Chapter 9
Integration of Ethylene and Gibberellin Signaling

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Abstract The phytohormones ethylene and gibberellin (GA) act synergistically to regulate a diversity of plant growth and development processes. In the presence of ethylene, the signaling mediated by ethylene receptors and CTR1 (CONSTITUTIVE TRIPLE RESPONSE1) is switched off, while EIN2 (ETHYLENE INSENSITIVE2) and EIN3 (ETHYLENE INSENSITIVE3) together mediate ethylene signaling. GA promotes plant growth by facilitating the degradation of the DELLA proteins, a family of nuclear growth repressors. Although the existence of crosstalk between ethylene and GA in the context of growth and development has long been known, its molecular basis is only now beginning to be understood. Both the synthesis and the signaling pathways controlled by ethylene and GA are reciprocally regulated. In this chapter, recent advances in the understanding of how they regulate germination, root and hypocotyl growth, apical hook development, and flowering initiation are reviewed. The significance of ethylene–GA crosstalk in the plant response to abiotic stress is described.

Keywords Crosstalk · Germination · Root development · Hypocotyl elongation · Apical hook formation · Flowering time · Abiotic stress

9.1 Introduction

The gibberellins (GAs) form a family of tetracylic diterpenoid plant hormones which impinge on various aspects of plant growth and development, from germination through stem, hypocotyl and root growth to the switch from vegetative to

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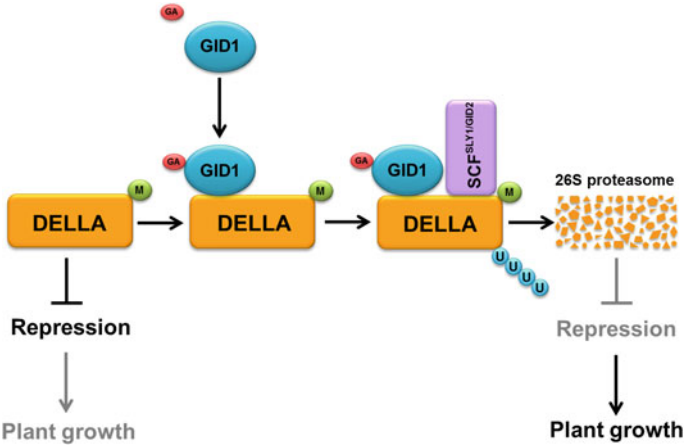

Fig. 9.1 The GA signaling pathway involving DELLA proteins. In the absence of GA, the DELLA proteins repress plant growth. The binding of GA to its receptor GID1 permits the interaction between GID1 and DELLA. The formation of the GID1-GA-DELLA complex enhances the interaction between DELLA and the $SCF^{SLY1/GID2}$ F-box component, leading to the polyubiquitination of DELLA and their targeting for degradation via the 26S proteasome pathway. *M*, post-translationally modified DELLA; *U*, ubiquitinated DELLA

reproductive growth (Sun and Gubler 2004; Jiang and Fu 2007; Gao et al. 2008, 2011). The analysis of GA-insensitive mutants in both *Arabidopsis thaliana* and rice has identified a number of GA signaling components, in particular, the GA receptor GID1 (GIBBERELLIN INSENSITIVE DWARF1), the DELLA proteins (the major repressors of GA signaling), and F-box-containing proteins such as SLY1 [SLEEPY1] in *A. thaliana* and GID2 [GIBBERELLIN INSENSITIVE DWARF2] in rice (Dill et al. 2004; Fu et al. 2004; Ueguchi-Tanaka et al. 2007; Itoh et al. 2008; Shimada et al. 2008). On this basis, a derepression model for GA signaling has been elaborated, according to which once bioactive GA has been perceived and bound by GID1, the resulting complex can drive the formation of a GA-GID1-DELLA protein complex capable of efficiently binding the E3 ubiquitin ligase $SCF^{SLY1/GID2}$ complex (Griffiths et al. 2006). This binding results in the polyubiquitination of the DELLA proteins, thereby directing their degradation via the 26S proteasome, and so relieving the growth suppression exerted by them (Fig. 9.1) (Gao et al. 2011; Daviere and Achard 2013).

The gas ethylene also regulates a wide range of growth and developmental processes. It is perceived by the ETR1 (ETHYLENE RESPONSE1) family of ethylene receptors (Hua et al. 1995, 1998; Hua and Meyerowitz 1998; Sakai et al. 1998; Guo and Ecker 2004). In its absence, ETR1 activates CTR1 (CONSTITUTIVE TRIPLE RESPONSE1), a Raf-like Ser/Thr protein kinase which suppresses ethylene signaling (Kieber et al. 1993; Huang et al. 2003; Mayerhofer et al. 2012). EIN2 (ETHYLENE INSENSITIVE2) acts downstream of CTR1 and represents a critical component of the ethylene signaling pathway (Alonso et al. 1999). In the presence of ethylene, its receptors become inactivated, in turn switching off CTR1

and permitting EIN2 to function. The basis for the EIN2-mediated transduction of the ethylene signal from the ethylene receptors associated with the endoplasmic reticulum to its downstream transcription factors EIN3 and EIL1 (EIN3-LIKE1) has been described independently by three research groups (Ju et al. 2012; Qiao et al. 2012; Wen et al. 2012). EIN3 and EIL1 mediate a wide array of the plant responses to ethylene (Chao et al. 1997; Solano et al. 1998; Stepanova and Alonso 2009). The levels of EIN3 and EIL1 in the nucleus are finely tuned by the Skp1-Cullin-F-box protein (SCF) E3 ubiquitin ligase isoforms EBF1 and EBF2 (Guo and Ecker 2003; Potuschak et al. 2003; Gagne et al. 2004).

The existence of crosstalk between ethylene and GA signaling has been recognized for a long time, but its molecular basis remains to be determined. Both ethylene and GA affect one another at the level of synthesis, signaling and gene expression (De Grauwe et al. 2007, 2008; Dugardeyn et al. 2008). In this chapter, we focus on the interaction between ethylene and GA signaling in the context of the regulation of germination, root growth, hypocotyl elongation, apical hook development, and floral induction (Fig. 9.2). In addition, we also describe their role in the plant response to abiotic stress (Fig. 9.3).

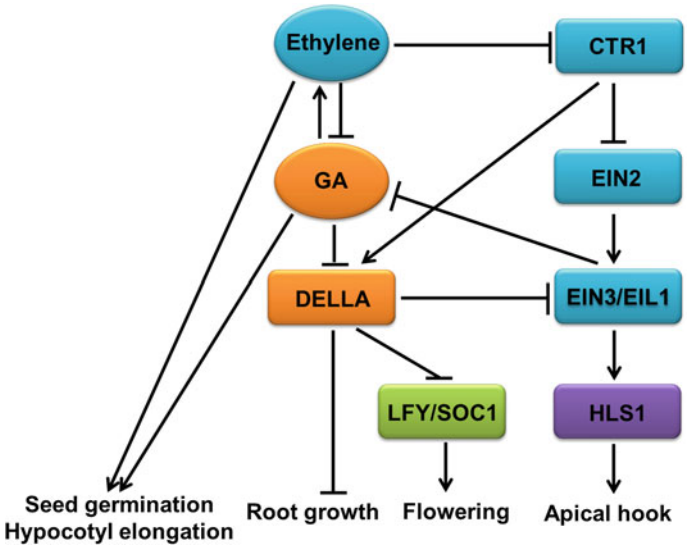

Fig. 9.2 The modulation of *A. thaliana* growth and development, as controlled by ethylene and GA (modified from Weiss and Ori (2007)). Ethylene and GA in concert promote germination and induce the elongation of the hypocotyl in the presence of light. Ethylene and GA also together regulate apical hook development in the absence of light by up-regulating *HLS1*. Ethylene stabilizes EIN3/EIL1, while GA relieves the DELLA-imposed repression on EIN3/EIL1. EIN3/EIL1 links the ethylene and GA pathways to activate *HLS1* transcription. Ethylene inhibits root growth by controlling the abundance/stability of DELLA. Activation of ethylene signaling reduces the level of bioactive GA, thereby promoting the accumulation of DELLA, which in turn delays flowering via the regulation of *LFY* and *SOC1* transcription. *CTR1* CONSTITUTIVE TRIPLE RESPONSE1; *EIN2* and *EIN3*, ETHYLENE INSENSITIVE2 and 3; *EIL1*, EIN3-LIKE1; *HLS1* HOOKLESS1; *LFY* LEAFY; *SOC1* SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1

