Review

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Feature Review

Auxin and ethylene: collaborators or competitors?

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The individual roles of auxin and ethylene in controlling the growth and development of young seedlings have been well studied. In recent years, these two hormones have been shown to act synergistically to control specific growth and developmental processes, such as root elongation and root hair formation, as well as antagonistically in other processes, such as lateral root formation and hypocotyl elongation. This review examines the growth and developmental processes that are regulated by crosstalk between these two hormones and explores the mechanistic basis for the regulation of these processes. The emerging trend from these experiments is that ethylene modulates auxin synthesis, transport, and signaling with unique targets and responses in a range of tissues to fine-tune seedling growth and development.

Importance of plant hormone crosstalk

Plant hormones are essential regulators of growth and development. Over the past decade, many of the plant hormone receptors, signaling components, and downstream transcriptional networks have been identified through genetic analyses. Similar progress in understanding the synthesis and transport of hormones has provided a more complete picture of the mechanisms controlling the distribution and abundance of these molecules. It is now possible to explore the more complex question of how a plant coordinates its growth with the simultaneous input of multiple hormones by using insights into individual hormone function and the plethora of signaling and synthesis mutants. One set of plant hormones with complex interactions is auxin and ethylene. The crosstalk between these two hormones, at both physiological and molecular levels, is receiving substantial study, bringing new understanding of how they are able to act either antagonistically or synergistically in a tissue-specific fashion to influence plant growth and development. This review focuses on our understanding of the molecular mechanisms that control physiological processes regulated by crosstalk between auxin and ethylene in seedling growth and development, and highlights areas where our understanding of the interactions is not yet clear.

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Auxin and ethylene signaling pathways

Identification of ethylene-insensitive mutants through *Arabidopsis* (*Arabidopsis thaliana*) molecular genetics opened a new era in dissecting plant hormone signaling [1,2]. The ethylene-induced triple response, which is characterized by short stature, a thickened hypocotyl, and enhanced apical hooks, was exploited to design an elegant genetic screen that readily identified insensitive mutants as tall seedlings in a lawn of short seedlings [1,2]. These screens isolated mutants that exhibited ethylene insensitivity (*ein* or *etr* mutants) [1–3], enhanced ethylene signaling characterized by a constitutive triple response (*ctr1*) [4,5], or ethylene overproduction (*eto1*) [2]. Equivalent signaling genes have been identified in tomato (*Solanum lycopersicum*) and rice (*Oryza sativa*), suggesting that ethylene is perceived similarly across the plant kingdom [6,7].

Physiological and genetic characterization of ethylene mutants has revealed a linear signaling pathway that begins with ethylene binding to and turning off the receptor proteins, including ETR1 and its tomato ortholog, NEVER-RIPE (NR) [8,9], as shown in Figure 1. CTR1, a protein kinase with sequence similarity to the catalytic domain of RAF protein kinase (a mitogen-activated protein kinase kinase kinase) is downstream from ETR1 and functions as a negative regulator of signaling [4,5]. EIN2 is an essential, positive modulator of ethylene signaling [10] that, either directly or indirectly, controls the activity of transcription factors including EIN3 and EIN3-like (EIL) proteins, whose targets include *ERF1* (*ETHYLENE RESPONSE FACTOR1*) and *EDF1* through 4 (*ETHYLENE RESPONSE DNA BINDING FACTOR 1* through *4*)[11–13]. As a result of this hierarchical transcriptional cascade, ethylene either positively or negatively regulates diverse genes encoding proteins that mediate the growth response to ethylene [14].

The auxin signaling pathway was identified through the examination of transcriptional changes in response to auxin treatment and isolation of mutant seedlings with altered response to auxin. Early work identified genes that are rapidly induced by auxin, including the *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*), *GRETCHEN HAGEN3* (*GH3*), and *SMALL AUXIN-UP RNA* (*SAUR*) gene families [15]. Subsequent studies identified auxin response factors (ARFs) that regulate the expression of

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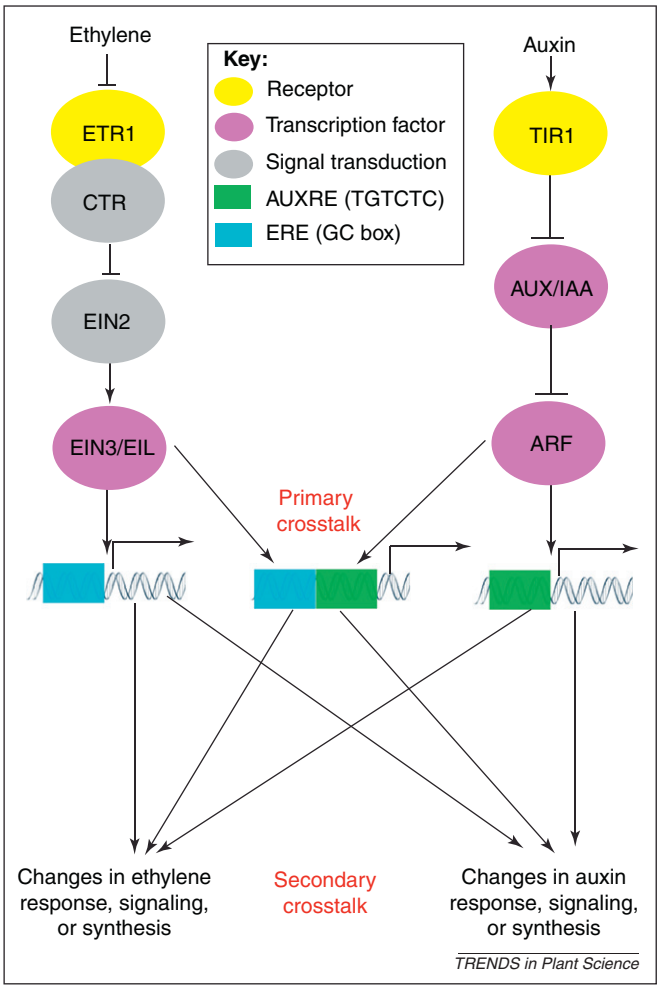

