The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes

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The length of the *Arabidopsis thaliana* life cycle depends on the timing of the floral transition. Here, we define the relationship between the plant stress hormone ethylene and the timing of floral initiation. Ethylene signaling is activated by diverse environmental stresses, but it was not previously clear how ethylene regulates flowering. First, we show that ethylene delays flowering in *Arabidopsis*, and that this delay is partly rescued by loss-of-function mutations in genes encoding the DELLAs, a family of nuclear gibberellin (GA)-regulated growth-repressing proteins. This finding suggests that ethylene may act in part by modulating DELLA activity. We also show that activated ethylene signaling reduces bioactive GA levels, thus enhancing the accumulation of DELLAs. Next, we show that ethylene acts on DELLAs via the CTR1-dependent ethylene response pathway, most likely downstream of the transcriptional regulator EIN3. Ethylene-enhanced DELLA accumulation in turn delays flowering via repression of the floral meristem-identity genes *LEAFY* (*LFY*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). Our findings establish a link between the CTR1/EIN3-dependent ethylene and GA–DELLA signaling pathways that enables adaptively significant regulation of plant life cycle progression in response to environmental adversity.

floral transition | *Arabidopsis thaliana* | *LFY* | gibberellin

Floral initiation is a major step in the plant life cycle (1). Accordingly, plants have evolved mechanisms for regulating the timing of floral initiation. These mechanisms permit an adaptively significant integrated response to multiple interacting factors (both internal and external to the plant). In essence, the endogenous developmental competence of plants to flower is integrated with environmental cues that signal the onset of conditions favorable for reproductive success (2).

In this article, we describe the role of the gaseous phytohormone ethylene in the regulation of floral initiation. Ethylene is already known to modulate *Arabidopsis* vegetative environmental growth responses (3–5). For example, adverse environmental conditions enhance ethylene production, and thereby restrain growth (3, 4). Ethylene is perceived by the ETR1 family of ethylene receptors (6–10). In the absence of ethylene, ETR1 activates CTR1, a Ser/Thr kinase (closely related to the RAF kinases) that is a negative regulator of ethylene signaling (11, 12). Downstream of CTR1 are several positive regulators of ethylene response: EIN2 (a membrane-associated protein whose function is not clear; ref. 13) and the EIN3 and EIN3-like (EIL) transcription factors (14, 15). EIN3 regulates ethylene-responsive genes (6, 15), whereas overexpression of EIN3 results in the constitutive activation of ethylene responses (14). Furthermore, ethylene response depends on EIN3 stability. In the absence of ethylene, EIN3 degradation is promoted by a specific Skp1-cullin-F box protein (SCF) E3 ubiquitin ligase ($SCF^{EBF1/EBF2}$) that targets EIN3 for destruction by the proteasome (16–18). However, despite this detailed understanding of mechanisms connecting ethylene perception to ethylene response, the mechanisms by which EIN3 modulates plant growth remain unclear. In addition, ethylene-mediated regulation of the floral transition (19) has not been systematically investigated.

In contrast, the phytohormone gibberellin (GA) is well known to play a prominent role in regulating the timing of the floral transition (20). GA-deficient mutants are dwarfed and late-flowering, and treatment of these plants with GA restores normal growth and flowering time (20). GA is perceived by a soluble receptor, GID1 (21, 22). Downstream of GID1 is a family of nuclear growth repressor proteins, the DELLAs (23–25). The DELLAs are a subfamily of the GRAS family of putative transcriptional regulators (20, 26), a subfamily that in *Arabidopsis* comprises GAI, RGA, RGL1, RGL2, and RGL3 (23, 27–29). DELLAs restrain plant growth, whereas GA promotes growth via relief of DELLA-mediated growth restraint (24, 25, 30, 31). The binding of GA to GID1 promotes an interaction between GID1 and DELLAs, and it has been proposed that this interaction subsequently enhances the affinity between DELLAs and a specific SCF E3 ubiquitin–ligase complex (involving the F-box protein AtSLY1/OsGID2), thus promoting the eventual destruction of DELLAs by the 26*S* proteasome (21, 32–34). The GA–DELLA system regulates the timing of floral initiation via effects on the levels of transcripts of the floral meristem identity genes *LEAFY* (*LFY*) and *SUPRESSOR OF OVEREXPRESSION CONSTANS 1* (*SOC1*). In particular, DELLAs delay flowering in short-day photoperiods (SDs) by repressing the up-regulation of *LFY* and *SOC1* transcripts (35–40). DELLAs subsequently regulate the development of flowers themselves, via transcriptional repression of the floral homeotic genes *APETALA3*, *PISTILLATA*, and *AGAMOUS* (41). Interestingly, the expression of *LFY* and *APETALA1* was not affected by DELLAs during flower development (41), indicating differential regulation of *LFY* by DELLAs during floral initiation and flower development.