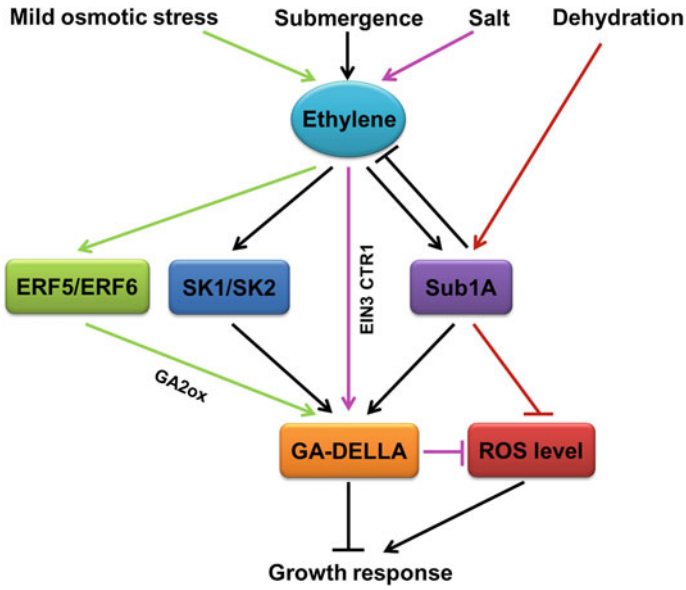

Fig. 9.3 The rice ethylene and GA regulatory network during an episode of abiotic stress (modified from Bailey-Serres and Voesenek (2010)). In deepwater rice, ethylene up-regulates *SK1* and *SK2* and elevates the level of GA, thereby inducing a rapid elongation of the stem internode, allowing the canopy to rise above the water's surface. In submergence-tolerant rice, ethylene activates *Sub1A*, which promotes DELLA accumulation, enhancing survival by limiting stem elongation and restricting assimilate consumption. *Sub1A* also promotes the tolerance of mild osmotic stress by preventing the build-up of damaging levels of reactive oxygen species. Mild osmotic and salinity stress activates ethylene signaling and stabilizes the DELLA proteins, which act to limit plant growth. *ERF1* ETHYLENE-RESPONSIVE FACTOR; *ROS* reactive oxygen species; *SK1* and *2* SNORKEL1 and 2; *Sub1A* Submergence 1A

9.2 Ethylene and GA Coordinately Promote Seed Germination

Seed of the *A. thaliana* GA-deficient mutant *ga1-3* (which synthesizes little or no *ent*-kaurene because it lacks a functional copy of *GA1*) is unable to germinate without the provision of exogenous GA. Ethylene influences germination in *A. thaliana* (Kepczynski and Kepczynska 1997), since the ethylene synthesis precursor 1-aminocyclopropane-1-carboxylic acid (ACC) can compensate for the absence of GA in the *ga1-3* seed, allowing it to germinate without GA supplementation in the light; its promotive effect is less marked in the dark (Karssen et al. 1989; Vriezen et al. 2004). Several ethylene response genes, including *ACO* (encoding *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE*), are up-regulated in imbibed *ga1-3* seed following the exogenous supply of GA_4 (Ogawa et al. 2003). The transcription of both the ethylene-inducible gene *HOOKLESS1*

(*HLS1*) (Lehman et al. 1996) and the ethylene receptor gene family's *ERS1* (*ETHYLENE RESPONSE SENSOR1*) (Hua et al. 1998) is similarly increased by the exogenous supply of GA_4 (Ogawa et al. 2003). The implication is that GA may activate ethylene synthesis and/or production, thereby promoting the ethylene responses in the imbibed *A. thaliana* seed.

The dominant *A. thaliana etr1-1* mutant is ethylene insensitive. Freshly harvested *etr1-1* seed germinates less readily than does wild-type (WT) seed (Beaudoin et al. 2000). Only bioactive GA_4 is present in mature WT seed at a concentration of 25 ng/g dry weight (DW), while the concentrations of GA_1, GA_4 and GA_7 are all higher in the *etr1-2* mutant seed than in WT seed. WT seed accumulates GA_1 over the first 18 h of germination, but thereafter its concentration declines, with a concomitant modest rise in the concentration of GA_4. Germinating and germinated WT seed contains 7–12 ng/g DW GAs, whereas *etr1-2* seed contains more GA_1 and GA_4 (Chiwocha et al. 2005). Thus, it appears that while GA may promote ethylene synthesis in imbibed *ga1-3* seed, the enhanced level of GA present in *etr1* seed indicates that ethylene may suppress GA synthesis. The indication is that a feedback mechanism regulates GA and ethylene synthesis, and that these two phytohormones synergistically influence germination (Fig. 9.2).

9.3 Ethylene Inhibits *A. thaliana* Root Growth by Interacting with DELLA

The DELLA proteins are members of the plant-specific GRAS family [named from the first three members of the family to be identified, namely GAI (GIBBERELLIN INSENSITIVE), RGA (REPRESSOR OF GA1-3), and SCARECROW] and are thought to represent transcriptional regulators. The *A. thaliana* DELLA proteins include GAI, RGA, RGL1 (RGA-LIKE1), RGL2, and RGL3 (Jiang and Fu 2007), all of which act to repress GA signaling (Dill et al. 2001; Silverstone et al. 2001; Fu and Harberd 2003). The root is a key organ because it is responsible for the acquisition of water and nutrients. The *ga1-3* mutant develops a shortened primary root, which can be made to grow to a WT length either by providing the plant with exogenous GA or by knocking out DELLA. These observations show that the GA-DELLA regulatory system operates in the context of seedling root growth (Fu and Harberd 2003). Ethylene also inhibits root growth. When *A. thaliana* seedlings are exposed to either ethylene or ACC, primary root growth is inhibited (Abeles et al. 1992), and this inhibition involves the DELLA proteins GAI and RGA (Achard et al. 2003) (Fig. 9.2). When ACC is not supplied to seedlings of DELLA protein-lacking mutants (*gai-t6*, *rga-24*, and *gai-t6 rga-24*), the root length is identical to that of the WT. Conversely, in the presence of ACC treatment, the WT roots are shorter than those of *rga-24* seedlings, and the double mutant *gai-t6 rga-24* roots grow even longer than those of seedlings of the two single mutants. These data suggest that GAI and RGA together mediate ethylene-induced root growth inhibition. In addition, GA treatment can overcome the ACC-induced inhibition of seedling root growth (Fig. 9.2).

GFP (green fluorescent protein)-RGA fusion protein is detectable in root cell nuclei, but rapidly disappears in response to GA treatment (Silverstone et al. 2001). Ethylene appears to inhibit root growth in *A. thaliana* by delaying the GA-mediated degradation of GFP-RGA (Achard et al. 2003) (Fig. 9.2). When *pRGA*::*GFP-RGA* containing transgenic plants are treated with GA, the GFP signal is markedly attenuated within 90 min at both the root tip and the root elongation zone and disappears completely by 3 h. However, when the same seedlings are grown in an atmosphere containing ethylene gas, the fusion protein becomes more stable, remaining readily detectable even 3 h after the GA treatment. Ethylene promotes many responses which are antagonized by CTR1, and the GFP-RGA fusion product is less readily degraded as a result of GA treatment in the mutant than in WT plants. The implication is that ethylene delays the GA-mediated degradation of GFP-RGA via a CTR1-dependent signaling pathway (Achard et al. 2003) (Fig. 9.2).