Figure 1. Model of auxin–ethylene crosstalk. In *Arabidopsis,* ethylene and auxin responses are initiated by binding the ETR1 and TIR1 receptors, respectively. Ethylene binds to and inhibits ETR1 activity, which in turn leads to inhibition of the CTR kinase, a negative regulator of EIN2 activity. EIN2 activates the EIN3 and EIN3-like (EIL) family of transcription factors, which in turn promote transcription of genes containing an ethylene-responsive element (ERE) in their promoter region. Auxin signaling is mediated by proteasome-dependent degradation of AUX/IAA transcriptional repressors, which release ARF transcription factors to activate transcription of genes with auxin responsive elements (AUXRE) in their regulatory region. Primary crosstalk occurs by activation of genes that contain both AUXRE and ERE in their promoter region, allowing both signaling pathways to directly regulate transcription. Secondary crosstalk occurs through expression of genes that are either auxin or ethylene responsive, but the activities of which control expression of genes that regulate the other hormones' synthesis, signaling, or response.

many auxin-responsive genes [16] and demonstrated that AUX/IAA proteins act as transcriptional repressors [17]. Development of auxin-responsive reporter lines using promoters of auxin induced genes to drive expression of genes encoding either β-glucuronidase (GUS) or green fluorescent protein (GFP) greatly facilitated understanding of the auxin response pathway. In particular, the synthetic promoter *PDR5* (*DR5* promoter):β-glucuronidase (hereafter referred to as *DR5:GUS*) is derived from a repeated regulatory element of the *GH3* promoter [17] and has been used as a reporter of ARF dependent transcription in a large number of studies. A brief summary of the auxin signaling pathway is shown in Figure 1.

Meanwhile, forward genetic screens of mutants that are less sensitive to the negative effect of auxin or auxin transport inhibitors on root elongation identified *auxin resistant 1* (*axr1*), *axr2*, *axr3*, *axr4* and *transport inhibitor resistant1* (*tir1*) mutants [18]. *TIR1* encodes an F-box protein, which functions as an auxin receptor, and mediates proteolytic cleavage of transcriptional repressors, including the AUX/IAA proteins [19–21]. The identification of receptor-mediated proteolytic cleavage uncovered a new paradigm in plant hormone signaling [18], which is also a mechanism used by other plant hormone-specific F-box proteins, regulating the degradation of transcription factors and other regulatory proteins, including those that function in ethylene signaling [22].

Auxin transport and initial evidence for auxin–ethylene crosstalk

The earliest genetic evidence that ethylene and auxin may act through convergent pathways to regulate root growth came from the identification of ethylene-insensitive mutants with defects in auxin transporters: *aux1* and *ethylene insensitive root 1/pinformed 2* (*eir1/pin2*) [23–25]. IAA influx into cells is mediated by auxin-uptake carriers encoded by the *AUX1* and *Like AUX1* (*LAX*) gene family [26,27]. Auxin moves out of plant cells through efflux proteins, including PIN and ATP binding cassette type B/P-glycoprotein/multidrug resistance (ABCB/PGP/MDR) proteins [28]. *PIN* gene products were initially linked to auxin transport by the phenotypes of *pin* mutants. The *pin1* mutant exhibits reduced auxin transport in inflorescences [29], and *pin2*, *pin3*, and *pin7* have reduced polar auxin transport in roots [30–32]. Mutations in *ABCB*/*MDR*/*PGP* genes alter indole 3-acetic acid (IAA) transport and associated phenotypes in seedlings [33–36].

Auxin is transported from cell to cell. In shoots, IAA, the predominant naturally occurring auxin, moves unidirectionally from the apex to the base [28]. In roots, auxin transport is more complex, with two distinct polarities. IAA moves toward the root tip, in the rootward direction (formerly referred to as acropetal transport), through the central cylinder and in the shootward direction (formerly referred to as basipetal transport) through the outer layers of root cells [37]. In *Arabidopsis* roots, both polarities of IAA movement control distinct processes and are mediated by unique suites of auxin transport proteins. Rootward movement of IAA from the shoot into the root apex has been implicated in the control of lateral root formation and elongation [32,38–40]. AUX1 [32,41,42], ABCB19 [35,36], and PIN1, PIN3, and PIN7 [32,43,44] participate in rootward auxin transport and lateral root initiation and/or elongation. Shootward movement of IAA from the root tip is required for the gravity response [31]. AUX1 [26,45], PIN2 [30,31,46], and ABCB4 [35] mediate this polarity of IAA transport, and mutants of these genes show altered gravitropic responses. This information on the localization and physiological function of auxin transport proteins provides a framework to understand the effect of ethylene on auxin transport and signaling, as well as the dependent developmental processes that are influenced by these two hormones.

Understanding of the basic features of auxin transport and the signal transduction pathways for auxin and ethylene sets the backdrop for considering how ethylene may affect auxin regulated growth and developmental processes. This review is divided into multiple sections each

focused on a distinct growth and developmental process in which such crosstalk has been observed and for which molecular mechanisms of auxin–ethylene interaction have been reported.

