Mechanisms of Cross Talk between Gibberellin and Other Hormones

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It has always been clear that different plant hormones affect overlapping processes, such that the output of plant hormone action depends on specific hormone combinations rather than on the independent activities of each. In the last two decades, numerous components of the signal transduction pathways of various plant hormones have been identified, leading to the elucidation of partial or entire signaling cascades. These findings have provided the tools to begin addressing the mechanisms underlying the cross talk among different hormone signal transduction pathways. Such cross talk involves diverse mechanisms, which act at both the hormone response and biosynthesis levels, creating a delicate response network. In this review, we describe how gibberellin (GA) interacts with other plant hormones, concentrating on its interactions with abscisic acid (ABA), auxin, ethylene, and cytokinin. Although evidence for interactions of GA with brassinosteroids (Bouquin et al., 2001) and jasmonate (Traw and Bergelson, 2003) also exists, we focus on studies addressing the mechanisms governing the cross talk.

GA BIOSYNTHETIC AND RESPONSE PATHWAYS

GAs regulate various developmental processes throughout the life cycle of the plant, from seed germination through leaf expansion, stem elongation, flower induction, and development to seed development (Sun and Gubler, 2004). As the interactions between GA and other hormones involve components from the GA biosynthetic and response pathways, we first briefly introduce a few relevant players in these pathways. For a more comprehensive description of these pathways, see recent reviews (Hedden and Phillips, 2000; Sun and Gubler, 2004; Hartweck and Olszewski, 2006; Lange and Lange, 2006; Razem et al., 2006). The GA biosynthetic pathway has been elucidated by a combination of biochemical and genetic approaches. The first few steps of the pathway, from farnesylgeranyl disphosphate to GA12-aldehyde, are common to all species. The final steps to produce active GAs are species specific but in most cases require activity of the GA 20-oxidase (GA20ox) and GA3ox enzymes. In contrast, the enzyme GA2ox antagonizes GA activity by deactivating GAs. The level of endogenous active GA is governed by feedback regulation, where active GAs suppress the expression of the GA2ox and GA3ox genes and promote the expression of the GA2ox gene.

Studies of GA signal transduction, using genetic approaches, have led to the identification of positive and negative signaling components (Sun and Gubler, 2004). The most extensively characterized among these are the DELLA proteins, a class of nuclear proteins that belong to the GRAS family of transcriptional regulators and act as suppressors of GA signaling. The molecular mechanism by which DELLA proteins suppress GA responses is not yet clear. The Arabidopsis (Arabidopsis thaliana) genome contains five DELLA genes (REPRESSOR OF ga1-3 [RGA], GA INSENSITIVE [GAI], RGA LIKE1 [RGL1], RGL2, and RGL3), whereas in the rice (Oryza sativa) genome, only one family member has been identified (SLENDER1 [SLRI]; Itoh et al., 2002).

A major breakthrough in our understanding of the GA-signaling cascade has been the recent discovery of the soluble GA receptor GA INSENSITIVE DWARF1 (GID1) in rice and Arabidopsis (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006; Griffiths et al., 2007). GA binding to GID1 triggers its interaction with the DELLA domain of the DELLA proteins (Griffiths et al., 2007). This interaction stimulates binding of the DELLA proteins to an SCF E3 ubiquitin ligase via specific F-box proteins (GID2/SLY), leading to polyubiquitination and degradation of the DELLA protein by the 26S proteosome (Sasaki et al., 2003; Dill et al., 2004; Griffiths et al., 2007).