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Abbreviations: GA, gibberellin; SCF, Skp1-cullin-F box protein; SD, short-day photoperiod; LD, long-day photoperiod; ACC, 1-aminocyclopropane-1-carboxylic acid.

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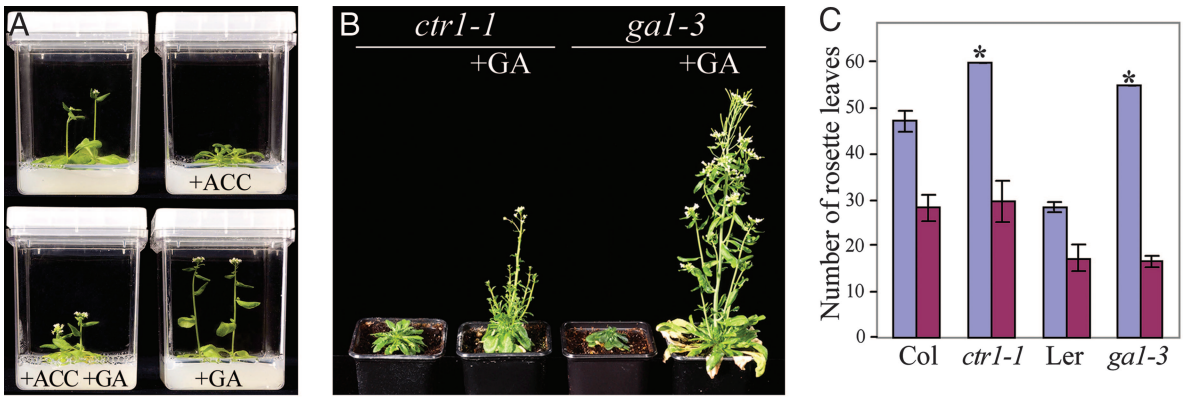

D

		GA_1	GA_4	GA_8	GA_9	GA_{20}	GA_{34}	GA_{19}	GA_{24}	GA_{53}
WT	Mean	65.4	295.5	n.d.	307.3	59.8	413.5	589.5	846.3	152.3
	s.d.	19.1	83.2	n.d.	87.7	17.2	50.6	110.2	96.7	18.7
ctr1-1	Mean	16.0	n.d.	n.d.	234.7	n.d.	298.3	153.3	2700.7	260.3
	s.d.	2.3	n.d.	n.d.	16.4	n.d.	10.7	26.7	228.2	14.5

Fig. 1. Ethylene delays flowering by reducing bioactive GA levels. (*A*) Representative (25-day-old) WT Ler plants (two plants per box are shown) grown in LDs on growth medium containing 10 μM ACC (+ACC) and/or 10 μM GA (+GA) (and control). All plants shown have bolted, except for plants growing on ACC. (*B*) Representative (5-week-old) *ctr1–1* and *ga1–3* mutant plants grown in SDs and treated with GA (+GA) or control. (*C*) Mean vegetative rosette leaf number (± SD; $n > 30$) of WT Col, *ctr1–1*, WT Ler, and *ga1–3* plants grown on soil in SDs and GA-treated (red) or control (blue). The asterisks represent plants that had not flowered by the end of the experiment (8 weeks). (*D*) Levels of GAs in WT Col and *ctr1–1* mutant plants (expressed as picograms per gram of fresh weight; ±SD; $n = 5$). n.d. indicates not detected.

Previous studies have indicated that ethylene can regulate vegetative growth by modulation of GA content (42). More recent evidence indicates that both ethylene and the phytohormone auxin can influence vegetative growth by modulation of DELLA levels (43–45). For example, ethylene inhibits *Arabidopsis* root growth at least in part by enhancing DELLA-dependent growth restraint (43). These observations have led to the proposal that DELLAs control plant growth in response to a plethora of internal and external cues, by integrating signals from different signaling pathways (3, 46, 47). However, although it is well known that adverse conditions promote the production of ethylene, the way in which adversity-generated ethylene affects the floral transition, a key step in the plant life cycle, is currently not well understood. In this study, we find that ethylene delays *Arabidopsis* flowering in a DELLA-dependent fashion. We show that activation of the ethylene signaling pathway reduces bioactive GA levels, thus promoting the accumulation of DELLAs. Accumulation of DELLAs in turn represses *LFY* and *SOC1*, thus delaying flowering. Our studies identify the "GA pathway" of floral control (1, 2) as a major regulator of flowering in response to environmental signals (see also ref. 48).

Results

Ethylene Delays Flowering via a DELLA-Dependent Signaling Pathway. We first found that *Arabidopsis* plants (WT) grown in the presence of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC; Fig. 1*A*), or in an ethylene-rich atmosphere (3), flowered late. Previous analyses have shown that ethylene signaling acts predominantly via CTR1 (11). We found that the *ctr1–1* loss-of-function mutation confers late flowering in long-day photoperiods (LDs) [supporting information (SI) Fig. 6]. Interestingly, the effect of *ctr1–1* on flowering time is particularly evident in SDs. *ctr1–1* plants were still in the vegetative growth phase after 2 months growth in SDs (although WT plants had already flowered (Fig. 1 *B* and *C*). Eventually, a few *ctr1–1* plants did flower after 2 months in SDs (data not shown).