9.4 Ethylene and GA Synergistically Induce the Elongation of the *A. thaliana* Hypocotyl

In *A. thaliana*, hypocotyl elongation is achieved predominantly via cell elongation and is tightly regulated by various phytohormones. GA promotes the process, whereas ethylene inhibits it (Cowling and Harberd 1999; Collett et al. 2000). Ethylene has been shown to stimulate hypocotyl elongation when seedlings are grown in a low nutrient medium (LNM) (Smalle et al. 1997). In this situation, most of the elongation occurs over the first three days following imbibition. Treatment with ACC extends the period of rapid growth for an additional day. In contrast, treatment with GA_3 has no effect on the duration of rapid growth, but does increase the growth rate between days two and three (Saibo et al. 2003). The effect of a combined application of ACC and GA_3 is at the very least additive, and in most cases synergistic, thereby resulting in the highest increase in hypocotyl length (Saibo et al. 2003; vandenBussche et al. 2007) (Fig. 9.2). Furthermore, the hypocotyl elongation in the *gai etr1-3* double mutant plant is completely insensitive to either phytohormone (De Grauwe et al. 2007).

The ethylene-mediated regulation is now known to depending on blue light and cryptochrome signaling, and that GA is required for the ethylene-stimulated hypocotyl elongation to occur (vandenBussche et al. 2007). Treatment with ACC in the presence of blue light up-regulates the transcription of the GA synthesis genes *GA20ox1* (*GA 20-oxidase*) and *GA3ox1* (*GA 3-oxidase*) and down-regulates that of the GA metabolism genes *GA2ox1* (*GA 2-oxidase*) and *GA2ox7*, as well as that of the GA synthesis gene *GA3ox2* (Vriezen et al. 2004; Vandenbussche et al. 2007). There is no substantial effect on the abundance of transcript of genes encoding either GA receptors or the DELLA proteins, although the accumulation of GFP-RGA fusion protein is enhanced in ACC-treated *pRGA*::*GFP-RGA* plants (Vandenbussche et al. 2007).

GA and ethylene have an effect on endoreduplication frequency (Traas et al. 1998; Gendreau et al. 1999). Ethylene induces endoreduplication in both light- and dark-grown hypocotyl, and the process involves the ethylene signaling pathway genes *CTR1* and *EIN2* (Gendreau et al. 1999). Hypocotyl cells in LNM-grown seedlings contain nuclei with three ploidy levels: 2C, 4C, and 8C (C is the DNA content of the haploid genome in the G1 phase). ACC treatment increases the 8C:4C ratio and a small fraction of cells undergo an additional round of endoreduplication to deliver about 2 % 16C nuclei. Similarly, GA_3 treatment increases the 8C:4C ratio, but no 16C nuclei are produced. A combined ACC and GA_3 treatment has the greatest enhancing effect on the 8C:4C ratio and on the representation of 4 % 16C nuclei (Saibo et al. 2003).

Although cell elongation is mainly responsible for hypocotyl growth, cell division also should be considered. Treatment with either ACC or GA_3 on their own increases cortical cell number by, respectively, 1.4 and 1.3, while in combination, they enhance cortical cell number by 2.5 (Saibo et al. 2003). However, the measurements of GUS activity generated by *CycB1;1*::*GUS* transgenics (the *CycB1;1* product is involved in cell division) imply that cell division occurs solely in the hypocotyl epidermal layers, which contributes to the development of stomata. Treatment with either ACC or GA increases stomata number in the hypercotyl by, respectively, 33 % and 21 %, while the combined treatment increases it by 55 % (Saibo et al. 2003).

9.5 Ethylene-Induced Apical Hook Development Is Dependent on GA

The apical hook is a transient curvature of the hypocotyl tip assumed to protect the cotyledons and the shoot apical meristem from mechanical damage as the seedling grows through the soil. The curvature is generated by asymmetric growth, specifically via differential cell division and elongation of the inner and outer sides of the hypocotyl (Lehman et al. 1996; Raz and Ecker 1999; Raz and Koornneef 2001). The formation of the apical hook involves three developmental phases: formation, maintenance, and opening (Raz and Ecker 1999; Abbas et al. 2013). Ethylene is known to be an important regulator of apical hook development. Dark-grown seedlings treated with exogenous ethylene or ACC produce a shortened root, a shortened and radially expanded hypocotyl, and an exaggerated apical hook (Abeles et al. 1992; Ecker 1995). GA is also involved in apical hook development. The *ga1-3* mutant does not form an apical hook in three-day-old etiolated seedlings, whereas in the absence of either GAI and/or RGA, a hook is formed by this mutant. These data indicate that GA opposes DELLA proteins repression and can promote the apical hook formation (Achard et al. 2003) (Fig. 9.2). In addition, the GA-DELLA regulatory system also affects apical hook maintenance, involving both ethylene and auxin signaling.

In the presence of either ethylene or ACC, the size of the apical hook is exaggerated in WT seedlings, but this is not the case in the *ga1-3* mutant (Achard et al. 2003). The *ctr1-1* mutant does not form a hook in the presence of inhibitor of GA synthesis paclobutrazol (PAC), consistent with the notion that GA is involved in apical hook formation, even in a constitutive ethylene signaling status (Vriezen et al. 2004). The development of the apical hook requires differential cell division and elongation at the hypocotyl apex (Raz and Koornneef 2001). Although GA and ethylene have little effect on cortical cell division in the hypocotyl, the influence of both ACC and GA on cell division during apical hook development is clear. PAC almost completely inhibits cell division in two- or three-day-old dark-grown seedlings, and *CycB1*;*1*::*GUS* transgenic seedlings harbor almost no GUS-stained cells. Thus, GA_3 counteracts the PAC effect and stimulates cell division. The combined application of ACC and GA_3 has the strongest stimulatory effect on cell division; but when compared with the effect of GA_3 on its own, the extent of the enhancement is not significant. The conclusion is that ethylene acts to enhance the effect of GA on cell division (Vriezen et al. 2004).

Similar to the effect on GFP-RGA level in the root and hypocotyl, ethylene also induces RGA accumulation in the nucleus within the apical hook. In addition, ACC treatment clearly up-regulates the transcription in the apical region of the GA-responsive gene *GASA1* (Herzog et al. 1995). GA homeostasis is regulated by feedback mechanisms, triggered by changes in GA-signaled GA synthesis and metabolism (Hedden and Phillips 2000). For example, the transcription of *GA1* is strongly up-regulated in PAC-treated seedlings. ACC treatment also markedly up-regulates *GA1* in the apical hook endodermis, which suggests that ACC, like PAC, attenuates the GA response and modulates the level of feedback over GA synthesis (Vriezen et al. 2004).

Gallego-Bartolome et al. (2011) have described a kinematic analysis of GA action during apical hook development. A comparison between the impact of PAC, ACC, and GA on WT (*A. thaliana* ecotype Landsberg *erecta*), the *gai-1* (*GA* signaling pathway gain-of-function mutant), *della* (a complex mutant which does not produce any of the five DELLA proteins), and the *ein2-1* (ethylene-insensitive mutant) (Guzman and Ecker 1990; Peng et al. 1997; Feng et al. 2008) demonstrates that GA plays a prominent role during the formation and opening the apical hook. PAC-treated WT seedlings fail to form a hook and gradually enter the opening phase, whereas the *della* mutant develops an exaggerated apical hook which is not induced to open by ACC treatment. Seedlings of *ein2-1* form no hook, although this failure can be partially reversed by GA treatment. The conclusion is that GA and ethylene probably act independently of one another during hook formation, whereas the two phytohormones cooperate to prevent hook opening. In further experiments, the same authors have established that GA activity in the endodermis is essential for normal hook development, at least during the late formation phase, whereas epidermal GA activity is non-essential.