While this relatively simple GA-signaling cascade involves three major players, a receptor, a DELLA protein, and an F-box protein, other studies have identified additional factors that affect GA responses (Hartweck and Olszewski, 2006). One of these is the GA response inhibitor SPIINDLY (SPY; Jacobsen and
The SPY protein resembles mammalian enzymes that modify proteins posttranslationally by a specific type of glycosylation, termed tetratricopeptide repeat-containing Ser and Thr-O-linked GlcNAc transferases (OGT). OGT transfers the single sugar moiety GlcNAc from UDP-GlcNAc to specific Ser/Thr residues via an O-linkage. This posttranslational modification can affect protein localization, phosphorylation, interaction with other proteins, and/or stability (Wells et al., 2001). While there is no direct biochemical evidence for the interaction of SPY with the DELLA proteins, genetic evidence suggests that SPY is required for DELLA’s GA response suppression activity (Hartweck and Olszewski, 2006; Silverstone et al., 2007).

**INTERACTION OF GA WITH OTHER HORMONES**

The mode of GA action in planta is still far from being understood, as numerous positive and negative functional interactions with other endogenous and environmental cues affect GA responses (Fig. 1). Nemhauser et al. (2006) have identified robust target genes that are affected specifically by a single hormone. However, in the case of GA-induced genes, no specific robust targets were identified. This may suggest that interactions with other hormones play major roles in GA action, which necessitates the existence of efficient and sensitive cross talk mechanisms among the corresponding signaling pathways. Recently, several studies have focused on the molecular machinery behind the interactions between GAs and other hormones, uncovering a complex network.

**MECHANISM OF THE ANTAGONISTIC INTERACTION BETWEEN GA AND ABA**

GA and ABA play antagonistic roles in the regulation of numerous developmental processes. Whereas GA is associated with the promotion of germination, growth, and flowering, ABA inhibits these processes. Moreover, the antagonistic relationship and the ratio between these two hormones regulate the transition from embryogenesis to seed germination (Razem et al., 2006). Several different mechanisms have been shown to underlie this antagonistic interaction in different developmental processes (Fig. 2). During cereal seed germination, the developing embryo releases GAs to the aleurone cells where they induce the transcription of several genes encoding hydrolytic enzymes, including α-amylase. These enzymes are then secreted to the endosperm and hydrolyze starch and proteins, supplying nutrients to the developing embryo. In contrast, ABA suppresses α-amylase expression. The GA-induced, ABA-suppressed transcription of α-amylase in the aleurone layer of cereal seeds was classically used as an experimental system to study the interaction between GA and ABA. The α-amylase promoter contains a GA response element, required for both its activation by GA and suppression by ABA (Rogers

**Figure 1.** GA interacts positively and negatively with other plant hormones throughout the plant’s life cycle. Some of the effects are shown. CK, Cytokinin.
and Rogers, 1992). Gubler et al. (1995) identified a GA-induced Myb-like protein (GAMyb) that binds to the GA response element box of the α-amylase promoter. Induction of GAMyb and α-amylase transcription was shown to be mediated by the DELLA protein SLR1, as both are up-regulated in slr1 mutants, even in the absence of GA. How does ABA affect this pathway? The induction of GAMyb and α-amylase by GA is suppressed by an ABA-induced Ser/Thr protein kinase, PKABA1. ABA and PKABA1 inhibited the up-regulation of GAMyb and α-amylase in slr1 mutants as well, suggesting that the inhibition of GAMyb and α-amylase by PKABA1 occurs downstream of DELLA (Gomez-Cadenas et al., 1999, 2001). However, a more recent study has shown that when PKABA1 is suppressed by RNAi, ABA still inhibits the GA-induced α-amylase expression. This finding indicates that ABA affects this process through an additional, PKABA1-independent pathway (Zentella et al., 2002). A candidate alternative ABA-signaling pathway to suppress GA responses in rice may involve two ABA-induced WRKY transcriptional regulators (Xie et al., 2006). These proteins suppress GA-induced α-amylase transcription, but their interaction with PKABA1 is not yet clear.

A different mechanism of interaction between GA and ABA in the regulation of root growth was described by Achard et al. (2006). In Arabidopsis, GA promotes and ABA suppresses root growth, and both effects seem to be mediated by the DELLA proteins. ABA application increased the stability of RGA and blocked its GA-induced degradation. Moreover, the quadruple-DELLA mutant (loss of GAI, RGA, RGL1, and RGL2) is relatively resistant to the growth-inhibitory effects of ABA. Therefore, while during cereal seed germination ABA seems to act downstream of DELLA, it affects Arabidopsis growth via DELLA. It is thus possible that distinct mechanisms of interaction between GA and ABA are utilized for different developmental decisions.