We next showed that GA abolished the effect of ACC and *ctr1–1* on flowering time in SDs (Fig. 1 *A–C*; also in LDs, as shown in SI Fig. 6). Thus, the defect in SD flowering conferred by ACC or *ctr1–1* was reminiscent of the defect in SD flowering conferred by the GA-deficiency mutation *ga1–3* (which can also be overcome by GA; Fig. 1 *B* and *C*; ref. 36). We therefore compared the endogenous GA contents of WT and *ctr1–1* plants. As shown in Fig. 1*D*, the levels of the biologically active ("bioactive") GAs, GA_4 and GA_1, were significantly reduced in LD-grown *ctr1–1* plants. These observations indicate that ethylene-mediated inhibition of CTR1 activity results in a reduction in bioactive GA levels and a consequent delay in floral initiation. Furthermore, the contents of some intermediate GAs (GA_{24} and GA_{53}; substrates of the GA 20-oxidase enzymes that catalyze the penultimate step in the production of bioactive GAs; ref. 49) were significantly increased in *ctr1–1*, suggesting that ethylene inhibits 20-oxidase activity (see *Discussion*).

The developmental effects of GA are caused by the destruction of DELLAs (34). Because *ctr1–1* contains reduced levels of bioactive GAs, and GA overcomes ACC-induced and *ctr1–1*-conferred delays in SD flowering, we tested the hypothesis that ethylene delays flowering via a DELLA-dependent mechanism. We found that lack of the DELLAs GAI and RGA (in *ctr1–1 gai-t6 rga-24*) substantially suppressed the late-flowering phenotype conferred by *ctr1–1* in SDs (Fig. 2 *A*, *B*, and *D*). Actually, *ctr1–1* plants lacking GAI and RGA bolted 1 week later in SDs than did WT plants. This slight remaining delay could be DELLA-dependent (these plants retained RGL1, RGL2, and RGL3) or DELLA-independent. As shown above, GA treatment accelerated the SD flowering time of *ctr1–1* and restored almost to normal the SD flowering time of *ctr1–1 gai-t6 rga-24* (Fig. 2 *B* and *D*). Similarly, the delayed flowering of *ctr1–1* in LDs was significantly reduced by GA treatment or lack of GAI and RGA (SI Fig. 7 *A* and *B*). Thus *ctr1–1* does indeed delay flowering via a DELLA-dependent mechanism.

Mutations in *SPINDLY* Accelerate the *ctr1–1* Floral Transition. The *SPINDLY* (*SPY*) gene encodes a negative regulator of GA

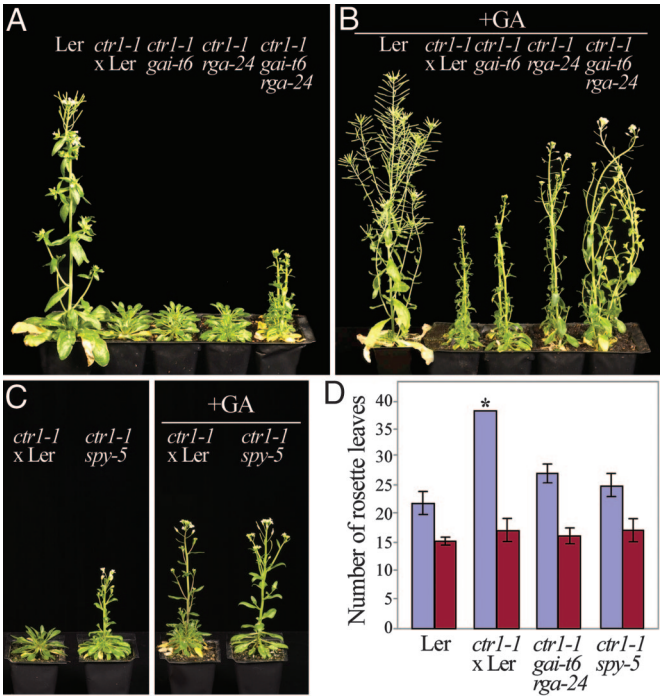

Fig. 2. Activation of GA signaling accelerates the flowering of *ctr1–1* plants. (*A* and *B*) Representative (5-week-old) WT Ler, *ctr1–1* × Ler, *ctr1–1 gai-t6*, *ctr1–1 rga-24*, and *ctr1–1 gai-t6 rga-24* mutant plants grown in SDs and treated with GA (*B*) or control (*A*). (*C*) Representative (5-week-old) *ctr1–1* × Ler and *ctr1–1 spy-5* mutant plants grown in SDs and treated with GA (+GA; *Right*) or control (*Left*). (*D*) Mean vegetative rosette leaf number (± SD; $n > 30$) of WT Ler, *ctr1–1* × Ler, *ctr1–1 gai-t6 rga-24*, and *ctr1–1 spy-5* plants grown on soil in SDs and treated with GA (red) or control (blue). The asterisk represents plants that had not flowered by the end of the experiment (8 weeks).