The *gai-1* mutant displays a reduced level of transcription of the ethylene synthesis genes *ACC SYNTHASE8* (*ACS8*) and *ACS5/ETO2* (Vogel et al. 1998; Yamagami et al. 2003), as well as of the ethylene-inducible gene *HLS1* (Lehman

et al. 1996), which all contribute to ethylene-induced hook development. Given that GAI and PIF5 (PHYTOCHROME INTERACTING FACTOR5) interact with one another in vivo, and that PIF5 binds the *ACS8* promoter in a GA-dependent manner, a possibility is that the DELLA proteins repress *ACS8* expression by inhibiting PIF5 activity. Moreover, GA-regulated *ACS* expression is associated with ethylene production in etiolated seedlings, and the *della* mutant produces more ethylene than does the WT plant (Gallego-Bartolome et al. 2011). GA also up-regulates *HLS1* expression (Gallego-Bartolome et al. 2011; Abbas et al. 2013). Analysis has shown that there is a temporal coincidence in the requirement of GA and HLS1 activity during hook development (Gallego-Bartolome et al. 2011).

EIN3/EIL1 are the primary transcription factors in the ethylene signal transduction pathway (Alonso et al. 2003), and overexpression of either *EIN3* or *EIL1* results in exaggerated hook curvature (Chao et al. 1997; An et al. 2010). An and her colleagues find that GA_3 enhances, whereas PAC represses, ethylene- and EIN3-overexpression-induced hook curvature in six-day-old etiolated seedlings, and *della* mutant exhibits exaggerated hook curvature, which requires an intact ethylene signaling pathway (An et al. 2012). *HLS1* encodes a protein with sequence similarity to N-acetyltransferase, and mutation in *HLS1* has no effect on the growth of the hypocotyl apex in the presence of exogenous ethylene (Lehman et al. 1996). HLS1 is also required for GA to have any effect on hook development (Gallego-Bartolome et al. 2011). The *hls1* mutation overrides the exaggerated hook curvature phenotype shown by *della*, and the *hls1 della* sextuple mutant forms no hook, which has been taken to imply that GA- and DELLA-regulated hook development is dependent on *HLS1* (An et al. 2012) (Fig. 9.2). Furthermore, ethylene and GA induce *HLS1* transcription in an EIN3/EIL1-dependent manner and neither can induce *HLS1* transcription in the *ein3 eil1* mutant. Other experiments have shown that EIN3 binds directly with the *HLS1* promoter to induce its expression (An et al. 2012). The DELLA proteins RGA and GAI may interact with the DNA-binding domains of EIN3/EIL1 in vivo and repress EIN3/EIL1-regulated *HLS1* expression (An et al. 2012) (Fig. 9.2).

9.6 Ethylene-Induced Flowering May Result in Part by a Modulation of DELLA Activity

A number of mechanisms have evolved to take account of endogenous signals and environmental cues to time the plant's transition from vegetative to reproductive growth, a critical moment in the life cycle of the flowering plant (Achard et al. 2007). That ethylene modulates the vegetative growth of *A. thaliana* in response to changes in the environment has been amply demonstrated (Abeles et al. 1992; Wang et al. 2002; Achard et al. 2006). However, its participation in the regulation of the switch from vegetative to reproductive growth is less clear (Thomas and Vince Prue 1997; Achard et al. 2007). On the other hand, GA is clearly important for controlling this

transition in *A. thaliana* (Mutasa-Gottgens and Hedden 2009; Srikanth and Schmid 2011). The *ga1-3* mutant is unable to flower under short-day (SD) conditions without exogenously supplied GA, and the flowering of the *gai* mutant is substantially delayed under SD conditions, a phenotype which cannot be rescued by GA_3 treatment (Wilson et al. 1992). GA promotes flowering in *A. thaliana* by activating floral meristem identity genes such as *LEAFY* (*LFY*) and *SUPPRESSOR OF OVEREXPRESSION CONSTANS1* (*SOC1*). The DELLA proteins act to delay flowering under SD conditions by repressing *LFY* and *SOC1* transcription (Moon et al. 2003; Achard et al. 2004, 2007; Mutasa-Gottgens and Hedden 2009).

Ethylene delays flowering in a DELLA-dependent manner (Fig. 9.2). In the presence of ACC or in an ethylene-rich atmosphere, flowering is delayed in WT *A. thaliana,* although the effect is less marked in both the *gai-t6 rga-24* double mutant and the quadruple DELLA mutant *gai-t6 rga-t2 rgl1-1 rgl2-1* (Achard et al. 2006). Floral transition is also delayed in the *gai eto2-1* double mutant, which overproduces ethylene, and even more strongly than in WT plants exposed to ethylene (De Grauwe et al. 2008). The loss-of-function *ctr1-1* mutant is late flowering under both long-day (LD) and SD conditions. Compared with WT plants, the level of GA_1 and GA_4 is significantly reduced in LD-grown *ctr1-1* mutant plants, while the content of intermediate GA species (such as GA_{24} and GA_{53}), which act as substrates for GA 20-oxidase in the synthesis of bioactive GA (Hedden and Phillips 2000), is significantly enhanced. Both *AtGA3ox1* and *AtGA20ox1* transcript abundance is higher in the *ctr1-1* mutant than in WT plants, but in the triple mutant *ctr1-1 gai-t6 rga-24*, the levels of transcript revert to those seen in the WT, thereby implicating DELLA function in the up-regulation of the two GA oxidase genes in *ctr1-1* (Achard et al. 2007).

The abundance of both *LFY* and *SOC1* transcript is lower in the *ctr1-1* mutant than in WT plants, but GA-treated *ctr1-1* plants or *ctr1-1* plants lacking both *GAI* and *RGA* exhibit a relatively normal *LFY* and *SOC1* transcript level. The inference is that the ethylene-mediated inhibition of CTR1 activity reduces the level of bioactive GA, resulting in a down-regulation of *LFY* and *SOC1*, and hence a delay in the switch to reproductive growth (Achard et al. 2007) (Fig. 9.2). The *gai-t6 rga-24* double mutant, however, rescues the late-flowering phenotype expressed by *ctr1-1* plants exposed to SD conditions. Similarly, the delayed flowering of *ctr1-1* grown under LD conditions can be negated either by GA treatment or by deleting *GAI* and *RGA*. SPINDLY (SPY), an *O*-linked *N*-acetylglucosamine transferase, acts as a negative regulator of GA signaling, and this has been demonstrated by showing that the loss-of-function *spy* mutation partially suppresses the late-flowering phenotype of the GA-deficient *ga1-2* mutant exposed to SD (Jacobsen and Olszewski 1993). However, the *ctr1-1 spy-5* double mutant flowers much earlier than the *ctr1-1* single mutant. Thus, the greater sensitivity to GA brought about by the lack of SPY at least partially suppresses the delay to floral transition conferred by the *ctr1-1* mutation (Achard et al. 2007). Ethylene activates ethylene responses by inhibiting $SCF^{EBF1/EBF2}$ activity, which in turn increases the stability of EIN3 and EIN3-LIKE proteins (Guo and Ecker 2003; Potuschak et al. 2003; Gagne et al. 2004). The constitutive ethylene response phenotype shown by the loss-of-function *ebf1-1 ebf2-1* mutant is

stronger than that of *ctr1-1*, and the flowering delay can be overridden by exogenously supplied GA. The level of EIN3 present is unaffected both by GA treatment of the *ebf1-1 ebf2-1* mutant, and by the absence of GAI and RGA (as in the *ctr1-1 gai-t6 rga-24* mutant), indicating that the DELLA proteins act downstream of CTR1/EIN3 in the ethylene-dependent regulation of flowering (Achard et al. 2007) (Fig. 9.2).