Root elongation

The best studied effect of ethylene, or the ethylene precursor 1-aminocyclopropane carboxylic acid (ACC), in roots is the inhibition of root elongation, which is synergistic with the effect of auxin on this process [47,48]. Kinematic analyses of root growth inhibition by auxin and ethylene using high temporal and spatial resolution revealed that IAA and ethylene reduce the expansion rate of the cells in the central elongation zone [49,50]. Figure 2 illustrates the effect of auxin and ethylene on primary root elongation. These observations were supported by the demonstration that mutants with enhanced ethylene signaling or synthesis have reduced root elongation [2,4]. Growth inhibition by elevated ethylene is reduced in ethylene-insensitive *Arabidopsis* mutants, including *etr1*, *ein2*, *ein3*, *eil1* [14,49,51,52], and the tomato mutants *Nr* and *Green-ripe* (*Gr*) [53,54]. The auxin overproducing mutant *superroot2* (*sur2*), transgenic plants overexpressing the *YUCCA* (*YUC*) gene, and wild-type plants treated with exogenous auxin also show extreme reduction in root elongation because of a reduction in the expansion rate of elongating cells [50,55–57]. As in ethylene-insensitive mutants, IAA-induced root growth inhibition is lost or substantially reduced in auxin-resistant mutants such as *tir1*, *axr2*, *axr3*, and *solitary-root* (*slr*) [58–62]. These observations indicate that auxin and ethylene have similar effects on root elongation, raising the question of whether there is any convergence in the signaling pathways regulating this response. The following sections explore separately the evidence for inhibition of root elongation by ethylene through modulation of auxin signaling, transport and synthesis.

Auxin signaling

Elevated levels of ethylene increase the auxin response in the root elongation zone, which is readily monitored by the auxin-inducible reporters *DR5:GUS* [14,42,52], *DR5:GFP* [32,52], *DR5:vYFP* [63] and *PIAA2* (*IAA2* promoter):*GUS* (hereafter referred to as *IAA2-GUS*) [49]. The effect of ethylene on the auxin response in the root tip is demonstrated by the elevated expression of *DR5:GFP* in Figure 2. A functional auxin signaling network is required for this response because *axr1*, *axr3-1* and *tir1* roots are insensitive to growth inhibition by ethylene [14,49,52]. The functional role of the ARF transcription factors in regulating the ethylene response remains unclear. The role of ARF19 and ARF7 was tested by two groups with contradictory results [52,64], indicating that the transcriptional networks controlling this process need further study.

The evidence is much less clear with regard to whether a fully functional ethylene signaling pathway is required for maximal auxin-dependent root growth inhibition. In response to ACC treatment, both *ein2* and *etr1* are resistant to growth inhibition and *DR5:GUS* induction [52]. By contrast, the *ein2* mutant (but not *etr1*) shows reduced growth inhibition on treatment with the synthetic auxin α-naphthaleneacetic acid (NAA), with normal NAA-induced

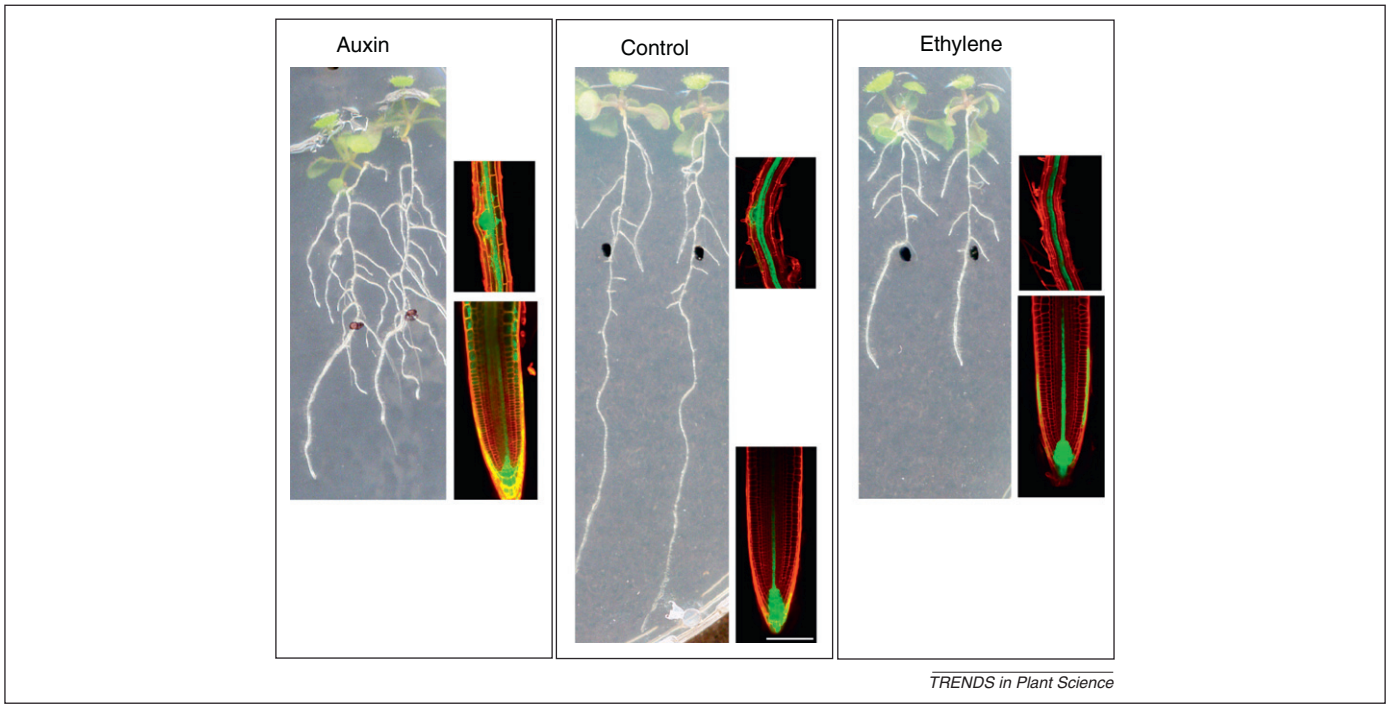

Figure 2. Auxin and ethylene alter root growth and development. Five-day-old *Arabidopsis* seedlings were transferred to medium containing 1 μM IAA or ACC and the tip of the roots at the time of transfer was marked by a black dot. When their roots were imaged five days later, both auxin and ethylene had decreased the rate of root elongation relative to an untreated control, as judged by the length of root that had formed below the black dots. This inhibition of growth was accompanied by elevated auxin accumulation and auxin-induced gene expression at the root tip, as visualized by the *DR5:GFP* reporter (green) in the insets shown in an overlay with propidium iodide (red) to reveal individual cells. By contrast, auxin treatment enhanced lateral root elongation and initiation of lateral roots particularly in the region of the primary root formed prior to transfer, whereas ethylene treatment prevented any root formation in the region formed after transfer. *DR5:GFP* was increased in the mature region of the root after auxin treatment, placed next to the region of the root from which images were captured, but decreased after ethylene treatment, consistent with the opposite roles of these hormones in controlling lateral root formation. Micrographs: scale bar = 100 μm.

