The Plant Cell, Vol. 10, 131, February 1998

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GA Signaling: Genes and GTPases

The rich history of efforts to understand the biosynthesis and biological activity of gibberellins (GAs) began with Mendel (his stem length gene *Le* encodes a GA 3β-hydroxylase that activates GAs [Lester et al., 1997; Martin et al., 1997]). In the years since Mendel unknowingly got the ball rolling, we have learned a great deal about GA biosynthesis (see, e.g., Kende and Zeevaart, 1997), and it is now well established that GAs trigger plant growth by promoting cell division and cell elongation (for reviews, see Jacobs, 1997; Kende and Zeevaart, 1997). By contrast, extensive investigations have yet to uncover any GA receptor(s), and many of the molecular details of GA signal transduction pathways remain to be defined (Hooley, 1994).

In addition to their effect on cell expansion, GAs play a role in many other plant processes, including seed germination, root development, shoot growth, flowering time, sex determination, and chlorophyll content (see, e.g., Dellaporta and Calderon-Urrea, 1993; Blazquez et al., 1997; Cho and Kende, 1997; Jacobs, 1997; Ogas et al., 1997). Each of these processes represents a potential target for improving the agronomic properties of crop plants, adding further impetus to studies of GA perception and signal transduction.

Several GA signaling intermediates have been identified through mutant analyses in a number of plant species (Swain and Olszewski, 1996; Ross et al., 1997), and the recent cloning of some of the corresponding genes is facilitating efforts to understand how different signaling components may interact.

In Arabidopsis, for example, one of the important players is *SPINDLY* (*SPY*), which acts as a negative regulator of GA responses (Jacobsen et al., 1996). The deduced amino acid sequence of SPY suggests that the protein is an N-acetyl glucosamine transferase that may glycosylate other molecules involved in GA signaling. Related glycosyl transferases from animals usually add Glc-NAc moieties to Ser/Thr-rich regions in their target proteins (see, e.g., Kreppel et al., 1997). However, it remains to be determined whether such modifications affect the signaling activity of target proteins directly or by blocking phosphorylation sites.

A second GA signaling intermediate from Arabidopsis is encoded by the *GA-INSENSITIVE* (*GAI*) gene, which has also been cloned (Peng et al., 1997). The original *gai* allele behaves genetically as a gain-of-function mutation (Peng and Harberd, 1993), and the recent molecular analyses confirm that this allele encodes a constitutively active mutant protein that has apparently lost its ability to respond to GA (Peng et al., 1997).

The deduced amino acid sequence of GAI is closely related to that of SCARECROW (SCR), which controls cell fate in Arabidopsis roots (Di Laurenzio et al., 1996). Sequence domains conserved in these two proteins suggest that GAI and SCR are members of a novel class of putative transcriptional regulators, termed the VHIID class (for a conserved Val-His-Ile-Ile-Asp motif), which appears to be unique to plants. Together with the genetic experiments, these data suggest that GAI is also a negative regulator of GA responses. GAI may act directly to repress the transcription of GA-induced genes or indirectly, by promoting the expression of such a repressor (Peng et al., 1997).

Another link between GA signaling and root development has been established recently with the identification of the Arabidopsis *PICKLE* (*PKL*) gene (Ogas et al., 1997). Mutations in this gene block the transition between embryonic and adult developmental programs in the primary root and also affect many aspects of shoot development that are influenced by GA (Ogas et al., 1997). Double mutant studies with *gai* suggest that *PKL* may operate in a *GAI*-independent GA signaling pathway.

Although the preceding synopsis illustrates how genetic approaches have helped to identify a number of GA signaling components, the relevant molecular interactions are still rather unclear. A different approach that tackles these interactions more directly focuses on the GA-mediated induction (and counteracting ABA-mediated repression) of gene expression in barley aleurone cells (for a review, see Jacobsen et al., 1995).

This experimental system has provided important information on a number of GA signaling intermediates including, most recently, cGMP (Penson et al., 1996), sugars (Perata et al., 1997), and the GA-inducible transcription factor GAMyb. GAMyb binds to a sequence element in the promoter of the barley aleurone α-amylase gene that is closely related to c-Myb and v-Myb consensus sequences (Gubler et al., 1995).

Two further advances in the investigation of GA signal transduction pathways are reported in this issue of THE PLANT CELL. **On pages 155–169, Silverstone et al.** report that the *RGA* gene (for repressor of *ga1-3*) encodes a new member of the VHIID family, and **on pages 245–253, Jones et al.** present data implying that heterotrimeric GTPases are involved in early stages of GA signal transduction in barley aleurone cells.

Silverstone et al. detected the *RGA* locus in a screen designed to identify negative regulators of GA signaling pathways (Silverstone et al., 1997). They began this screen by mutagenizing seed

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from plants carrying *ga1-3*, a null allele of the *GA1* gene. Because *GA1* encodes the enzyme copalyl diphosphate synthase (also known as *ent*-kaurene synthase A), which carries out the first committed step in GA biosynthesis (Sun and Kamiya, 1994; Kende and Zeevaart, 1997), *ga1-3* mutant plants are virtually devoid of endogenous GAs and are severely dwarfed. Any mutations that suppress the dwarf (and other) phenotypes of *ga1-3* may be allowing GA perception and signal transduction to occur despite the extremely low levels of endogenous GAs in this mutant; on this basis, the corresponding wild-type genes would be expected to encode negative regulators of GA signaling.

