similar function to that in STATs.

Recently, the RGA and GAI orthologs in barley and rice have also been shown to be encoded by the *SLN* locus (PM Chandler, A Marion-Poll, F Gubler, personal communication) and the *SLENDER RICE* (*SLR*) locus ([58]; J Yamaguchi, personal communication), respectively. A loss-of-function mutation in *SLN* or *SLR* resulted in a 'slender' phenotype in barley and in rice, respectively, presumably because of a constitutive derepression of GA signaling. These data show that the activities of RGA and GAI orthologs are highly conserved in both dicots and monocots.

GA-regulated genes

To identify components involved in GA-mediated internode elongation, early GA-induced genes were isolated from the intercalary meristem of the deepwater rice (reviewed in [59]). These include a leucine-rich repeat receptor-like transmembrane protein kinase (*OsTMK*, [60•]) and a putative transcription factor *Os-GRF1* [61•]. Reverse-genetic and/or antisense approaches may help researchers to understand the function of these genes in GA signaling.

RGA and GAI appear to be transcriptional regulators, but their downstream targets are unknown. Only a few genes that are regulated by GA at the transcriptional level have been isolated [62]. The α-amylase genes in barley aleurone are well studied (reviewed in [6•,63]) and could be candidate targets for SLN. Induction of transcription of these genes by GA requires the transcription factor GAMYB [64,65]. A zinc-finger transcriptional repressor, HRT, was found to inhibit GA-induced amylase expression in barley aleurone cells [66]. Further studies are needed to clarify the relationships among SLN, GAMYB and HRT in controlling the expression of α-amylase genes.

The transcription of genes involved in cell elongation, such as the tonoplast intrinsic protein, γ-TIP, in *Arabidopsis* [62] and expansins in rice [59], is also induced by GA. Genes encoding enzymes (i.e. GA 20-oxidases and 3β-hydroxylases) that are involved in the synthesis of bioactive GAs are downregulated (reviewed in [42•,67,68]) and 2-oxidase genes for deactivation of GA are upregulated by exogenous GA treatment [42•,69•]. Several GA-induced genes in specific tissues have also been identified. In *Arabidopsis*, GA induces flowering and trichome initiation by activating a floral meristem identity gene, *LEAFY*, and an activator gene for trichome formation, *GLABROUS1* [70,71]. A vacuolar H^+-ATPase gene is expressed in a GA-dependent manner in the micropylar region before radical emergence during germination of tomato seeds [72•]. This gene may

be involved in weakening the endosperm cap or facilitating protein reserve mobilization.

Conclusions

Genetic analyses and the predicted gene functions of *SPY*, *RGA*, *GAI* and *SHI* suggest that they encode repressors of GA signaling, whereas SLY and perhaps PKL are activators of GA response. Heterotrimeric G-proteins and GAMYB have also been implicated as positive regulators of GA response. The current model for GA signaling in *Arabidopsis* proposes that SPY acts as an OGT and activates RGA and GAI to repress the expression of GA-induced genes. The GA signal derepresses the GA-response pathway by inhibiting these proteins. Because SHI, PKL and GAMYB are also nuclear proteins, they might interact with RGA and GAI in protein complexes to repress (e.g. SHI) or activate (e.g. PKL, GAMYB) GA-induced genes. Further epistasis analyses and biochemical studies are needed to place all of the known components, including genes and second messengers, in the GA-signaling pathway. Identification of the elusive GA receptor(s) and additional factors in GA signaling will require more sophisticated mutant screens and biochemical approaches.

Update

Raventos *et al.* [73••] have taken an alternative approach in isolating GA signaling mutants. Transgenic *Arabidopsis* plants containing both the GA-responsive *GASA1*-promoter::*GUS* and *GASA1*-promoter::luciferase genes were mutagenized, and mutants that showed altered expression of both reporters were isolated. *GASA* overexpressors and *GASA* underexpressors were named *goe* and *gue* mutants, respectively. Three *goe* mutants were characterized further by GA and paclobutrazol treatments, and by RNA-blot analyses. Some, but not all, of these mutants showed increased or reduced sensitivity to GA and paclobutrazol, and/or altered expression of the *GA-20-oxidase* gene. They did not, however, exhibit a straightforward 'GA-overdose' phenotype, and their physiological characteristics are complex. Future work is required to elucidate the roles of these genes in GA signaling.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bethke PC, Jones RL: **Gibberellin signaling.** *Curr Opin Plant Biol* 1998, **1**:440-446.