DR5:GUS induction in both *etr1* and *ein2* [52]. In another report, *ein2*, *ein3* and *eil1* show wild-type responses to treatment with a range of IAA concentrations [14].

Several studies have examined the interactions between auxin and ethylene at the level of transcription. Using auxin and ethylene receptor or signaling mutants, the abundance of transcripts of genes encoding flavonoid biosynthetic enzymes and auxin transport proteins has been examined. In both cases, ACC-dependent transcript changes are lost in the ethylene signaling mutants *ein2* and *etr1*, but IAA-induced changes are not [32,63]. Similarly, in *tir1*, auxin-induced gene expression changes are lost but ACC-induced transcription and repression of root branching are maintained [32,63]. These results suggest that, although auxin and ethylene may both enhance expression of certain genes, these transcriptional effects may operate through independent signaling pathways, resulting in a model in which transcriptional targets may contain both ethylene and auxin response elements and crosstalk between these pathways may occur at the level of target genes. This possibility is illustrated in Figure 1 and is consistent with primary transcriptional crosstalk. Consistent with this possibility, the upstream regulatory regions of many of these genes induced by auxin and ethylene have been shown to contain putative auxin response element (AuxRE) and ethylene response element (ERE) sequences, which are sites of ARF and EIN3/EIL binding, respectively [63].

To more globally dissect the interactions between auxin and ethylene at the level of transcription, a genome-wide analysis of dark-grown *Arabidopsis* roots was performed using wild type, *ein2* and *aux1* with and without auxin and ethylene treatment [14]. This experiment not only uncovered numerous genes that were regulated only by auxin or ethylene, but also found that 33% of ethylene-regulated genes and 23% of auxin-regulated genes were in both data sets [14]. Of the transcripts that exhibited changes in expression after auxin treatment, 38% required EIN2 function for this induction and 28% of ethylene-altered transcripts required AUX1 [14]. The authors concluded that ethylene and auxin may mostly act independently to control transcription, and that auxin–ethylene crosstalk occurs at the level of each hormone influencing the synthesis of the other, perhaps by transcriptional control of hormone biosynthetic genes [14].

Auxin transport

Ethylene modulates root elongation through altering auxin transport. The first genetic evidence suggesting this relationship was the isolation of auxin transport mutants (*aux1* and *pin2/eir1*) in screens for reduced growth inhibition by ethylene [23–25]. The ethylene response in the *aux1* mutant could be restored by the synthetic auxin NAA, which bypasses IAA influx proteins, but not by IAA, which is poorly taken up in the absence of AUX1 [47], suggesting that an elevation in intracellular auxin concentration is needed for the ethylene response. In addition, the enhanced auxin signaling in root tips after ethylene treatment is lost in *aux1* and *pin2*, as observed by examination of *IAA2:GUS*, *DR5:GUS* and *DR5:GFP* expression [14,42,49,52]. Although growth inhibition by ethylene is also reduced in *aux1* and *pin2*, the *pin1*, *pin4* and *pin7* mutants all show normal growth responses to ethylene [52]. ACC increases endogenous promoter driven protein reporter fusions including *PIN2:GUS*, *PIN2:GFP* and *AUX1:YFP*, and *AUX1* transcripts, whereas these transcript changes are lost in *ein2* and *etr1* [32,52]. Furthermore, in *aux1*, the ethylene-induced increases in *ERF1* transcripts are lost [49], as is the ethylene-induced expression of the *PEBS:GUS* ethylene-responsive reporter [14]. Together, these results support a model in which ethylene enhances auxin transport in the elongation zone, leading to reduced cell expansion as a result of the elevated IAA levels [52].

Auxin synthesis

An interesting reciprocal regulation of auxin and ethylene synthesis has been reported. Elevated levels of auxin lead to increased ethylene synthesis [65]. This effect is mediated by increased transcription of the genes that drive ethylene synthesis, including specific members of the ACC synthase (ACS) family, which catalyze the rate-limiting step in ethylene synthesis [14,66]. Furthermore, ethylene may also positively regulate auxin synthesis. Free IAA levels increase in the root tip after treatment with 100 μM ACC; this response is lost in *etr1* or after treatment with the ACC synthesis inhibitor 1-aminoethoxyvinyl-glycine (AVG) [49,52]. The rate of *de novo* IAA synthesis has also been measured after treatment with relatively high doses of ACC. In one case, both 10 and 100 μM ACC increased IAA synthesis [49]; however, in another case,100 μM ACC did not have any significant effect on auxin synthesis [52]. Nevertheless, reductions in free IAA and/or IAA synthesis were detected by both groups in the presence of AVG [49,52], leading to the hypothesis that ethylene may stimulate localized auxin biosynthesis. However, the effect of AVG on IAA synthesis may be direct, rather than working through altered ethylene levels. A recent report indicates that AVG blocks endogenous auxin biosynthesis by inhibiting the activity of tryptophan aminotransferase, which catalyzes the conversion of tryptophan to indole-3-pyruvic acid [67]. The demonstration that constitutive ethylene signaling resulted in a fivefold increase in auxin concentration in the *ctr1* root apex compared with wild type [68] suggests that ethylene stimulates localized auxin biosynthesis or alters auxin transport leading to accumulation in that tissue.