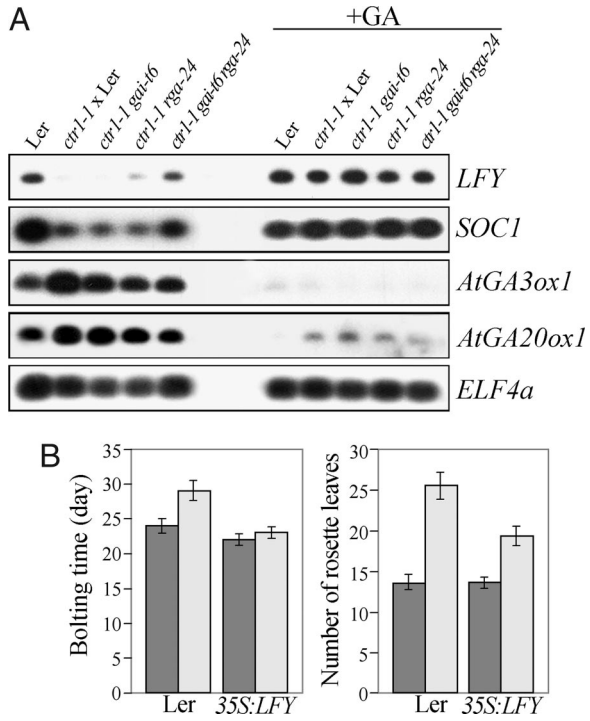

Fig. 3. Ethylene delays flowering via DELLA-dependent repression of *LFY* and *SOC1* transcript levels. (*A*) Levels of floral meristem identity *LFY* and *SOC1*, and GA biosynthesis *AtGA3ox1* and *AtGA20ox1* gene transcripts in SD, soil-grown, GA-treated WT Ler, *ctr1–1* × Ler, *ctr1–1 gai-t6*, *ctr1–1 rga-24*, and *ctr1–1 gai-t6 rga-24* mutant plants (and controls). *ELF4a* transcripts provide loading control. (*B*) Flowering time (time at which 50% of plants had bolted) expressed as time to bolt and number of rosette leaves (± SD; $n > 15$) of WT Ler and *35S:LFY* overexpression plants grown in LDs on growth medium containing 10 μM ACC (light gray) or control (dark gray).

signaling, and loss-of-function *spy* mutations partially suppress the phenotype of the GA-deficient *ga1–2* mutant (50). We found that LD-grown *ctr1–1 spy-5* plants bolted at a similar time to WT controls (or *ctr1–1 gai-t6 rga-24* plants; data not shown). In SDs, *ctr1–1 spy-5* plants bolted 10 days later than WT plants but much earlier than *ctr1–1* single-mutant plants (Fig. 2 *C* and *D*). Thus the elevated GA responses conferred by lack of either GAI and RGA or SPY at least partially suppress the delay in floral transition conferred by the *ctr1–1* mutation.

Ethylene Delays Floral Transition via DELLA-Dependent Repression of the Floral Activator Genes *LFY* and *SOC1*. GA promotes SD flowering by activating the floral meristem-identity genes *LFY* and *SOC1* (36–39), via a mechanism that is DELLA-dependent (40). Because *ctr1-1*, like *ga1–3*, exhibits DELLA-dependent delays in flowering time, we investigated the possibility that *ctr1–1* might delay SD flowering by maintaining relatively low levels of *LFY* and *SOC1* transcripts. We determined relative transcript levels at the time when WT plants bolted and found that *ctr1–1* plants had relatively low levels of both *LFY* and *SOC1* transcripts. Relatively normal *LFY* and *SOC1* transcript levels were observed in GA-treated *ctr1–1* plants or *ctr1–1* plants lacking both GAI and RGA (Fig. 3*A* and SI Fig. 7*C*). These observations suggest that ethylene inhibits the up-regulation of *LFY* and *SOC1* transcript levels via a DELLA-dependent mechanism, thus delaying the floral transition. Consistent with this hypothesis, transgenic overexpression of *LFY* (in a weak overexpression line; *35S:LFY*; ref. 51) overcame the effect of ACC on floral transition (completely with respect to time of bolting, partially with respect to rosette leaf number; Fig. 3*B*).