2. Harberd NP, King KE, Carol P, Cowling RJ, Peng J, Richards DE: **Gibberellin inhibitor of an inhibitor of...?** *BioEssays* 1998, **20**:1001-1008.

3. Phillips A: **Gibberellins in *Arabidopsis*.** *Plant Physiol Biochem* 1998, **36**:115-124.

4. Ogas J: **Plant hormones: dissecting the gibberellin response pathway.** *Curr Biol* 1998, **8**:165-167.

5. • Thornton TM, Swain SM, Olszewski NE: **Gibberellin signal transduction presents...the SPY who *O*-GlcNAc'd me.** *Trends Plant Sci* 1999, **4**:424-428.

This review summarizes the components in GA signaling that have been identified recently. It also includes a section on OGTs in animals, which is helpful when speculating how SPY might function at a molecular level in GA signaling in plants.

6. • Lovegrove A, Hooley R: **Gibberellin and abscisic acid signalling in aleurone.** *Trends Plant Sci* 2000, **5**:102-110.

This review provides updated and detailed information on both GA and abscissic-acid signaling in aleurone cells.

7. Hooley R, Beale MH, Smith SJ: **Gibberellin perception at the plasma membrane of *Avena fatua* aleurone protoplasts.** *Planta* 1991, **183**:274-280.

8. Gilroy S, Jones RL: **Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts.** *Plant Physiol* 1994, **104**:1185-1192.

9. Lovegrove A, Barratt DHP, Beale MH, Hooley R: **Gibberellin-photoaffinity labelling of two polypeptides in plant plasma membrane.** *Plant J* 1998, **15**:311-320.

10. Keith B, Brown S, Srivastava LM: ***In vitro* binding of gibberellin A_4 to extracts of cucumber measured by using DEAE-cellulose filters.** *Proc Natl Acad Sci USA* 1982, **79**:1515-1519.

11. Nakajima M, Takita K, Wada H, Mihara K, Hasegawa M, Yamaguchi I, Murofushi N: **Partial purification and characterization of a gibberellin-binding protein from seedlings of *Azukia angularis*.** *Biochem Biophys Res Commun* 1997, **241**:782-786.

12. Neer EJ: **Heterotrimeric G proteins: organizers of transmembrane signals.** *Cell* 1995, **80**:249-257.

13. Hooley R: **A role for G proteins in plant hormone signalling?** *Plant Physiol Biochem* 1999, **37**:393-402.

14. Jones HD, Smith SJ, Desikan R, Plakidou-Dymock S, Lovegrove A, Hooley R: **Heterotrimeric G proteins are implicated in gibberellin induction of α-amylase gene expression in wild oat aleurone.** *Plant Cell* 1998, **10**:245-253.

15. Penson SP, Schuurink RC, Fath A, Gubler F, Jacobsen JV, Jones RL: **cGMP is required for gibberellic acid-induced gene expression in barley aleurone.** *Plant Cell* 1996, **8**:2325-2333.

16. Gilroy S: **Signal transduction in barley aleurone protoplasts is calcium dependent and independent.** *Plant Cell* 1996, **8**:2193-2209.

17. Ritchie S, Gilroy S: **Calcium-dependent protein phosphorylation may mediate the gibberellic acid response in barley aleurone.** *Plant Physiol* 1998, **116**:765-776.

18. Ross JJ, Murfet IC, Reid JB: **Gibberellin mutants.** *Physiol Plant* 1997, **100**:550-560.

19. • McCourt P: **Genetic analysis of hormone signaling.** *Annu Rev Plant Physiol Plant Mol Biol* 1999, **50**:219-243.

This review summarizes different genetic approaches for isolating hormone-response mutants and for identifying components of hormone signal transduction pathways.

20. Mitsunaga S, Tashiro T, Yamaguchi J: **Identification and characterization of gibberellin-insensitive mutants selected from among dwarf mutants of rice.** *Theor Appl Genet* 1994, **87**:705-712.