The best evidence for ethylene-regulated auxin synthesis comes from a screen for weak ethylene-insensitive (*wei*) mutants that identified several genes encoding proteins that function in ethylene-induced auxin synthesis [69,70]. These include the alpha and beta subunits of anthranilate synthase, ASA1/WEI2/TIR7 and ASB1/WEI7, respectively, which catalyze the first committed step of tryptophan biosynthesis and thereby contribute to the availability of IAA precursor [69]. The *WEI8* gene encodes a tryptophan aminotransferase that functions in the indole-3-pyruvic acid branch of IAA synthesis, and its cloning led to the identification of the tryptophan aminotransferase of Arabidopsis related (TAA1/TAR) gene family [70]. A *wei8 tar2* double mutant has significant reductions in free IAA, *DR5:GUS* expression at the root tip in the presence and

absence of ACC, and root growth inhibition [70]. Together, these results suggest that enhancing the synthesis of one another is one of the mechanisms by which ethylene and auxin synergistically inhibit root elongation.

Lateral root development

In contrast to root elongation, which is synergistically inhibited by auxin and ethylene, these two hormones act antagonistically on lateral root initiation. These opposite effects of auxin and ethylene are evident in the seedling images shown in Figure 2. Auxin stimulates lateral root formation and elongation, with the most profound effects in the region of the primary root formed before auxin treatment [71], resulting in overall increases in root biomass. Mutants and inhibitors that reduce auxin transport reduce lateral root initiation and emergence [38,40,72]. However, the role of auxin in the regulation of lateral root formation is more complex than initially thought. A role of auxin in pre-priming sites of lateral root formation in the region close to the root tip has been identified [73,74] and several recent articles have demonstrated that an increase in the local accumulation of auxin is sufficient to drive lateral root formation [44,75]. Localized IAA accumulation by sporadic expression of the *iaaM* gene under the control of a *cre-lox* system specified the site of pericycle cell differentiation into lateral root founder cells [75]. When primary roots are bent or mechanically stimulated, lateral roots initiate at the site of bending [44,73,76,77], and localized formation of auxin maxima coincides with the earliest events in lateral root initiation (as demonstrated with the auxin-responsive promoter line DR5::venusYFP) [44]. Other reports have argued that, in regions of roots close to the apex, auxin inhibits lateral root initiation [78]. These effects are influenced by light and media conditions [79,80] and the type of auxin used, with NAA and IAA having very different effects [32]. What is clear from these studies is that the role of auxin in lateral root initiation, particularly near the tip, is complex, but in most situations auxin is a positive regulator of lateral root development.

By contrast, treatment with ethylene or ACC reduces lateral root initiation in both *Arabidopsis* and tomato, with the most profound effects on the primary root that elongates after exposure to elevated ethylene, in the region formed below the black dot in Figure 2 [42,54,71]. Similarly, *Arabidopsis ctr1* and *eto1* mutants and the tomato *epinastic* (*epi*) mutant have reduced lateral root formation [42,81]. By contrast, dominant negative *etr1* and *Nr* receptor mutants, as well as the ethylene-insensitive *ein2* and *Gr* mutants, form an elevated number of lateral roots [42,54]. The *etr1* and *Nr* receptor mutants are also completely insensitive to the effect of ACC treatment on lateral root formation [42,54], whereas *etr1* and *ein2* form more lateral roots after exogenous auxin with a wild-type response [78]. This ACC insensitivity in lateral root formation is more profound than the insensitivity to root elongation, suggesting that the effects of ethylene on primary and lateral root growth can be uncoupled [42,54].

Given that ethylene has been reported to negatively regulate auxin transport, particularly in shoot tissues [82], the hypothesis that ethylene acts by reducing auxin transport in roots was tested [42,54]. Surprisingly, elevated levels of ethylene, either through treatment with ACC or in the *eto1* mutant, resulted in elevated rootward and shootward auxin transport, and *etr1* and *ein2* mutations abolished this effect [42,54,71]. Although an increase in the *DR5:GFP* signal was observed in the root tip on ACC treatment, in the rootward side of the differentiation zone of the root, where the lateral root develops (~5 mm from the root tip) [83], the opposite effect was observed, with ACC reducing the *DR5:GFP* signal (Figure 2). Interestingly, treatment with the ethylene synthesis inhibitor AVG reversed the expression pattern, reducing fluorescence in the root tip and increasing it in the mature root [32]. These changes in auxin-responsive gene expression are mirrored by increases in free IAA levels in the root tip after ACC or ethylene treatment [49,52] and by reductions in free IAA levels quantified in whole roots treated with ACC or in the *eto1* mutant [42]. The changes in free IAA and auxin-induced gene expression are consistent with the opposite effects of ethylene and auxin on lateral root formation in this region of the root and the synergistic effects of these hormones in the root tip elongation zone.

The requirement for specific auxin transport proteins in ethylene-elevated rootward auxin transport and reduced lateral root formation was tested using single or double mutants in genes encoding auxin influx and efflux proteins. AUX1, LAX3, PIN3 and PIN7 are necessary for the positive effect of ACC on transport and its negative effect on root development [32]. By contrast, neither PIN2 nor ABCB19 affects the response to ACC in either lateral root development or auxin transport [32]. The abundance of *AUX1*, *PIN3* and *PIN7* transcripts increased after ACC treatment in an EIN2- and ETR1-dependent manner [32]. These transcripts are also elevated by IAA treatment in wild type but not in *tir1*; ACC-dependent increases in gene expression and decreases in lateral root formation were observed in the *tir1* mutant. These results suggest that transcription of *AUX1*, *PIN3* and *PIN7* is independently regulated by auxin and ethylene [32].