GA Biosynthesis Gene Transcripts Are Up-Regulated in *ctr1–1*. The *in planta* levels of bioactive GAs are subject to tight regulatory control, in particular at the level of accumulation of gene transcripts encoding GA biosynthesis enzymes. For example, the *AtGA3ox1* (*GA4*) and *AtGA20ox1* (*GA5*) genes encode, respectively, GA 3β-hydroxylase and GA 20-oxidase enzymes that catalyze the final steps in the production of bioactive GAs (49, 52, 53). Increased DELLA accumulation (as in the GA-deficient *ga1–3* mutant) results in increased levels of these transcripts, because of perturbation of a GA-activated DELLA-dependent negative feedback loop (52, 53). We found that *ctr1–1* plants accumulated higher levels of *AtGA3ox1* and *AtGA20ox1* transcripts than WT controls (Fig. 3*A* and SI Fig. 7*C*). In contrast, *AtGA3ox1* and *AtGA20ox1* transcripts accumulated to a level similar to that of WT in *ctr1–1* plants lacking GAI and RGA (*ctr1–1 gai-t6 rga-24*), thus implicating DELLA function in the up-regulation of these transcripts in *ctr1–1* (Fig. 3*A*). We also found that the elevated *AtGA3ox1* and *AtGA20ox1* transcript levels in *ctr1–1* are reduced 2 days after GA treatment (Fig. 3*A*; the small amount of remaining *AtGA20ox1* transcripts observed in GA-treated *ctr1–1* plants might represent nascent transcripts). Thus the delayed SD flowering and elevated *AtGA3ox1* and *AtGA20ox1* transcript levels that are characteristic of *ctr1–1* both likely result from increased DELLA accumulation (consequent on a reduction in the level of bioactive GAs).

Ethylene Delays Floral Transition via an EIN3-Dependent Mechanism. Ethylene activates ethylene-responses by inhibiting the activity of $SCF^{EBF1/EBF2}$, thus increasing the stability of EIN3 and the EIN3-like proteins (16–18). We next showed that the ethylene-

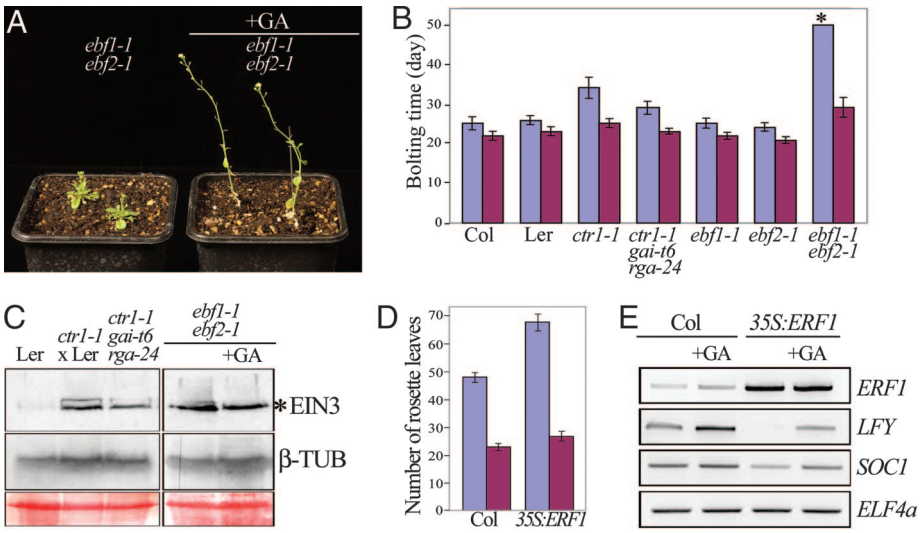

Fig. 4. Ethylene regulation of floral transition is EIN3-dependent. (*A*) Representative (30-day-old) *ebf1–1 ebf2–1* mutant plants grown in LDs and treated with GA (+GA; *Right*) or control (*Left*). (*B*) Flowering time (time at which 50% of plants had bolted) of selected lines as indicated (± SD; $n > 30$) grown in soil in LDs in the presence (red) or absence (blue) of GA treatment. The asterisk represents plants that had not bolted by the end of the experiment (50 days). (*C*) Immunodetection of EIN3 in 2-week-old selected lines as indicated. *ebf1–1 ebf2–1* plants were treated with GA (+GA) or not. The asterisk marks EIN3 at the expected molecular size. β-tubulin (β-TUB; *Middle*) and Ponceau red (*Bottom*) staining of the membrane after transfer serve as a sample-loading controls. (*D*) Mean vegetative rosette leaf number (±SD; $n > 15$) of WT Col and *35S:ERF1* plants grown on soil in SDs (8-h photoperiod), GA-treated (red) or control (blue). (*E*) Levels of *ERF1* and floral meristem identity *LFY* and *SOC1* gene transcripts (determined by RT-PCR) in SDs, soil-grown, GA-treated WT Col, and *35S:ERF1* plants (and controls). *ELF4a* transcripts provide loading control.