21. •• Chandler PM, Robertson M: **Gibberellin dose–response curves and the characterization of dwarf mutants of barley.** *Plant Physiol* 1999, **120**:623-632.

The authors characterized three recessive classes of barley dwarf mutants by determining the response curves of the maximal leaf-elongation rate to different GA_3 concentrations. The *grd* (for GA-responsive dwarf) mutants showed normal GA responses and are probably GA-biosynthesis mutants. The *gse* mutants were much less responsive to GA_3 than wild-type plants in both leaf elongation and α-amylase production by the aleurone. Therefore, *GSE* might encode a negative regulator in GA response. The *elo* (for elongation) mutants showed no leaf elongation in response to GA_3, but normal GA-induced amylase production in aleurone. Because *elo* is epistatic to the constitutive GA-response mutant *sln*, the *ELO* genes probably function specifically in leaf elongation.

22. Steber CM, Cooney S, McCourt P: **Isolation of the GA-response mutant *sly1* as a suppressor of *ABI1-1* in *Arabidopsis thaliana*.** *Genetics* 1998, **149**:509-521.

23. Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A: **Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the α-subunit of GTP-binding protein.** *Proc Natl Acad Sci USA* 1999, **96**:10284-10289.
•• This paper reports the isolation of the rice *D1* locus by map-based cloning. RNA blot and sequence analyses using several *d1* alleles indicated that *D1* encodes the putative α-subunit of G protein. A cosmid clone containing this coding sequence was able to complement a *d1* mutant.

24. Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, Sasaki T, Asahi T, Iwasaki Y: **Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice.** *Proc Natl Acad Sci USA* 1999, **96**:7575-7580.
•• This paper reports that antisense inhibition of expression of the putative α-subunit of the G protein in transgenic rice caused shortening of internodes, leaves and panicles, and the formation of small seeds. The map location of this gene is closely linked to the *D1* locus on chromosome 5. In addition, the authors showed that mutations in five *d1* alleles are located in the coding region of the α-subunit of the G protein.

25. Ogas J, Cheng J-C, Sung ZR, Somerville C: **Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant.** *Science* 1997, **277**:91-94.

26. Ogas J, Kaufmann S, Henderson J, Somerville C: **PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*.** *Proc Natl Acad Sci USA* 1999, **96**:13839-13844.
• This paper reports the isolation of *PKL* by positional cloning. *PKL* encodes a putative CHD3 protein, which is a chromatin-remodeling factor. Ribonuclease protection assays showed that the *PKL* gene in wild-type plants is expressed ubiquitously in all tissues. Expression of *LEC1*, an activator of embryo identity, is specifically de-repressed in roots of the *pkl* mutant. The level of the *LEC1* mRNA accumulation is not, however, enhanced by treatment with paclobutrazol.

27. van Tuinen A, Peters AHLJ, Kendrick RE, Zeevaart JAD, Koornneef M: **Characterization of the *procera* mutant of tomato and the interaction of gibberellins with end-of-day far-red light treatments.** *Physiol Plant* 1999, **106**:121-128.
• This paper reports that the *procera* (*pro*) mutation restored most of the phenotype of the GA-deficient *gib-1* mutant to that of wild-type plants, except that the leaves were still not serrated. The *pro* mutation also conferred resistance to tetcyclasis, a GA-biosynthesis inhibitor, but the GA content of the double mutant was as low as that of the *gib-1* mutant. It was concluded that the *pro* mutation causes an increased sensitively to GA. The authors also found that GA is required for stem growth after end-of-day far-red light treatment, but that *pro* has no effect on stem growth under these conditions.

28. Jacobsen SE, Binkowski KA, Olszewski NE: **SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in *Arabidopsis*.** *Proc Natl Acad Sci USA* 1996, **93**:9292-9296.

29. Silverstone AL, Ciampaglio CN, Sun T-p: **The *Arabidopsis RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway.** *Plant Cell* 1998, **10**:155-169.

30. Jacobsen SE, Olszewski NE: **Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction.** *Plant Cell* 1993, **5**:887-896.

31. Robertson M, Swain SM, Chandler PM, Olszewski NE: **Identification of a negative regulator of gibberellin action, HvSPY, in barley.** *Plant Cell* 1998, **10**:995-1007.