Alterations in the abundance of auxin transport proteins in response to elevated ethylene could alter auxin transport from either the shoot or the root tip into the zone of lateral root formation; both have been suggested to deliver auxin that drives lateral root formation [38–40,73]. Because ACC increased auxin accumulation at the root tip and depleted it in more distal root regions, a role of rootward IAA transport in response to ACC treatment seemed more logical [32]. Additionally, PIN3 and PIN7 are not detectable in the peripheral cell layers where shootward transport occurs, and mutant analysis clearly demonstrated their involvement. To better understand the regulation of rootward transport by ACC, the localization patterns of auxin transport proteins were examined with and without ACC treatment. *AUX1:YFP*, *PIN3:GFP* and *PIN7:GFP* fluorescence increased with ACC treatment and decreased with AVG treatment in the root apex [32]. By contrast, in the mature region of the root, ACC decreased *AUX1:YFP* fluorescence and increased both *PIN3:GFP* and *PIN7:GFP* fluorescence [32]. AVG treatment had opposite effects, increasing *AUX1:YFP* and the abundance of lateral root primordia, and decreasing *PIN3:GFP* and *PIN7:GFP*. ACC treatment prevented the previously reported increases in *AUX1:YFP* at

the position of root formation [44]. ACC also enhanced *PIN3:GFP* and *PIN7:GFP* fluorescence all along the root, blocking the previously described reduced accumulation immediately below the point of bending in untreated roots and thereby preventing the formation of a local auxin maximum [32,44]. These results have been integrated into a model (Figure 3) in which the control of lateral root development by ethylene involves changes in auxin transport and accumulation patterns [32]. This model suggests that ACC inhibits lateral root development by blocking changes in the abundance of local auxin transport protein needed to form local auxin maxima that drive lateral root formation. ACC treatment stimulates PIN3 and PIN7 transcription, leading to increased protein abundance and enhanced rootward auxin transport [32]. The consequence of this global increase in auxin transport is the loss of auxin accumulation sites in the mature region of the root that will develop into LRP. In the absence of ethylene, this localized IAA accumulation occurs because of depletions in auxin transport proteins below the site of root formation. In the presence of ACC, increases in PIN3 and PIN7 expression along the root enhance IAA transport, resulting in redistribution of auxin away from the mature region of the root, which then limits lateral root formation.

Root and shoot gravitropism

An additional auxin-dependent process that is modulated by ethylene is gravitropism. Ethylene or ACC reduces the

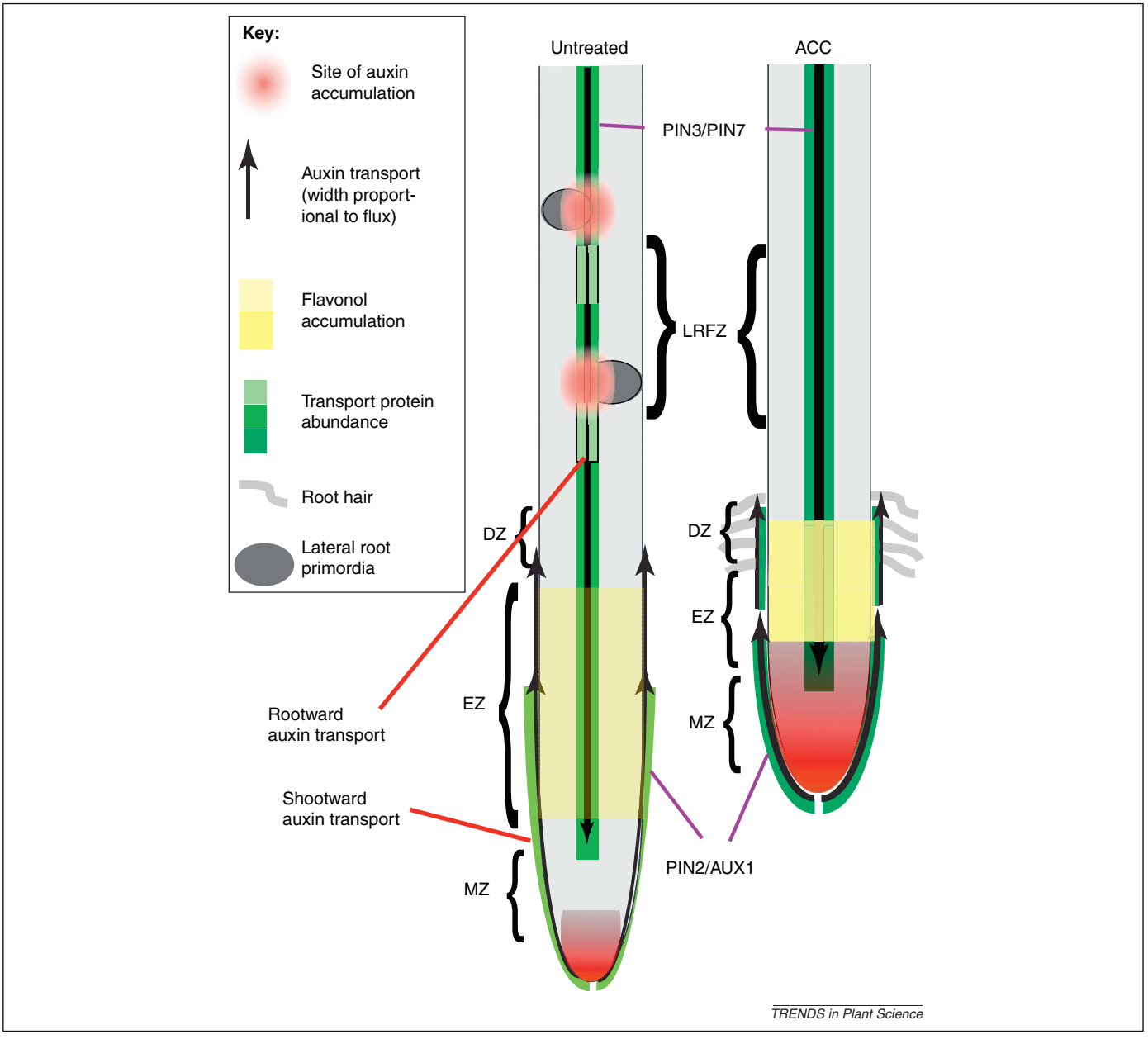

Figure 3. Auxin and ethylene synergistically inhibit root elongation and antagonistically regulate lateral root development. In untreated roots, lateral root formation occurs in the lateral root-forming zone (LRFZ) as a result of localized auxin maxima. This accumulation might occur as a result of depletion of PIN3 and PIN7 proteins that prevents auxin movement away from these points, enhancing auxin accumulation. Ethylene increases rootward auxin transport by increasing transcription and translation of PIN3 and PIN7 in the central cylinder, which may then deplete the lateral root-forming zone of auxin while increasing auxin accumulation in the root apex or meristematic zone (MZ). This effect may be responsible for the negative regulation of lateral root formation by ethylene. At the root tip, increases in transcription and translation of AUX1 and PIN2 enhance shootward transport of the auxin into the elongation zone (EZ), thereby reducing primary root elongation.