The complexity of the interaction between GA and ABA and its possible organ-specific mechanism were recently demonstrated in Arabidopsis. Both ABA and GA induce the accumulation of microRNA159 (miR159), which targets the MYB33 mRNA. Interestingly, MYB33 promotes ABA responses in seeds and GA responses in flowers. Thus, these two antagonistic hormones exert their action through a common mediator, MYB33, and desensitize their signaling through the same homeostatic mechanism, miR159, at different developmental stages (Achard et al., 2004; Reyes and Chua, 2007). Whether miR159 and MYB33 are part of the mechanism of interaction between the two hormones or are just used by both in a development-specific manner is not yet clear.

**AUXIN INTERACTS POSITIVELY WITH GA**

The activities of GA and auxin overlap with respect to the regulation of cell expansion and tissue differentiation. Auxin affects GA signaling as well as GA biosynthesis (Fig. 3). In Arabidopsis, GA stimulation of root elongation has been shown to require auxin. GA-induced root elongation was inhibited by the removal of the shoot apex that is a major auxin source, and this effect was reversed by auxin application. Moreover, application of the auxin-transport inhibitor 1-N-naphthylphthalamic acid or mutation in the auxin-efflux regulator AtPIN1 suppressed the effect of GA on root elongation and on RGA degradation in the

![Figure 2. Network of interactions between GA and ABA. ABA suppresses GA responses through DELLA-dependent and -independent pathways. Interactions mediated by changes in protein activity or stability are in gray and those mediated by gene expression are in black. Numbers in parentheses indicate the respective reference as follows: 1, Achard et al., 2004; 2, Achard et al., 2006; 3, Gomez-Cadenas et al., 1999; 4, Gomez-Cadenas et al., 2001; 5, Gubler et al., 1995; 6, Reyes and Chua, 2007; 7, Rogers and Rogers, 1992; 8, Xie et al., 2006.]

![Figure 3. Network of interactions between GA and auxin. Auxin promotes GA responses by destabilizing DELLA and by promoting the expression of GA biosynthetic genes. Interactions mediated by changes in protein activity or stability are in gray and those mediated by gene expression are in black. Numbers in parentheses indicate the respective reference as follows: 1, Frigerio et al., 2006; 2, Fu and Harberd, 2003; 3, O’Neill and Ross, 2002; 4, Ross et al., 2000; 5, Wolbang and Ross, 2001.]

root cells. GA-induced RGA degradation was also inhibited in the mutant axr1 in which auxin signaling is compromised. These results suggest that auxin promotes the degradation of DELLA in root cells in response to GA, which is a prerequisite for GA-induced root elongation (Fu and Harberd, 2003). Thus, the DELLA protein RGA seems to act as an integrator of GA and auxin signals in the root.

In addition to its requirement for GA signaling in the root, auxin also affects GA production in the stem by positively regulating the expression of GA biosynthetic genes (Nemhauser et al., 2006). Decapitation of pea (Pisum sativum) and tobacco (Nicotiana tabacum) shoot apices reduced the level of active GAs in the stems, and this effect was reversed by auxin application (Ross et al., 2000; Wolbang and Ross, 2001). Auxin was shown to induce the expression of the GA biosynthetic gene GA20ox in tobacco and Arabidopsis, whereas in pea, the hormone induced the expression of GA3ox and suppressed the expression of GA2ox, which is involved in GA deactivation (O’Neill and Ross, 2002; Frigerio et al., 2006). Loss of the DELLA genes GAI and RGA had no effect on the induction of GA2ox by auxin. Thus, auxin induces GA biosynthesis through a DELLA-independent pathway or via other DELLA proteins. Addressing this problem in rice, which contains only a single DELLA, or in Arabidopsis plants completely lacking DELLA activity may help distinguish between these possibilities. The effect of auxin on GA biosynthesis was shown to transduce via the degradation of auxin signaling suppressors Aux/IAA proteins (for review, see Teale et al., 2006) and the resulting activation of the transcription factor AUXIN RESPONSE FACTOR7 (ARF7). Moreover, loss of the auxin receptor TIR1, an F-box protein that mediates Aux/IAA degradation and the consequent ARF activation, suppressed auxin regulation of GA biosynthetic gene expression (Frigerio et al., 2006). Therefore, auxin positively interacts with GA either at the biosynthesis level or by promoting DELLA degradation.