32. Kreppel L, Blomberg MA, Hart GW: **Dynamic glycosylation of nuclear and cytosolic proteins.** *J Biol Chem* 1997, **272**:9308-9315.

33. Das AK, Cohen PTW, Barford D: **The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein–protein interactions.** *EMBO J* 1998, **17**:1192-1199.

34. Silverstone AL, Mak PYA, Casamitjana Martínez E, Sun T-p: **The new *RGA* locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*.** *Genetics* 1997, **146**:1087-1099.

35. Scott DB, Jin W, Ledford HK, Jung H-S, Honma MA: **EAF1 regulates vegetative-phase change and flowering time in *Arabidopsis*.** *Plant Physiol* 1999, **120**:675-684.
• *EAF1* is likely to play an inhibitory role in the autonomous pathway because the recessive *eaf1* mutation leads to an early flowering phenotype in both long-day and short-day (SD) conditions. In SD, the *eaf1* mutant displays a phenotype that is similar to that of the *phyB* and *spy* mutants. The authors propose that EAF1 may affect the GA content or GA response during germination and flowering because the *eaf1* mutation confers increased resistance of seeds to paclobutrazol.

36. Koornneef M, Elgersma A, Hanhart CJ, van Loenen MEP, van Rijn L, Zeevaart JAD: **A gibberellin insensitive mutant of *Arabidopsis thaliana*.** *Physiol Plant* 1985, **65**:33-39.

37. Borner A, Plaschke J, Korzun V, Worland AJ: **The relationships between the dwarfing genes of wheat and rye.** *Euphytica* 1996, **89**:69-75.

38. Winkler RG, Freeling M: **Physiological genetics of the dominant gibberellin-nonresponsive maize dwarfs, *Dwarf8* and *Dwarf9*.** *Planta* 1994, **193**:341-348.

39. Fujioka S, Yamane H, Spray CR, Katsumi M, Phinney BO, Gaskin P, MacMillan J, Takahashi N: **The dominant non-gibberellin-responding dwarf mutant (*D8*) of maize accumulates native gibberellins.** *Proc Natl Acad Sci USA* 1988, **85**:9031-9035.

40. Talón M, Koornneef M, Zeevaart JAD: **Accumulation of C-19-gibberellins in the gibberellin-insensitive dwarf mutant *gai* of *Arabidopsis thaliana* (L.) Heynh.** *Planta* 1990, **182**:501-505.

41. Webb SE, Appleford NEJ, Gaskin P, Lenton JR: **Gibberellins in internodes and ears of wheat containing different dwarfing alleles.** *Phytochemistry* 1998, **47**:671-677.

42. Yamaguchi S, Kamiya Y: **Gibberellin biosynthesis: its regulation by endogenous and environmental signals.** *Plant Cell Physiol* 2000, **41**:251-257.
• This review provides updated information on the regulation of the GA-biosynthetic pathway by the endogenous developmental program, the GA-response pathway, and environmental cues.

43. Peng J, Harberd NP: **Derivative alleles of the *Arabidopsis* gibberellin-insensitive (*gai*) mutation confer a wild-type phenotype.** *Plant Cell* 1993, **5**:351-360.

44. Wilson RN, Somerville CR: **Phenotypic suppression of the gibberellin-insensitive mutant (*gai*) of *Arabidopsis*.** *Plant Physiol* 1995, **108**:495-502.

45. Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP: **The *Arabidopsis GAI* gene defines a signalling pathway that negatively regulates gibberellin responses.** *Genes Dev* 1997, **11**:3194-3205.

46. Pysh LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN: **The *GRAS* gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the *SCARECROW-LIKE* genes.** *Plant J* 1999, **18**:111-119.
• The authors found 19 *GRAS* gene family members in the *Arabidopsis* database. The highly conserved VHIID and SAW motifs were proposed to be essential for the activity of the GRAS proteins. Several GRAS proteins also contain the NLS and the LXXLL motif, suggesting that they could be nuclear proteins. The expression pattern of ten *GRAS* genes in siliques, shoots and roots was examined by RNA-blot analysis. *In situ* hybridization showed that *SCL3* displayed a similar expression pattern to that of *SCR*, which is expressed primarily in the endodermis of roots.

47. Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN: **The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root.** *Cell* 1996, **86**:423-433.