early phase of both the root [63,84,85] and shoot gravity response [86–88]. However, some additional studies failed to see any effect of ethylene on the shoot gravity response [89,90]; this may be due to the absence of kinetic data at early time points after seedlings are reoriented relative to the gravity vector, or may reflect differences in the mechanism of gravity perception and response in roots and shoots. Roots sense gravity locally in the columella cells of the root cap [91], and auxin is redistributed from the root tip to one side of the root after gravitational stimulation where the elevated auxin levels inhibit growth on the lower side of the root leading to downward growth [92]. In shoots, gravity is perceived in the starch sheath parenchyma tissues that run the length of the hypocotyl [93] and stimulates lateral auxin transport in multiple tissues along the hypocotyl [94]. This redistribution of auxin leads to elevated auxin accumulation on the lower side of the hypocotyl that stimulates growth and leads to upward bending [94].

Gravitropism is most studied in roots, and in this tissue the negative effect of exogenous ethylene is more consistent [63,85]. Inhibition of root gravitropism by treatment with ACC is illustrated in Figure 4. Alterations in the shootward flow of auxin, either reduced transport or enhanced transport, perturb the root gravity response [92]. For instance, the reduction in shootward auxin transport in *eir1/agravitropic1* (*agr1*) and *pinoid-9* (*pid-9*) or perturbation of transport by auxin transport inhibitors reduces or abolishes root gravitropic curvature [30,31,95]. Similarly, an enhancement in the shootward transport of auxin is accompanied by a reduction in the rate of the gravity response in *roots curl in NPA1* (*rcn1*) and *transparent testa4* (*tt4*) mutant roots [96,97]. Ethylene alters the shootward IAA transport [42] on which gravitropism relies for differential growth [31], suggesting that ethylene can modulate gravitropism by altering auxin transport. Consistent with ethylene targeting auxin transport, mutants with defects in genes encoding auxin transport proteins have defects in gravitropism and ethylene responses. These mutants include the auxin influx mutant *aux1* [98] and the auxin efflux mutants *agr1/eir1/pin2/wav6* [25,30,46,99]. Mutations that alter auxin signaling, including *axr1* [100], *tir1-1* [52,101], *axr2* [102], *axr3* [52,59] and *diageotropica* (*dgt*) of tomato [103,104], are also associated with reduced ethylene sensitivity and altered gravitropic curvature. Taken together, these results provide evidence that auxin and ethylene can act in concert to regulate the root gravity response.

However, mutants that are ethylene resistant but auxin sensitive, show a more variable gravitropic phenotype. Both *ein2* and *etr1* show a wild-type root gravity response in the absence of ethylene [47,85]. By contrast, in the presence of elevated ethylene, which reduces gravitropic response in wild type, the ethylene-insensitive mutants, *etr1*, *ein2* and *ACC-related long-hypocotyls* (*alh1*) exhibit an enhanced root gravity response in the presence of auxin compared with wild type [85,105]. Many ethylene-insensitive plants do not exhibit a gravitropism phenotype when ethylene is limiting, such as when plants are grown along the surface of agar plates. However, when plants are grown in soil, ethylene levels are likely to be much higher because of more limited diffusion. This idea is supported by