induced delay in floral transition works via CTR1/EIN3-dependent signaling. The F-box specificity components of $SCF^{EBF1/EBF2}$ are encoded by the genes *EBF1* and *EBF2* (16–18). Loss-of-reduced-function *ebf1–1* and *ebf2–1* mutations, especially in the *ebf1–1 ebf2–1* double-mutant combination, confer stabilization of EIN3 in the absence of ethylene (16–18). We found that although flowering of *ebf1–1* or *ebf2–1* single mutants was not significantly delayed, *ebf1–1 ebf2–1* double mutants exhibited a clearly detectable delay in bolting time (Fig. 4 *A* and *B* and SI Fig. 8). Thus the severe *ctr1*-like vegetative growth phenotype of *ebf1–1 ebf2–1* plants (17) is accompanied by a *ctr1*-like delay in flowering. Although *ctr1–1* plants bolted ≈10 days later than WT controls, *ebf1–1 ebf2–1* plants had still not bolted by ≈25 days after the mean bolting time of WT in LDs (Fig. 4*B*). Thus, conditions that stabilize EIN3 (perhaps in addition to other effects of the *ebf1–1 ebf2–1* combination) correlate with a severe delay in flowering. Furthermore, we found that *ein3–1* mutants were insensitive to ethylene-induced late flowering (SI Table 1).

As shown above, GA treatment or lack of GAI and RGA overcomes the delayed flowering that is characteristic of *ctr1–1*. Similarly, we found that GA treatment overcomes the delayed flowering of *ebf1–1 ebf2–1* plants grown in LDs (Fig. 4 *A* and *B*). However, GA treatment did not restore a normal growth phenotype or floral transition to *ebf1–1 ebf2–1* plants grown in SDs (data not shown), indicating that there are aspects of the growth phenotype conferred by the double *ebf1–1 ebf2–1* mutation that are not GA-responsive.

The phenotype conferred by *ctr1–1* and *ebf1–1 ebf2–1* results from stabilization of EIN3 (16–18). It was therefore possible that GA treatment or lack of GAI and RGA causes destabilization of EIN3 in these lines, thus overcoming the EIN3-dependent delay in flowering. However, we showed that EIN3 levels are changed neither by lack of GAI and RGA, nor by GA treatment. As shown previously (16, 17), EIN3 accumulates to immunodetectable levels in *ctr1–1* and *ebf1–1 ebf2–1* mutants (Fig. 4*C*). We found that EIN3 levels were substantially maintained in *ctr1–1* mutants that additionally lacked GAI and RGA or in *ebf1–1 ebf2–1* mutants treated with GA (Fig. 4*C*). Because EIN3 was not detected in WT controls (Fig. 4*C*), our observations suggest that EIN3 accumulation delays flowering via effects on DELLA stability (rather than vice versa). Consistent with this hypothesis, we found that transgenic overexpression of *ETHYLENE RESPONSE 1* (*ERF1*, a gene that is transcriptionally activated by EIN3; ref. 15) from a *35S:ERF1* construct conferred a constitutive ethylene response (15), a delay in floral initiation in SDs similar to that conferred by *ctr1–1* (Fig. 4*D*), and an associated reduction in *LFY* and *SOC1* transcript levels (Fig. 4*E*). We also found that GA treatment suppressed the delayed SD flowering of *35S:ERF1* plants and restored almost to normal the levels of *LFY* and *SOC1* transcripts in those plants (Fig. 4 *D* and *E*). Thus, our experiments indicate that ethylene-mediated EIN3 accumulation delays flowering (at least in part) by activating *ERF1*, which we propose promotes DELLA accumulation by reducing GA content.

Discussion

Ethylene production is commonly stimulated by adverse biotic or abiotic stress conditions (3, 5, 54, 55), and elevated ethylene levels frequently delay flowering (19). However, the mechanism by which ethylene delays flowering was not previously understood. Several distinct genetic pathways are known to promote flowering by activating floral meristem-identity genes (e.g., the photoperiod pathway, the autonomous pathway, the GA pathway), whereas other pathways inhibit the activity of floral meristem-identity genes (56). The experiments described in this article define the relationship between these previously defined floral pathways and the stress hormone ethylene.

We initially showed that ethylene treatments caused a delay in the flowering of *Arabidopsis*, a similar delay was conferred by the constitutive ethylene-response *ctr1–1* mutation, and the delaying effects of both ethylene and *ctr1–1* were increased in SDs. Because the GA pathway has a greater effect on the flowering time of *Arabidopsis* in SDs than in LDs (36) our observations immediately suggested that the effect of ethylene on flowering depends more on the GA pathway than it does on the other

flowering pathways. Accordingly, we found that the delayed SD flowering of *ctr1–1* could be corrected by exogenous GA, and that *ctr1–1* contains reduced levels of bioactive GAs (Fig. 1). We also found that exogenous GA substantially rescues the vegetative growth phenotypes (vegetative rosette size, petiole length, etc.) of *ctr1–1* (data not shown).