**DEVELOPMENTAL AND ENVIRONMENTAL CIRCUMSTANCES DETERMINE WHETHER INTERACTIONS BETWEEN GA AND ETHYLENE ARE POSITIVE OR NEGATIVE**

The interaction between GA and the stress-related gaseous hormone ethylene is rather complex, as both negative and positive reciprocal effects have been demonstrated (Fig. 4). Ethylene inhibits growth in a GA-antagonistic manner. Achard et al. (2003) have shown that at least part of the inhibitory effect of ethylene on growth and its interaction with GA in this regard is mediated by the DELLA proteins. GA promotes seedling root elongation in Arabidopsis, and this effect is inhibited by ethylene. However, in gai rga double mutants, GA stimulated root elongation also in the presence of ethylene, suggesting that ethylene acts through these DELLA proteins in this process. In agreement with this, ethylene inhibited RGA degradation in root-cell nuclei in response to GA. The effect of ethylene on RGA stability was mimicked by the loss of its signaling suppressor CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), suggesting that ethylene’s RGA-stabilizing signal is transduced via a CTR1-dependent pathway (for review, see Guo and Ecker, 2004). Negative interaction between ethylene and GA was also shown in mature plants. The induction of several GA-responsive genes by GA was enhanced in the Arabidopsis ethylene-resistant mutant ctr1 or when plants were pretreated with the ethylene perception inhibitor 1-methylcyclopropene. Thus, ethylene inhibits GA response in mature Arabidopsis plants (De Graauwe et al., 2007). Furthermore, ethylene delayed the transition to flowering in Arabidopsis under short days, and this effect was suppressed by GA treatment and in gai rga double mutant (Achard et al., 2007). While in seedlings, ethylene was shown to affect DELLA stability directly, during the transition to flowering, it affected GA biosynthesis. The resulting reduction in the level of biologically active GAs repressed the expression of two central flowering genes, LEAFY and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 and the consequent transition to flowering. This effect of ethylene on the accumulation of active GA is transduced via CTR1 and the downstream transcriptional regulator ETHYLENE INSENSITIVE3. This study suggests that the antagonistic interaction between ethylene and GA mediates the timing of the decision to flower in response to changing environmental conditions.
While ethylene is classically considered to be a growth inhibitor that antagonizes GA responses, positive interactions have also been described. In etiolated seedlings, ethylene induces a triple response that includes the formation of an apical hook and the inhibition of hypocotyl and root growth. The apical hook is formed by asymmetric elongation of the inner and outer sides of the hypocotyl and helps the young seedling grow through the soil. Induction of the apical hook by ethylene requires GA activity (Achard et al., 2003; Vriezen et al., 2004). Ethylene-treated hypocotyls of the GA-deficient mutant ga1-3 did not form an apical hook, but the response to ethylene was restored by GA treatment or when ga1-3 was combined with the loss of both RGA and GAI. A synergistic promotive effect of GA and ethylene was also shown in light-grown Arabidopsis seedlings (Saibo et al., 2003); while GA is the major factor controlling cell elongation in light-grown hypocotyls, ethylene enhances this effect. Whether this positive interaction is mediated by the DELLA proteins, i.e. ethylene reduces DELLA stability, is not yet known. These studies indicate that GA and ethylene stimulate each other’s activities reciprocally under specific circumstances.