With a number of *rga* mutants in hand, the authors were able to refine their initial mapping experiments (Silverstone et al., 1997) and place *RGA* close to the top of chromosome 2 in the Arabidopsis genetic map. However, because they found that the region surrounding the *RGA* locus has few known markers, Silverstone et al. were obliged to use the genomic subtraction technique (Sun et al., 1992) to clone *RGA.* Once they had done so, they realized very quickly that RGA and GAI are closely related. In fact, Peng et al. (1997) cloned the same gene on the basis of its similarity to *GAI* (they called it *GRS1* [for GAI-related sequence]).

Silverstone et al. have also identified several additional VHIID family members by searching for sequences related to SCR, GAI, and RGA in the available plant DNA sequence databases. Curiously, two of these sequences, which were initially identified on the basis of their ability to functionally complement yeast mutants with deficiencies in nitrogen metabolism (Truong et al., 1997), turn out to be identical to RGA and GAI. Although the significance of this unusual observation remains to be determined, it may be related to the presumed role of the VHIID proteins as transcriptional regulators.

The growing collection of full-length and partial VHIID sequences is forming the basis for a functional analysis of RGA and the other VHIID proteins. Silverstone et al. have begun this analysis by focusing on domains present only in RGA and GAI and on other domains that are more broadly conserved in the VHIID family. For example, they show that one of the former, a putative nuclear localization signal, is functional in RGA by demonstrating that an RGA–green fluorescent protein (GFP) fusion protein cannot be detected in the cytoplasm of cells bombarded with the corresponding DNA construct; it is apparent only in the nuclei of these cells.

Two other domains that are conserved in RGA and GAI may be involved more specifically in GA signal transduction. These are the Ser/Thr-rich domains located toward the N termini of the two proteins, which are potential targets of SPY activity, and a nearby stretch of acidic amino acids. This "DELLA" domain is partially deleted in the constitutively active *gai* allele mentioned above, raising the possibility that it may be directly involved in GA perception and/or GAI deactivation (Peng et al., 1997).

On the basis of their data and recent studies of GAI (Peng et al., 1997) and SPY (Jacobsen et al., 1996), Silverstone et al. present a revised working model of the GA signal transduction pathway(s) in Arabidopsis. One fundamental feature of this model is that SPY probably functions to regulate the activities of RGA and GAI. For example, the authors suggest that in the absence of GA, SPY may activate GAI and/or RGA. The active versions of these two proteins could then repress genes that play a role in GA-mediated developmental processes. Conversely, in the presence of GA, GAI and RGA could be inactivated, perhaps following the removal of the GlcNAc moiety thought to be added by SPY.

Whether or not SPY acts on them, it seems likely that RGA and GAI operate fairly late in the GA response pathway to affect the transcription of GA-regulated genes. By contrast, the second paper in this issue to address GA signaling focuses on the early phases of the GA signal transduction pathway(s) including events that may occur very soon after GA perception.

In this paper, Jones et al. use the barley aleurone system to explore the role of heterotrimeric G proteins in GA signaling. GTP binding proteins have been implicated in a variety of signal transduction pathways in higher plants, and on the basis of investigations of their cellular localization and function in plants and animals, it seems likely that they may interact with other signaling components at the plasma membrane (see, e.g., Neuhaus et al., 1997; for reviews, see Ma, 1994; Quail, 1995).

Jones et al. show that the effects of Mas7, a potent activator of GDP/GTP exchange by heterotrimeric G proteins, on both α-amylase gene expression and secretion closely mimic those of the bioactive GA, GA_1. Moreover, Mas7 stimulates expression of an α-amylase::β-*glucuronidase* (*GUS*) reporter construct in isolated aleurone protoplasts. In the reciprocal experiments, the authors report that the nonhydrolyzable GTP analog GDP-β-S prevents the GA_1-mediated activation of the α-amylase–GUS reporter.

These results provide compelling evidence that heterotrimeric GTPases play a role in early GA-mediated signal transduction events in barley aleurone. To further investigate what this role may be, Jones et al. have cloned novel G-α and G-β GTPase subunits from barley aleurone cells. With these genes in hand, the authors can begin structure–function analyses that may eventually help to establish a link between G proteins and the GA-mediated induction of GAMyb expression.

Although the research articles by Silverstone et al. and Jones et al. add significantly to our understanding of GA signaling pathways in plants, there are a number of questions that remain to be addressed. For example, does SPY

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interact directly with GAI and RGA? Is there a SPY antagonist and, if so, how does it function? Are homologs of *SPY*, *GAI*, and *RGA* expressed in barley aleurone, and do they function similarly? Conversely, is the role of GAMyb confined to the aleurone, or does it (and its potential homologs in other plant species) control additional GA-dependent processes? Finally, how do signaling components defined biochemically in barley aleurone cells impinge on the activity of those defined genetically in Arabidopsis and other plant species? With all of these questions open, this is clearly an exciting time to be investigating the molecular mechanisms of GA signaling.

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