9.7 The Interaction of Ethylene and GA Signaling in Plant Response to Abiotic Stress

Environmental stresses, such as drought, soil salinity, and flooding, can all depress plant growth and seed production, and a suite of protective mechanisms, many of which involve phytohormones, have been evolved to cope with these stresses. By modifying the level, distribution, and/or signal transduction activity of these phytohormones, a plant is able to adjust its physiology and biochemistry quite rapidly, a critical requirement for its survival (Colebrook et al. 2014). Abscisic acid (ABA) and ethylene are the two most frequently encountered phytohormones associated with the abiotic stress response. In *A. thaliana*, the response to salinity is triggered (at least in part) by an accumulation of ABA, which activates a variety of ABA signaling pathways (Zhu 2002; Shinozaki et al. 2003). Salinity inhibits growth (at least in part) via the ABI1-dependent, ABA-mediated enhancement of DELLA (Achard et al. 2006). In deepwater rice, submergence stimulates the degradation of ABA, thereby enhancing the plant's responsiveness to GA and promoting the elongation of the internode (Hoffmann-Benning and Kende 1992). A brief account follows describing what is known regarding the interaction between ethylene and GA signaling during the abiotic stress response.

Prolonged flooding results in the development of hypoxic conditions in the soil, which has a dramatic impact on levels of respiration and photosynthesis, on redox homeostasis, and on intracellular pH. Paddy rice, and particularly deepwater rice, has developed various strategies to prevent the build-up of anoxia in their tissue (Xu et al. 2006; Fukao et al. 2006). Deepwater rice plants respond rapidly to a rising water level by elongating their stem internodes to maintain the upper canopy above the water's surface. This process is strongly regulated by ethylene, the rapid accumulation of which in submerged tissue (via physical entrapment and active synthesis) triggers shoot elongation, adventitious root formation, and alterations in carbohydrate metabolism (Steffens et al. 2006; Xu et al. 2006; Hattori et al. 2009). GA appears to be involved in some of these ethylene-mediated responses (Steffens et al. 2006; Xu et al. 2006). Ethylene also coordinates the balance of GA and ABA content during submergence (Xu et al. 2006). In deepwater rice, adventitious roots are induced to grow from a node, and their development in time replaces or at least supports the main root system which will have become increasingly dysfunctional as a result of prolonged hypoxia (Steffens et al. 2006). The growth of these

secondary roots is mediated by ethylene (Suge 1985; Lorbiecke and Sauter 1999), while GA has little or no effect on their development. Nevertheless, a combined treatment of ethylene and GA results in a synergistic promotive effect, while the application of a competitive inhibitor of ethylene abolishes root growth. The inference is that ethylene perception is required for adventitious root growth, and that the resulting ethylene-induced growth of adventitious roots is strongly promoted by GA (Steffens et al. 2006).

GA's involvement in the regulation of the ethylene-mediated growth response of deepwater rice lies not only in inducing adventitious root growth but also in promoting shoot growth. Hattori et al. (2009) have identified that the genes *SNORKEL1* (*SK1*) and *SK2* are responsible for allowing the rapid elongation of the stem internode. These genes are specific to deepwater rice germplasm, and are up-regulated by flooding. The constitutive expression of *SK1* and *SK2* in non-deepwater rice has a positive effect on stem internode elongation even under non-flooded conditions. SK1 and SK2 both possess a single APETALA2/ethylene response factor (AP2/ERF) domain. Their transcription is inducible by exogenously supplied ethylene, but not by any other phytohormone. The *SK1* and *SK2* promoter regions harbor core sequences for EIN3-binding site and can bind to the rice EIN3-like protein (OsEIL1b). The suggestion is that *SK1* and *SK2* serve as ethylene response factors and form part of an ethylene signaling pathway which positively regulates stem internode elongation in deepwater rice (Fig. 9.3).

Physiological experiments show that ethylene, ABA, and GA are all involved in the submergence response (Kende et al. 1998). Ethylene has been identified as an initiation factor for internode elongation (Raskin and Kende 1984; Kende et al. 1998). When plants are submerged, although ethylene accumulates in both deepwater and non-deepwater rice, it only induces stem internode elongation in the former type. The exogenous supply of GA induces stem internode elongation in deepwater rice plants even when they are not submerged, while in flooded plants, the level of GA_1 at the nodes is heightened in deepwater rice, but not in non-deepwater rice. Stem internode elongation is repressed in deepwater rice by treatment with the GA synthesis inhibitor uniconazole, and this repression can be lifted by supplying the plant with GA. The indications are that GA positively regulates stem internode elongation in deepwater rice, operating through the action of *SK1* and *SK2* (Hattori et al. 2009) (Fig. 9.3). The probable scenario therefore is that under submergence, the response of deepwater rice is to rapidly accumulate ethylene, which has the effect of up-regulating *SK1* and *SK2*; additionally, this pair of genes may, directly or indirectly, promote GA synthesis and/or GA signal transduction, in order to promote stem internode elongation, thereby allowing the apex of the plant to remain above the water's surface where it can access the oxygen necessary to escape hypoxic stress (Hattori et al. 2009, 2011) (Fig. 9.3).

Flash flooding also has the effect of submerging the plant, but in this case, the water subsides after a period of some days. Submergence-tolerant rice cultivars respond to temporary submergence by restricting the growth of their shoot and limiting its respiration, recommencing normal growth once the water has subsided (Singh et al. 2001; Das et al. 2005; Fukao et al. 2006; Xu et al. 2006). A major

quantitative trait locus designated *Submergence 1* (*Sub1*) has been identified as harboring three distinct genes (*Sub1A*, *Sub1B*, and *Sub1C*) responsible for this mode of submergence tolerance (Fukao et al. 2006; Xu et al. 2006). Each of the *Sub1* genes encodes a protein featuring an ERF-like DNA-binding domain. Genotypic variation at *Sub1* locus confers distinctions in submergence tolerance. *Sub1B* and *Sub1C* are present in both tolerant and non-tolerant paddy cultivars, whereas *Sub1A* is only found in tolerant ones. Under submergence, the level of ethylene rises rapidly in each of a pair of near isogenic paddy rice lines, one carrying *Sub1B* and *Sub1C* and the other carrying *Sub1A, Sub1B*, and *Sub1C*, but it rises to a higher level in the former (non-tolerant) line. The constitutive expression of *Sub1A* in a non-tolerant cultivar results in a marked improvement in submergence tolerance (Xu et al. 2006). There is an indication that the abundance of *Sub1A* transcript in node and internode regions in some rice accessions containing *Sub1A* is positively correlated with the degree of submergence tolerance. *Sub1A* transcription is inducible by low levels of ethylene (up to 100 ppm) but this treatment has the effect of reducing endogenous ethylene production in submerged plants via negative feedback regulation (Singh et al. 2010). The inference is that the presence of *Sub1A* is necessary and sufficient to confer submergence tolerance (Xu et al. 2006) (Fig. 9.3).

Under normal conditions, the non-tolerant and tolerant isogenic lines develop at a similar rate and their stem elongation response to GA_3 treatment is similar. In submerged plants, the tolerant line responds positively to GA_3 supply with negative implication for its survival. The exposure of the non-tolerant line to PAC reduces its stem elongation, thereby enhancing its survival. The suggestion is that GA-induced stem elongation has a negative impact on tolerance to prolonged submergence (Setter and Laureles 1996; Das et al. 2005). Rice plants engineered to constitutively express *Sub1A* display a classic GA-insensitive phenotype: germination is delayed, the plants are semi-dwarfed, flowering is late, maturity is slow, and grain set is reduced. The seedling GA_3 dose response curve indicates that the ectopic expression of *Sub1A* compromises the plant's response to GA, thereby having a negative effect on GA-dependent processes. SLR1 and SLR1 LIKE1 (SLRL1) are both nuclear-localized GRAS proteins which function as suppressors of GA signaling in rice. The transcription of both *SLR1* and *SLRL1* is submergence-inducible in both submergence-tolerant and non-tolerant plants, but the level of inducibility is greater and its onset is more rapid in the tolerant ones. Engineered constitutive *Sub1A* expressors display an elevated basal level of *SLR1* and *SLRL1* transcription under normal growing conditions, but higher levels when the plants are submerged. These observations confirm that the submergence-induced expression of *Sub1A* enhances the transcription and translation of *SLR1* and *SLRL1*, thereby restraining GA-mediated underwater elongation, prolonging submergence endurance, and sustaining the plant's capacity to regrow once the flooding has subsided (Fukao and Bailey-Serres 2008) (Fig. 9.3).