Ethylene plays a central role in regulating the plant’s developmental reaction to stress. GA has been shown to be positively involved in ethylene activity under conditions of oxygen deficiency. The elongation of deepwater-rice internodes requires the activity of GA and ethylene (Sauter et al., 1995). Reduced O$_2$ level during rice submergence was suggested to induce ethylene production, which in turn inhibited ABA synthesis. This changed the balance between ABA and GA, resulting in GA-induced stem elongation. In *Rumex palustris*, submergence induces the expression of GA$_3$ox, suggesting that ethylene induces GA synthesis (Benschop et al., 2006). Thus, during submergence, ethylene affects the GA-to-ABA ratio by inhibiting ABA production and inducing GA synthesis. Ethylene also induced adventitious root growth in submerged rice. While GA had no effect on its own on this process, it acted synergistically with ethylene to promote root growth (Steffens et al., 2006).

Depending on the developmental process and environmental conditions, ethylene thus interacts both positively and negatively with GA. Furthermore, the interaction between these two hormones operates at both the biosynthesis and signal transduction levels, exhibits reciprocal effects of these hormones on one another, and involves both additive and synergistic effects.

**RECIProCAL INTERACTION BETWEEN GA AND CYTOKININ**

GA and cytokinin exert antagonistic effects on numerous developmental processes, including shoot and root elongation, cell differentiation, shoot regeneration in culture, and meristem activity (Greenboim-Wainberg et al., 2005; Jasinski et al., 2005). Several recent studies have shown development-dependent reciprocal interactions between the two hormones, where cytokinin inhibits the production of GA and promotes its deactivation and GA inhibits cytokinin responses (Fig. 5).

High cytokinin and low GA signals are required for normal shoot apical meristem (SAM) function (Sakamoto et al., 2001; Jasinski et al., 2005; Yanai et al., 2005). SAM regulators from the KNOTTED1-like homeobox (KNOXI) protein family were shown to induce expression of the cytokinin-biosynthesis gene *ISOPENTENYL TRANSFERASE* and accumulation of the hormone in the meristem (Jasinski et al., 2005; Yanai et al., 2005). Proteins from this family were also shown to negatively control GA level in the SAM by binding to the GA20ox promoter and directly repressing its transcription (Sakamoto et al., 2001; Hay et al., 2002; Chen et al., 2004). KNOXI and cytokinin both induced the expression of the gene encoding the GA-deactivating enzyme GA2ox at the base of the SAM, perhaps to block biologically active GAs transported to the SAM from flanking tissues (Jasinski et al., 2005). Thus, KNOXI proteins control the balance between cytokinin and GA in the SAM by inducing cytokinin production, directly inhibiting GA synthesis, and indirectly promoting GA deactivation. Genome-wide expression profiling of cytokinin-treated Arabidopsis seedlings revealed that cytokinin inhibits the expression of GA20ox and GA3ox and promotes that of RGA and GAI, further expanding upon the negative interactions between these hormones (Brenner et al., 2005).