48. Sanchez-Fernandez R, Ardiles-Diaz W, van Montagu M, Inze D, May MJ: **Cloning of a novel *Arabidopsis thaliana RGA*-like gene, a putative member of the VHIID-domain transcription factor family.** *J Exp Botany* 1998, **49**:1609-1610.

49. Fridborg I, Kuusk S, Moritz T, Sundberg E: **The *Arabidopsis* dwarf mutant *shi* exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein.** *Plant Cell* 1999, **11**:1019-1031.
•• This paper shows that overexpression of the *SHI* gene in *Arabidopsis* leads to elevated levels of active GAs and a GA-insensitive dwarf phenotype. The predicted SHI protein contains acidic and glutamine-rich sequences, and a putative zinc-finger region. These results suggest that *SHI* might function as a transcription factor that represses GA signaling.

50. Peng J, Richards DE, Moritz T, Cano-Delgado A, Harberd NP: **Extragenic suppressors of the *Arabidopsis gai* mutation alter the dose–response relationship of diverse gibberellin responses.** *Plant Physiol* 1999, **119**:1199-1207.
• This paper illustrates that *gar2-1* and *spy-7* partially suppress the effect of the *gai* mutation on final plant height and flowering time, and that the effects of these mutations were additive. Both mutations also resulted in

paclobutrazol resistance during seed germination. However, *gai* ; *gar2-1* seeds are more resistant to paclobutrazol than *gai* ; *spy-7* seeds. The authors also demonstrated that the elevated bioactive GA levels in *gai* were restored partially by *gar2-1* and *spy-7* individually, and were completely returned to wild-type levels by combining *ga2-1* and *spy-7*.

51. Lubas WA, Frank DW, Krause M, Hanover JA: ***O*-linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats.** *J Biol Chem* 1997, **272**:9316-9324.

52. Hart GW: **Dynamic *O*-linked glycosylation of nuclear and cytoskeletal proteins.** *Annu Rev Biochem* 1997, **66**:315-335.

53. Snow DM, Hart GW: **Nuclear and cytoplasmic glycosylation.** *Int Rev Cytol* 1998, **181**:43-74.

54. Perkins JH: *Geopolitics and the Green Revolution*. New York: Oxford University Press; 1997.

55. •• Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F *et al.*: **'Green Revolution' genes encode mutant gibberellin response modulators.** *Nature* 1999, **400**:256-261.

Cloning of the *Rht* genes in wheat and the *d8* gene in maize revealed that these genes are functional orthologs of *RGA* and *GAI* in *Arabidopsis*. Several semi-dominant *Rht* and *D8* alleles encode an amino-terminal truncated protein, which presumably acts as a constitutive repressor in GA signaling. This paper illustrated that *GAI*/*RGA*/*Rht*/*d8* genes are common negative regulators of GA signaling in both dicots and monocots. The gain-of-function mutations in these genes cause a semi-dwarf phenotype, which was crucial to increase grain yield in wheat during the 'Green Revolution'.

56. • Silverstone AL, Sun T-p: **Gibberellins and the green revolution.** *Trends Plant Sci* 2000, **5**:1-2.

This article highlights the recent cloning of the *RGA* and *GAI* orthologs in crops, including the so-called 'Green Revolution' gene *Rht* in wheat.

57. Darnell JE Jr: **STATs and gene regulation.** *Science* 1997, **277**:1630-1635.

58. Ogawa M, Kusano T, Katsumi M, Sano H: **Rice gibberellin-insensitive gene homolog, *OsGAI* encodes a nuclear-localized protein capable of gene activation at transcriptional level.** *Gene* 2000, **245**:21-29.

59. Kende H, van der Knaap E, Cho HT: **Deepwater rice: a model plant to study stem elongation**. *Plant Physiol* 1998, **118**:1105-1110.

60. • van der Knaap E, Song WY, Ruan DL, Sauter M, Ronald PC, Kende H: **Expression of a gibberellin-induced leucine-rich repeat receptor-like protein kinase in deepwater rice and its interaction with kinase-associated protein phosphatase.** *Plant Physiol* 1999, **120**:559-569.