The abundance of neither *SLR1* nor *SLRL1* transcript is increased by the ethylene treatment of a submergence non-tolerant plant, but not so for a tolerant one. At the protein level, the treatment suppresses the production of SLR1 in the non-tolerant plant but not in the tolerant one, while the abundance of SLRL1 is little changed in the former, but markedly increased in the latter. The indication is therefore that

ethylene induces *Sub1A* expression, which has the effect of up-regulating *SLR1* and *SLRL1* transcription and translation (Fukao and Bailey-Serres 2008). The ethylene does not affect the growth of non-submerged tissue, whether or not *Sub1A* is present. However, a combined ethylene plus GA_3 treatment promotes the GA-mediated elongation of the aerial part of the non-tolerant plant, but significantly attenuates the response in the tolerant one. The interpretation is that in the absence of *Sub1A*, ethylene enhances GA responsiveness by lowering the level of both SLR1 and SLRL1. On the other hand, ethylene-induced *Sub1A* expression stimulates the transcription and translation of *SLR1* and *SLRL1*, which results in the suppression of GA-mediated growth (Fukao and Bailey-Serres 2008) (Fig. 9.3). The overall picture is that submergence tolerance in paddy rice is modulated by *Sub1A*, the product of which dampens ethylene production and responsiveness, resulting in a restriction in ethylene-promoted GA responsiveness achieved via the accumulation of SLR1 and SLRL1; the ultimate result is to limit stem internode elongation and assimilate consumption during a submergence episode.

It is notable that both the *SK* genes and *Sub1A* encode ERFs (ETHYLENE-RESPONSIVE FACTORs) and are associated with GA and ethylene action; however, they confer opposite functions in regulating stem internode elongation in response to flooding: while *Sub1A* restricts shoot elongation during submergence, the *SK* genes stimulate it (Fig. 9.3). In addition, a novel rice ERF gene *OsEATB* (for ERF protein associated with tillering and panicle branching) also mediates crosstalk between ethylene and GA to regulate rice internode elongation. Ethylene treatment sharply down-regulates *OsEATB* expression while increases *OsCPS2* expression (a key GA synthesis gene encodes ent-kaurene synthase A). *OsEATB* suppresses the internode elongation process through the restriction of GA biosynthesis, specifically down-regulating the expression of *OsCPS2* (Qi et al. 2011). *OsEATB* expression is also negatively regulated by ABA and abiotic stress, but further investigations of the molecular crosstalk of the related signaling pathways are still required.

At the end of a submergence episode, rice plants often experience dehydration stress due to reduced hydraulic conductivity in leaf sheaths (Setter et al. 2010). Individuals which harbor *Sub1A* recover by developing new leaves once the water has subsided (Fukao and Bailey-Serres 2008), and also show markedly enhanced recovery from drought at the vegetative stage (Fukao et al. 2011). During a drought episode, *Sub1A* contributes to limiting the extent of water loss, and also promotes the recommencement of leaf growth and development once the stress has been lifted. The abundance of *Sub1A* transcript is markedly enhanced by drought stress, which increases expression of genes associated with acclimation to dehydration. The presence of *Sub1A* during a period of drought stress is associated with a reduced accumulation of ROS (reactive oxygen species), which limits the stress-induced damage to a range of cellular components, and so aids in survival (Fukao et al. 2011) (Fig. 9.3). Therefore, in addition to providing robust submergence tolerance, *Sub1A* improves survival of rapid dehydration following submergence subsidence and water deficit during drought (Fukao et al. 2011) (Fig. 9.3).

The GA and ethylene associated brake on growth imposed by abiotic stress is mirrored in *A. thaliana* (Achard et al. 2006, 2008; Dubois et al. 2013). In response

to water deprivation, the growth of the leaf is halted via the rapid up-regulation of the two transcription factors *ERF5* and *ERF6* (Hruz et al. 2008; Dubois et al. 2013). The growth under osmotic stress of *erf5 erf6* mutant leaves is less restricted than that of WT leaves, but the mutant shows no added tolerance to mild salinity stress. The transcription of either *ERF5* or *ERF6* in WT plants is up-regulated by mild osmotic stress and several other stresses including drought (Hruz et al. 2008; Dubois et al. 2013), but it is not up-regulated by mild salinity stress. *ERF6* over-expressors are dwarfed in stature and form dark green leaves and stunted rosettes, and they are highly sensitive to mild osmotic stress. The inference is that *ERF6* negatively regulates leaf growth under mild osmotic stress. The *GA2ox6* (which encodes an enzyme involved in GA inactivation) activated by mild osmotic stress is rapidly induced in *ERF6* overexpressors; meanwhile, in the *erf5 erf6* double mutant, the induction of *GA2ox6* is delayed (Dubois et al. 2013), which indicates that *ERF6* negatively regulates the level of GA by inducing *GA2ox6* expression. Overexpressing *GA20ox1* (which encodes the rate-limiting GA synthesis enzyme) suppresses the retarded growth phenotype conferred by *ERF6* overexpression. In addition, *ERF6* stabilizes RGA (the major DELLA protein species expressed in the developing leaf). These data confirm that, under mild osmotic stress, an elevated level of *ERF6* reduces the level of endogenous GA through the suppression of *GA2ox6*. The resulting stabilizing effect on the DELLA proteins eventually helps to negatively regulate leaf growth (Dubois et al. 2013). As mild osmotic stress triggers the accumulation of ACC, it is likely that *ERF5* and *ERF6* integrate ethylene and GA signaling to regulate plant growth and the mild osmotic stress response (Dubois et al. 2013) (Fig. 9.3).

High levels of soil salinity restrict the ability of the root to take up water; once the salt has entered the root, it compromises many aspects of cell physiology and slows growth (Hasegawa et al. 2000; Munns and Tester 2008). Achard et al. (2006) have shown that the seedlings of the *A. thaliana gai-t6 rga-t2 rgl1-1 rgl2-1* quadruple DELLA mutant are less inhibited by salinity (100 mM NaCl) than WT seedlings. WT plants respond to salinity by reducing the endogenous level of GA_1 and GA_4, and salt treatment promotes the accumulation of GFP-RGA in *pRGA*::*GFP-RGA* transgenic plants. Thus, one way in which salinity stress (as well as other forms of stress) can slow growth is by reducing the endogenous level of GA, which consequently favors the accumulation of DELLA proteins. Extreme salt concentrations kill plants, compared with WT and the quadruple DELLA mutant, the *ga1-3* and *gai* mutants show increased tolerance of extreme salt concentration (200 mM NaCl). Thus, DELLA proteins can contribute the survival of salt toxicity. Treatment with ethylene allows the WT plant to withstand a higher level of salinity stress (Wang et al. 2002), while the *ctr1-1* mutant shows an enhanced rate of survival when challenged by salinity stress, possibly because of the constitutive activation of ethylene signaling pathways (Guo and Ecker 2003; Potuschak et al. 2003); their increased tolerance is overridden in mutants which also lack *GAI* and *RGA*. Thus, salinity slows growth through the activation of ethylene signaling, the effects of which are at least partly integrated with GA signaling at the level of DELLA function (Fig. 9.3). Meanwhile, the accumulation of DELLAs up-regulates a suite of

genes encoding ROS detoxification enzymes, thereby limiting ROS-induced damage and enhancing salinity tolerance (Achard et al. 2006). The restraint imposed on growth and respiration allows the plant to ration its carbohydrate reserves during the stress period, so that energy is available when growth can resume (Fukao and Bailey-Serres 2008). A common regulatory mechanism mediating both the restraint on growth and survival of adverse conditions is likely to exist. Phytohormonal signaling pathways (ethylene, GA) may be involved in this mechanism, integrated at the level of DELLA function.