![Figure 5. Network of reciprocal interactions between GA and cytokinin. Two major players control the balance between GA and cytokinin. KNOXI proteins control the balance between the two hormones in the SAM by inducing cytokinin production, directly inhibiting GA synthesis, and indirectly promoting GA deactivation. SPY regulates the balance between the response pathways of these two hormones via suppression of GA signal and promotion of cytokinin responses. Interactions mediated by changes in protein activity or stability are in gray and those mediated by gene expression are in black. Numbers in parentheses indicate the respective reference as follows: 1, Brenner et al., 2005; 2, Chen et al., 2004; 3, Greenboim-Wainberg et al., 2005; 4, Hay et al., 2002; 5, Jasinski et al., 2005; 6, Sakamoto et al., 2001; 7, Silverstone et al., 2007; 8, Yanai et al., 2005. IPT, ISOPENTENYL TRANSFERASE.](image-url)
Whereas SAM activities require high cytokinin and low GA signals, later stages of cell maturation and elongation require the opposite: low cytokinin and high GA signals. A reverse antagonistic interaction, in which GA inhibits cytokinin, has also been demonstrated. Greenboim-Wainberg et al. (2005) have shown that GA, or a mutation in the GA-signaling suppressor SPY, inhibit cytokinin responses in Arabidopsis. Several lines of evidence suggest SPY acts directly to promote cytokinin responses and that GA suppresses cytokinin responses via SPY. This suggests that in the absence of GA, SPY represses GA signaling and promotes cytokinin responses, but when cellular GA levels increase, the hormone suppresses SPY activity and, therefore, cytokinin responses. How GA suppresses the cytokinin response via SPY is not yet clear. Recent results have suggested that GA has no effect on SPY activity toward the inhibition of GA responses (Silverstone et al., 2007). It is possible, however, that GA inhibits cytokinin primary responses TYPE-A RESPONSE REGULATORS, it was suggested that SPY interacts with, and perhaps modifies (via O-GlcNac modification), elements of the cytokinin phosphorelay cascade (for review of the cytokinin-signaling pathway, see Hutchison and Kieber, 2002; Kakimoto, 2003; Ferreira and Kieber, 2005). However, elucidation of the mechanism by which SPY affects the cytokinin pathway still requires intensive study.

Despite the pronounced effect of spy on cytokinin responses, the spy phenotype is much less severe than that of the triple mutant of the cytokinin receptors (Higuchi et al., 2004). This could result from functional redundancy with either GA-related or nonrelated components. The Arabidopsis genome contains one additional OGT gene, SECRET AGENT (SEC; Hartweck et al., 2002). While sec mutations do not show any obvious phenotypic alteration, the sec spy double mutant is lethal (Hartweck et al., 2002). As high GA levels or signal do not cause lethality, this lethality could result from an effect of the double mutant on both GA and cytokinin pathways.

Interestingly, GA and spy suppress phenotypes caused by KNOXI overexpression (Hay et al., 2002). Although GA and spy may simply restore the inhibition effect of KNOXI on GA biosynthesis, it is also possible that they inhibit KNOXI-induced cytokinin responses or that SPY is required directly for KNOXI activity, the latter representing another possible level of interaction between GA and cytokinin.

Hence, cytokinin and GA act mostly in an antagonistic manner. The reciprocal interaction is regulated at both the biosynthesis and signal transduction levels.

CONCLUSION AND PERSPECTIVES

GA interacts with almost all other plant hormones, in some cases reciprocally, whereby GA affects but is also being affected by the other hormone. The direction and type (positive or negative) of the interaction depends on the biological process, tissue, developmental stage, and/or environmental conditions. The network likely features further levels of complexity, as interactions between more than two hormones to regulate specific developmental processes have been documented. For example, GA, auxin, and ethylene interact to promote elongation of light-grown seedlings (Saibo et al., 2003), and GA, cytokinin, and auxin are all involved in SAM development (Shani et al., 2006). This naturally results in a seemingly infinite number of possible combinations for regulation, which may contribute to the plant’s ability to cope with a constantly changing environment with high flexibility (Brady and McCourt, 2003). It is clear, however, that the current knowledge is just the tip of the iceberg in a complex network of interactions between the various plant hormones.

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LITERATURE CITED


CORRECTIONS


Pislariu C. and Dickstein R. An IRE-Like AGC Kinase Gene, MtIRE, Has Unique Expression in the Invasion Zone of Developing Root Nodules in Medicago truncatula.

On Page 687, the first full sentence in the right column should read as follows: “Plants were inoculated with rhizobia carrying a constitutive lacZ gene, and nodulated roots were harvested at 15 dpi.”


Weiss D. and Ori N. Mechanisms of Cross Talk between Gibberellin and Other Hormones.

In Figure 3 of this Update, the inhibition bar from Aux/IAA to DELLA should be an arrow. The corrected figure is below.