Os-TMK was isolated as a GA-induced gene in the intercalary meristem of deepwater rice internodes. A recombinant protein containing a maltose binding protein and the kinase domain of Os-TMK was shown to be able to autophosphorylate serine and threonine residues. The authors isolated the kinase-associated type-2C protein phosphatase in rice (Os-KAPP). This KAPP was phosphorylated by the kinase domain of Os-TMK *in vitro*.

61. • van der Knaap E, Kim JH, Kende H: **A novel gibberellin-induced gene from rice and its potential regulatory role in stem growth.** *Plant Physiol* 2000, **122**:695-704.

By differential display, *Os-GRF1* was shown to be induced by GA in the intercalary meristem of deepwater rice internodes. This gene encodes a 43 kD protein with several features of transcription factors, including a putative NLS and a protein interaction domain present in SWI2/SNF2. A transient assay in onion cells showed that the Os-GRF1::GUS fusion protein was localized in the nucleus. Overexpression of Os-GRF1 in *Arabidopsis* greatly inhibited primary stem elongation, and caused a curly rosette leaf phenotype and abnormal gynoecia. GA treatments did not rescue these defects. Seed germination and flowering time were not, however, affected.

62. Huttly AK, Phillips AL: **Gibberellin-regulated plant genes.** *Physiol Plant* 1995, **95**:310-317.

63. Skadsen RW: **Physiological and molecular genetic mechanisms regulating hydrolytic enzyme gene expression in cereal grains.** *Physiol Plant* 1998, **104**:486-502.

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

65. Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen JV: **Target genes and regulatory domains of the GAMYB transcriptional activator in cereal aleurone.** *Plant J* 1999, **17**:1-9.

66. Raventos D, Skriver K, Schlein M, Karnahl K, Rogers SW, Rogers JC, Mundy J: **HRT, a novel zinc finger, transcriptional repressor from barley.** *J Biol Chem* 1998, **273**:23313-23320.

67. Lange T: **Molecular biology of gibberellin synthesis.** *Planta* 1998, **204**:409-419.

68. Hedden P, Proebsting WM: **Genetic analysis of gibberellin biosynthesis.** *Plant Physiol* 1999, **119**:365-370.

69. • Thomas SG, Phillips AL, Hedden P: **Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation.** *Proc Natl Acad Sci USA* 1999, **96**:4698-4703.

This paper reports the cloning of a GA 2-oxidase cDNA from runner bean using a functional screening method. The enzyme property was examined *in vitro* using *E. coli*-produced recombinant protein. Three *Arabidopsis* homologs (AtGA2ox1–3) were also isolated. AtGA2ox1 and AtGA2ox2 were expressed in the upper stems, flowers and siliques, but AtGA2ox3 mRNA was undetectable in these organs. Interestingly, expression of both AtGA2ox1 and ATGA2ox2 was downregulated in the GA-deficient mutant, and was induced after GA treatment.

70. Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D: **Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter.** *Plant Cell* 1998, **10**:791-800.

71. Perazza D, Vachon G, Herzog M: **Gibberellins promote trichome formation by up-regulating *GLABROUS1* in *Arabidopsis*.** *Plant Physiol* 1998, **117**:375-383.

72. • Cooley MB, Yang H, Dahal P, Mella RA, Downie AB, Haigh AM, Bradford KJ: **Vacuolar H$^+$-ATPase is expressed in response to gibberellin during tomato seed germination.** *Plant Physiol* 1999, **121**:1339-1347.

The gene (*LVA-P1*, for *Lycopersicon* vacuolar ATPase-proteolipid 1) that encodes the 16-kD c subunit of the vacuolar H$^+$-ATPase (V-ATPase) was shown to be induced by GA in the tomato GA-biosynthesis mutant, *gib-1*. In germinating seeds, *LVA-P1* mRNA accumulated at a higher level in the micropylar region before radicle emergence. Immunoblot and tissue-printing analyses showed that the a and b subunits of V-ATPase were also induced after GA treatment, and that the b subunit of V-ATPase accumulated to a higher level in the micropylar in GA-treated seeds. Thus, the V-ATPase holoenzyme might be present in the micropylar region in response to GA treatment.

73. •• Raventos D, Meier C, Mattsson O, Jensen AB, Mundy J: **Fusion genetic analysis of gibberellin signaling mutants.** *Plant J* 2000, **22**:427-438.