9.8 Conclusions and Perspectives

Ethylene and GA clearly participate in the plant growth, development, and the response to abiotic stress, but many aspects remain to be uncovered. For example, the molecular basis of the interaction between ethylene and GA during germination still needs elucidation. A number of questions are outstanding, such as: do ethylene and GA affect root cell division, cell differentiation, or stem cell fate? Where and how is signaling transduced? Other than GA, are there other hormones involved in ethylene-mediated flowering? How are developmentally separated hormone-mediated responses integrated with DELLA function to bring together several signaling pathways in the response to abiotic stress? Characterization of the many interactions involved and the identification of the roles played by the various phytohormones and gene products will require an integration of cell biological and system biology approaches. The goal is to gain an understanding of how plant growth and development is quantitatively modulated in the face of abiotic stress.

References

Abbas M, Alabadi D, Blazquez MA. Differential growth at the apical hook: all roads lead to auxin. Front Plant Sci. 2013;4:441.

Abeles FB, Morgan PW, Saltveit ME Jr. Ethylene in plant biology. San Diego: Academic Press; 1992.

Achard P, Vriezen WH, Van Der Straeten D, Harberd NP. Ethylene regulates *Arabidopsis* development via the modulation of DELLA protein growth repressor function. Plant Cell. 2003;15:2816–25.

Achard P, Herr A, Baulcombe DC, Harberd NP. Modulation of floral development by a gibberellin-regulated microRNA. Development. 2004;131:3357–65.

Achard P, Renou JP, Berthome R, Harberd NP, Genschik P. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol. 2008;18:656–60.

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, et al. The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc Natl Acad Sci USA. 2007;104:6484–9.

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, et al. Integration of plant responses to environmentally activated phytohormonal signals. Science. 2006;311:91–4.

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science. 1999;284:2148–52.

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM, Ecker JR. Five components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in *Arabidopsis*. Proc Natl Acad Sci USA. 2003;100:2992–7.

An F, Zhang X, Zhu Z, Ji Y, He W, Jiang Z, et al. Coordinated regulation of apical hook development by gibberellins and ethylene in etiolated *Arabidopsis* seedlings. Cell Res. 2012;22:915–27.

An F, Zhao Q, Ji Y, Li W, Jiang Z, Yu X, et al. Ethylene-induced stabilization of ETHYLENE INSENSITIVE3 and EIN3-LIKE1 is mediated by proteasomal degradation of EIN3 binding F-box 1 and 2 that requires EIN2 in *Arabidopsis*. Plant Cell. 2010;22:2384–401.

Bailey-Serres J, Voesenek LA. Life in the balance: a signaling network controlling survival of flooding. Curr Opin Plant Biol. 2010;13:489–94.

Beaudoin N, Serizet C, Gosti F, Giraudat J. Interactions between abscisic acid and ethylene signaling cascades. Plant Cell. 2000;12:1103–15.

Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell. 1997;89:1133–44.

Chiwocha SD, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross AR, et al. The etr1-2 mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. Plant J. 2005;42:35–48.

Colebrook EH, Thomas SG, Phillips AL, Hedden P. The role of gibberellin signalling in plant responses to abiotic stress. J Exp Biol. 2014;217:67–75.

Collett CE, Harberd NP, Leyser O. Hormonal interactions in the control of *Arabidopsis* hypocotyl elongation. Plant Physiol. 2000;124:553–62.

Cowling RJ, Harberd NP. Gibberellins control *Arabidopsis* hypocotyl growth via regulation of cellular elongation. J Exp Bot. 1999;50:1351–7.

Das KK, Sarkar RK, Ismail AM. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. Plant Sci. 2005;168:131–6.

Daviere JM, Achard P. Gibberellin signaling in plants. Development. 2013;140:1147–51.

De Grauwe L, Vriezen WH, Bertrand S, Phillips A, Vidal AM, Hedden P, Van Der Straeten D. Reciprocal influence of ethylene and gibberellins on response-gene expression in *Arabidopsis thaliana*. Planta. 2007;226:485–98.

De Grauwe L, Chaerle L, Dugardeyn J, Decat J, Rieu I, Vriezen WH, et al. Reduced gibberellin response affects ethylene biosynthesis and responsiveness in the *Arabidopsis* gai eto2-1 double mutant. New Phytol. 2008;177:128–41.

Dill A, Jung HS, Sun TP. The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci USA. 2001;98:14162–7.

Dill A, Thomas SG, Hu J, Steber CM, Sun TP. The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell. 2004;16:1392–405.

Dubois M, Skirycz A, Claeys H, Maleux K, Dhondt S, De Bodt S, et al. Ethylene response Factor6 acts as a central regulator of leaf growth under water-limiting conditions in *Arabidopsis*. Plant Physiol. 2013;162:319–32.

Dugardeyn J, Vandenbussche F, Van Der Straeten D. To grow or not to grow: what can we learn on ethylene-gibberellin cross-talk by in silico gene expression analysis? J Exp Bot. 2008;59:1–16.

Ecker JR. The ethylene signal transduction pathway in plants. Science. 1995;268:667–75.

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, et al. Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature. 2008;451:475–9.

Fu X, Harberd NP. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. Nature. 2003;421:740–3.

Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP. The *Arabidopsis* mutant sleepy1gar2-1 protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. Plant Cell. 2004;16:1406–18.

Fukao T, Bailey-Serres J. Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci USA. 2008;105:16814–9.

Fukao T, Yeung E, Bailey-Serres J. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell. 2011;23:412–27.

Fukao T, Xu K, Ronald PC, Bailey-Serres J. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell. 2006;18:2021–34.

Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, Yanagisawa S, et al. *Arabidopsis* EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. Proc Natl Acad Sci USA. 2004;101:6803–8.

Gallego-Bartolome J, Arana MV, Vandenbussche F, Zadnikova P, Minguet EG, Guardiola V, Van Der Straeten D, et al. Hierarchy of hormone action controlling apical hook development in *Arabidopsis*. Plant J. 2011;67:622–34.

Gao XH, Huang XZ, Xiao SL, Fu XD. Evolutionarily conserved DELLA-mediated gibberellin signaling in plants. J Integr Plant Biol. 2008;50:825–34.

Gao XH, Xiao SL, Yao QF, Wang YJ, Fu XD. An updated GA signaling 'Relief of Repression' regulatory model. Mol Plant. 2011;4:601–6.

Gendreau E, Orbovic V, Hofte H, Traas J. Gibberellin and ethylene control endoreduplication levels in the *Arabidopsis thaliana* hypocotyl. Planta. 1999;209:513–6.

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, et al. Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. Plant Cell. 2006;18:3399–414.

Guo H, Ecker JR. Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell. 2003;115:667–77.

Guo H, Ecker JR. The ethylene signaling pathway: new insights. Curr Opin Plant Biol. 2004;7:40–9.

Guzman P, Ecker JR. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell. 1990;2:513–23.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol. 2000;51:463–99.

Hattori Y, Nagai K, Ashikari M. Rice growth adapting to deepwater. Curr Opin Plant Biol. 2011;14:100–5.

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, et al. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature. 2009;460:1026–30.

Hedden P, Phillips AL. Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci. 2000;5:523–30.

Herzog M, Dorne AM, Grellet F. GASA, a gibberellin-regulated gene family from *Arabidopsis thaliana* related to the tomato GAST1 gene. Plant Mol Biol. 1995;27:743–52.

Hoffmann-Benning S, Kende H. On the role of abscisic acid and gibberellin in the regulation of growth in rice. Plant Physiol. 1992;99:1156–61.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinform. 2008;2008:420747.