Endogenous plant bioactive GA levels are regulated by a negative feedback mechanism that controls the levels of gene transcripts encoding GA biosynthesis enzymes (49, 52, 53). Thus, the elevated *AtGA3ox1* and *AtGA20ox1* transcript levels observed in *ctr1–1* are presumably a consequence of the reduced bioactive GA levels observed in that mutant. The reason these elevated transcript levels do not restore normal bioactive GA levels is not clear, especially given the relatively high accumulation of the GA 20-oxidase substrates GA_{24} and GA_{53}. Perhaps CTR1 activity regulates the activities of the 20-oxidase (and 3β-hydroxylase) enzymes themselves.

The reduced bioactive GA level in *ctr1–1* presumably causes accumulation of DELLAs, thus enhancing DELLA activity. Accordingly, we have shown that DELLA activity is substantially responsible for the late flowering of *ctr1–1* (because lack of GAI and RGA largely suppresses the late flowering of *ctr1–1*; Fig. 2 *A*, *B*, and *D*). In fact, ethylene likely regulates DELLA accumulation by modulating both endogenous bioactive GA levels and the relative stability of DELLAs in response to GA (43). The GA–DELLA pathway activates flowering via up-regulation of the floral meristem-identity genes *LFY* and *SOC1* (36–40). Accordingly, we found that, at the time when WT and *ctr1–1 gai-t6 rga-24* plants were just beginning to bolt, the later-flowering *ctr1–1* plants displayed reduced levels of *LFY* and *SOC1* transcript accumulation with respect to WT or *ctr1–1 gai-t6 rga-24* plants (Fig. 3*A*). Furthermore, we found that *35S*:*LFY* plants flower earlier than controls in the presence of the ethylene-precursor ACC. Taken together, these observations indicate that ethylene promotes the accumulation of DELLAs, the consequent inhibition of *LFY* and *SOC1* up-regulation, and a resultant delay in flowering.

At the seedling stage of development, ethylene signaling works primarily via the linear CTR1/EIN3 pathway (6). We determined whether the CTR1/EIN3 pathway also affects floral initiation by investigating the combined effects of the *ebf1–1* and *ebf2–1* mutations on flowering time. We found that *ebf1–1 ebf2–1* plants exhibited a severe *ctr1–1*-like phenotype (a phenotype that is more severe than that displayed by WT plants treated continuously with high levels of ethylene; ref. 6) and a delay in flowering that was restored to normal by treatment with exogenous GA (Fig. 4 *A* and *B*). We also showed that the level of the EIN3 protein in *ebf1–1 ebf2–1* plants was unaffected by treatment with GA (Fig. 4*C*) or by lack of the DELLAs GAI and RGA (in *ctr1–1 gai-t6 rga-24*; Fig. 4*C*). Furthermore, the late flowering conferred by transgenic overexpression of *ERF1* (a gene that is normally transcriptionally activated by EIN3) was suppressed by GA treatment (Fig. 4*D*). Taken together, these observations indicate that the GA–DELLA pathway acts downstream of CTR1 (and likely also downstream of EIN3) in the ethylene-dependent regulation of flowering.

A previous report (57) indicates that WT plants bolt 1–6 days earlier than the ethylene insensitive mutants *ein3–1*, *ein2–1* and *etr1*. We similarly observed a relative delay in *etr1–3* flowering time, but no delay in the flowering of *ein3–1* (SI Table 1). However, in contrast to what was observed with WT, we also observed that the flowering time of both *etr1–3* and *ein3–1* was not further delayed by ACC treatment (and also unchanged by ACC plus GA treatments; SI Table 1). Thus, as expected, *etr1–1* and *ein3–1*, because they confer ethylene insensitivity, also abolish the ethylene/DELLA-dependent delay in flowering. The (slight) delay in flowering exhibited by untreated ethylene-insensitive mutants (57) is presumably caused by an unknown mechanism that is distinct from the ethylene-mediated DELLA-dependent mechanism described here.

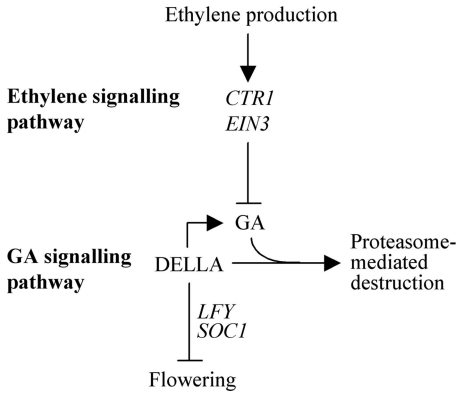

Fig. 5. Model for integration of the ethylene and GA–DELLA signaling pathways in the regulation of floral transition. Activation of ethylene signaling reduces bioactive GA levels, thus promoting the accumulation of DELLAs. DELLA accumulation in turn slows the plant life cycle and delays flowering. Ethylene production activates ethylene signaling by inhibiting CTR1 and increasing EIN3 levels via the $SCF^{EBF1/EBF2}$ ubiquitin pathway. Accumulation of DELLAs delays floral transition (via regulation of *LFY* and *SOC1* transcript levels) and increases the abundance of GA-biosynthesis gene transcripts via a negative feedback loop.

Thus, our observations indicate the existence of a previously unknown mechanism whereby environmental stress regulates the timing of a key plant life-cycle step (the floral transition) via a connection between the ethylene and GA–DELLA signaling pathways (Fig. 5). This mechanism is distinct from the recently proposed mechanism in which abscisic acid regulates floral transition by modulation of the flowering CA-dependent autonomous pathway (58). The ethylene-dependent mechanism of floral regulation comprises the following events. First, activation of ethylene production by environmental stress enhances ethylene responses via the linear CTR1–EIN3-dependent pathway (see also ref. 3). Second, activation of ethylene responses results in reduced bioactive GA levels, thus causing increased accumulation of DELLAs. Third, increased DELLA accumulation delays the initiation of the floral transition by inhibiting up-regulation of the floral inducers *LFY* and *SOC1*.

Our observations indicate intriguing similarities between *Arabidopsis* plants grown in SDs and in environments that induce ethylene signaling. In both conditions, the GA pathway becomes the predominant regulator of floral induction. Thus, the same signaling pathway has been recruited to facilitate appropriate response to these two distinct environmental regulators of floral transition.

Methods

***Arabidopsis* Lines.** Mutant lines were derived from Landsberg *erecta* (Ler) (*ga1–3*; *spy-5*; DELLA mutants) or Columbia (Col) (ethylene signaling mutants) backgrounds. *ga1–3*, *ctr1–1* × Ler, *ctr1–1 gai-t6*, *ctr1–1 rga-24*, *ctr1–1 gai-t6 rga-24*, *ctr1–1*, *etr1–3*, *ein3–1*, *ebf1–1*, *ebf2–1*, *ebf1–1 ebf2–1*, and *35S*:*LFY* were as described (refs. 3, 17, 43, 45, and 51 and *SI Text*). *35S*:*ERF1* was from the European *Arabidopsis* Stock Centre (Loughborough, U.K.; ref. no. N6143).

Flowering Time Experiments. Seeds were surface-sterilized and placed on GM medium [Murashige and Skoog medium 1×, pH 5.7 (M0255 Duchefa), 1% saccharose, 0.9% agar; containing 10 μM ACC and/or 10 μM GA_3, as indicated] at 4°C for 5 days (43). After 1 month at 20°C (16-h photoperiod), a representative (from among 10) was photographed. Soil-grown plants were

sown in 20°C, 16-h photoperiod (LD) or 10-h photoperiod (SD; except for the *35S:ERF1* experiment where SD was 8 h) and sprayed with 100 μM GA_3 (or water control) twice a week. Flowering time was measured temporally or expressed as the number of vegetative leaves produced before flowering.

RT-PCR Analysis. Total RNA was extracted (Trizol reagent; GIBCO/BRL, Carlsbad, CA) from apical meristem/young leaves of 3-week-old soil-grown plants (20°C; 10-h photoperiod except Fig. 4*E* where the photoperiod was 8 h; GA treatment and controls as described above; Figs. 3*A* and 4*E* and SI Fig. 7*C*). cDNA synthesis/PCR amplification were as described (43). For results in Fig. 3*A* and SI Fig. 7*C*, RT-PCRs (18 cycles) were blotted and probed with the corresponding full-length PCR amplified random-labeled ^{32}P-labeled fragment (Promega, Madison, WI). Primers for PCR amplification/probe preparation are in *SI Text*. For data in Fig. 4*E*, RT-PCRs (28 cycles) were loaded onto an agarose/ethidium bromide gel.

Immunodetection of EIN3. Protein extractions (2 week-old plants) and immunoblot analyses were as described (16, 43). Equivalent amounts were ground up in liquid nitrogen, homogenized in 2× SDS/PAGE sample buffer, separated by 10% PAGE, and blotted onto nitrocellulose. Immunodetection used an anti-EIN3 antibody and peroxydase-conjugated goat anti-rabbit IgG (Southern Biotech, Birmingham, AL), visualized by chemiluminescence (Amersham Biosciences, Piscataway, NJ). The blot was subsequently stripped with 0.2 M glycine, pH 2.5 and reprobed with anti-β-tubulin.

GA Determinations. GA determinations were performed on soil-grown mature vegetative rosettes of equivalent developmental age: WT, 16 days old; *ctr1–1*, 25 days old grown at 20°C, 16 h photoperiod, just before bolting, essentially as described (3). GAs from 500 mg (fresh weight) of tissue were purified and analyzed by GC/MS-selected reaction monitoring (JSM-Mstation 700; JEOL, Tokyo, Japan), using $^2H_2$-GAs (L. Mander, Australian National University, Canberra, Australia) as internal standards. Where indicated as not detected, endogenous GAs were not detected, whereas $^2H_2$–GA standards were detected.

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