Hua J, Meyerowitz EM. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell. 1998;94:261–71.

Hua J, Chang C, Sun Q, Meyerowitz EM. Ethylene insensitivity conferred by *Arabidopsis* ERS gene. Science. 1995;269:1712–4.

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, et al. EIN4 and ERS2 are members of the putative ethylene receptor gene family in *Arabidopsis*. Plant Cell. 1998;10:1321–32.

Huang Y, Li H, Hutchison CE, Laskey J, Kieber JJ. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. Plant J. 2003;33:221–33.

Itoh H, Ueguchi-Tanaka M, Matsuoka M. Molecular biology of gibberellins signaling in higher plants. Int Rev Cell Mol Biol. 2008;268:191–221.

Jacobsen SE, Olszewski NE. Mutations at the SPINDLY locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell. 1993;5:887–96.

Jiang C, Fu X. GA action: turning on de-DELLA repressing signaling. Curr Opin Plant Biol. 2007;10:461–5.

Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, et al. CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in *Arabidopsis*. Proc Natl Acad Sci USA. 2012;109:19486–91.

Karssen CM, Zagorski S, Kepczynski J, Groot SPC. Key role for endogenous gibberellins in the control of seed germination. Ann Bot. 1989;63:71–80.

Kende H, van der Knaap E, Cho HT. Deepwater rice: a model plant to study stem elongation. Plant Physiol. 1998;118:1105–10.

Kepczynski J, Kepczynska E. Ethylene in seed dormancy and germination. Physiol Plant. 1997;101:720–6.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. Cell. 1993;72:427–41.

Lehman A, Black R, Ecker JR. HOOKLESS1, an ethylene response gene, is required for differential cell elongation in the *Arabidopsis* hypocotyl. Cell. 1996;85:183–94.

Lorbiecke R, Sauter M. Adventitious root growth and cell-cycle induction in deepwater rice. Plant Physiol. 1999;119:21–30.

Mayerhofer H, Panneerselvam S, Mueller-Dieckmann J. Protein kinase domain of CTR1 from *Arabidopsis thaliana* promotes ethylene receptor cross talk. J Mol Biol. 2012;415:768–79.

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, et al. The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J. 2003;35:613–23.

Mutasa-Gottgens E, Hedden P. Gibberellin as a factor in floral regulatory networks. J Exp Bot. 2009;60:1979–89.

Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651–81.

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell. 2003;15:1591–604.

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, et al. The *Arabidopsis* GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev. 1997;11:3194–205.

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, et al. EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. Cell. 2003;115:679–89.

Qi W, Sun F, Wang Q, Chen M, Huang Y, Feng YQ, et al. Rice ethylene-response AP2/ERF factor OsEATB restricts internode elongation by down-regulating a gibberellin biosynthetic gene. Plant Physiol. 2011;157:216–28.

Qiao H, Shen Z, Huang SS, Schmitz RJ, Urich MA, Briggs SP, et al. Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. Science. 2012;338:390–3.

Raskin I, Kende H. Regulation of growth in stem sections of deep-water rice. Planta. 1984;160:66–72.

Raz V, Ecker JR. Regulation of differential growth in the apical hook of *Arabidopsis*. Development. 1999;126:3661–8.

Raz V, Koornneef M. Cell division activity during apical hook development. Plant Physiol. 2001;125:219–26.
Saibo NJ, Vriezen WH, Beemster GT, Van Der Straeten D. Growth and stomata development of *Arabidopsis* hypocotyls are controlled by gibberellins and modulated by ethylene and auxins. Plant J. 2003;33:989–1000.
Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, et al. ETR2 is an ETR1-like gene involved in ethylene signaling in *Arabidopsis*. Proc Natl Acad Sci USA. 1998;95:5812–7.
Setter TL, Laureles EV. The beneficial effect of reduced elongation growth on submergence tolerance of rice. J Exp Bot. 1996;47:1551–9.
Shimada A, Ueguchi-Tanaka M, Nakatsu T, Nakajima M, Naoe Y, Ohmiya H, et al. Structural basis for gibberellin recognition by its receptor GID1. Nature. 2008;456:520–3.
Shinozaki K, Yamaguchi-Shinozaki K, Seki M. Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol. 2003;6:410–7.
Silverstone AL, Jung HS, Dill A, Kawaide H, Kamiya Y, Sun TP. Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis*. Plant Cell. 2001;13:1555–66.
Singh HP, Singh BB, Ram PC. Submergence tolerance of rainfed lowland rice: search for physiological marker traits. J Plant Physiol. 2001;158:883–9.
Singh N, Dang TT, Vergara GV, Pandey DM, Sanchez D, Neeraja CN, et al. Molecular marker survey and expression analyses of the rice submergence-tolerance gene SUB1A. Theor Appl Genet. 2010;121:1441–53.
Smalle J, Haegman M, Kurepa J, Van Montagu M, Straeten DV. Ethylene can stimulate *Arabidopsis* hypocotyl elongation in the light. Proc Natl Acad Sci USA. 1997;94:2756–61.
Solano R, Stepanova A, Chao Q, Ecker JR. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev. 1998;12:3703–14.
Srikanth A, Schmid M. Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci. 2011;68:2013–37.
Steffens B, Wang J, Sauter M. Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. Planta. 2006;223:604–12.
Stepanova AN, Alonso JM. Ethylene signaling and response: where different regulatory modules meet. Curr Opin Plant Biol. 2009;12:548–55.
Setter TL, Bhekasut P, Greenway H. Desiccation of leaves after de-submergence is one cause for intolerance to complete submergence of the rice cultivar IR 42. Funct Plant Biol. 2010;37:1096–104.
Suge H. Ethylene and gibberellin: regulation of internodal elongation and nodal root development in floating rice. Plant Cell Physiol. 1985;26:607–14.
Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol. 2004;55:197–223.
Thomas B, Vince Prue D. Photoperiodism in plants. 2nd ed. San Diego: Academic press; 1997.
Traas J, Hulskamp M, Gendreau E, Hofte H. Endoreduplication and development: rule without dividing? Curr Opin Plant Biol. 1998;1:498–503.
Ueguchi-Tanaka M, Nakajima M, Motoyuki A, Matsuoka M. Gibberellin receptor and its role in gibberellin signaling in plants. Annu Rev Plant Biol. 2007;58:183–98.
Vandenbussche F, Vancompernolle B, Rieu I, Ahmad M, Phillips A, Moritz T, et al. Ethylene-induced *Arabidopsis* hypocotyl elongation is dependent on but not mediated by gibberellins. J Exp Bot. 2007;58:4269–81.
Vogel JP, Woeste KE, Theologis A, Kieber JJ. Recessive and dominant mutations in the ethylene biosynthetic gene ACS5 of *Arabidopsis* confer cytokinin insensitivity and ethylene overproduction, respectively. Proc Natl Acad Sci USA. 1998;95:4766–71.
Vriezen WH, Achard P, Harberd NP, Van Der Straeten D. Ethylene-mediated enhancement of apical hook formation in etiolated *Arabidopsis thaliana* seedlings is gibberellin dependent. Plant J. 2004;37:505–16.

Wang KL, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. Plant Cell. 2002;14 (Suppl):S131–51.

Weiss D, Ori N. Mechanisms of cross talk between gibberellin and other hormones. Plant Physiol. 2007;144:1240–6.

Wen X, Zhang C, Ji Y, Zhao Q, He W, An F, et al. Activation of ethylene signaling is mediated by nuclear translocation of the cleaved EIN2 carboxyl terminus. Cell Res. 2012;22:1613–6.

Wilson RN, Heckman JW, Somerville CR. Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol. 1992;100:403–8.

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, et al. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature. 2006;442:705–8.

Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, Theologis A. Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. J Biol Chem. 2003;278:49102–12.

Zhu JK. Salt and drought stress signal transduction in plants. Annu Rev Plant Biol. 2002;53:247–73.