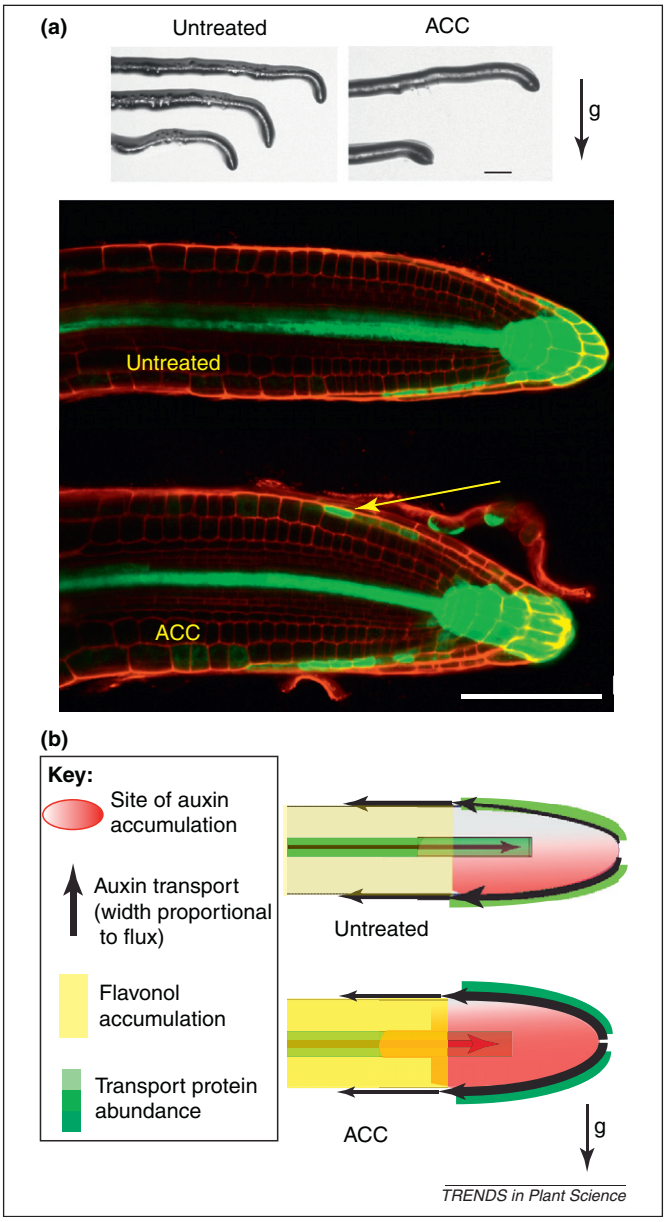

Figure 4. Ethylene negatively regulates the gravity response. Treatment of roots with either ethylene or its biosynthetic precursor, ACC, reduces root gravitropic curvature. (**a**) The images of roots include both root tip angles at low magnification and confocal images of roots eight hours after reorientation at 90° relative to the gravity vector. The angle of gravity is indicated by an arrow. The confocal image shows individual cells after propidium iodide staining (red) and the asymmetric expression of the auxin-responsive *DR5:GFP* reporter across the untreated root (green). By contrast, ACC treatment leads to *DR5:GFP* fluorescence on the upper side (as indicated by the arrow), which then minimizes the auxin gradient across the root, reducing the gravitropic curvature. Scale bars = 100 μm. (**b**) The model indicates the presence of elevated auxin transport protein synthesis and enhanced auxin accumulation in the root tip, and increases in flavonol accumulation in the elongation zone of ethylene-treated seedlings. Together, these changes should lead to enhanced auxin transport into the elongation zone and decreased auxin efflux out of this region, and to the accumulation of flavonoids. Together these two events reduce the formation of a gradient of auxin across a gravity-stimulated root tip, which is needed for gravitropic curvature.

the finding that the increases in the number of lateral roots of the ethylene-insensitive *Nr* mutant relative to wild type is increased more when roots are growing in soil, relative to roots grown on agar medium [54]. Furthermore, when *Arabidopsis* seedlings are grown in plates that are wrapped to prevent diffusion of ethylene gas, root

gravitropism is impaired relative to that in plants grown under conditions where diffusion is not limited [85].

The demonstration that exogenous application of ACC enhances shootward auxin transport [42,52] could explain the observed ACC inhibition of root gravitropism. Ethylene-elevated shootward transport of auxin would prevent the formation of the auxin gradient required for the early phase of the gravitropic response (Figure 4b). In particular, in the presence of ethylene, auxin may be elevated on both sides of the root rather than simply on the lower side, as shown using the *DR5:GFP* reporter construct in Figure 4. This model suggests an explanation for the lack of the gravity defect in ethylene-insensitive mutants such as *etr1* and *ein2*, in which the ethylene-induced stimulation of auxin synthesis and transport is absent [14,42,49,52] and hence the shootward transport and gravity response are unaffected.

The above model also explains the roles of ethylene and auxin in the altered growth of mechanically impeded roots. During mechanical impedance, root morphology is affected because of enhanced ethylene signaling [106]. Not surprisingly, the change in ethylene response is coupled with a change in root auxin response. Mechanical impedance also induces expression of the *ASA1* and *ASB1* genes, whose products catalyze the first committed step of the biosynthesis of tryptophan, an auxin precursor [106,107]. Analyses of the auxin-responsive reporters *DR5-GUS* and *IAA2-GUS* revealed the formation of an auxin gradient, with greater accumulation on the lower side of the mechanically impeded roots [106]. Taken together, these results suggest that localized enhancement of ethylene signaling in mechanically impeded roots stimulate auxin production in the root tip and promote its asymmetric redistribution, which is an absolute requirement for the root gravity response [92]. Consistent with this hypothesis, the *aux1* mutant is insensitive to the mechanical impedance response [106]. Further support for this idea comes from a recent report demonstrating that tomato root penetration in soil was completely blocked by ethylene signaling inhibitors, which also had altered auxin-dependent gene expression at the root apex [108].

Recent evidence suggests that ethylene-induced inhibition of the root gravity response might be tied to the synthesis of flavonoids, which act as endogenous negative regulators of auxin transport [63,85]. The *tt4* mutant makes no flavonoids and exhibits a delayed root gravity response and insensitivity to ACC-mediated inhibition of root gravitropism [63,85]. Flavonoids are endogenous auxin transport inhibitors [109], and the *tt4* mutant has elevated auxin transport [63,97,110,111]. The active flavonoid in the regulation of shootward auxin transport and root gravitropism is quercetin, given that plants with a *tt7* mutation that are blocked in the step preceding quercetin synthesis exhibit phenotypes identical to *tt4*, which lacks flavonoids [63]. In addition, both ACC and IAA strongly induce flavonoid biosynthesis through changes in transcription of the key genes in the flavonoid biosynthetic pathway, implying that ACC modulates the shootward transport of auxin through a flavonoid-regulated pathway, and alters the capability of the roots to create a proper auxin gradient after they perceive gravity stimulation [63,85]. The effect of elevated flavonoid synthesis after ethylene treatment on auxin transport at the root tip is shown in Figure 4, in which flavonoid synthesis in the root elongation zone prevents auxin efflux from the root tip.

Like roots, the initial gravitropic curvature of shoots had been shown to be inhibited by elevated ethylene [87,88,112]. In addition, the phenotypes of ethylene signaling mutants support the importance of ethylene in this process. For instance, hypocotyls of the ethylene-insensitive mutant *ein2-1* show a severely reduced response to gravity [88], which is in contrast to roots of this mutant, which have normal gravitropic responses. [47,85]. Similarly, the ethylene-insensitive tomato mutant *Nr* exhibits delays in shoot gravitropic curvature at an early time point, whereas *epi*, an ethylene-overproducing mutant, shows a reduced gravitropic response [87]. Ethylene affects the gravity response only at early time points after gravity stimulation in wild type, whereas the shoot gravity response of the *epi* mutant exhibits a significant delay at all time points compared with wild type [87]. Interestingly, the ethylene receptor mutant *etr1* shows a wild-type gravity response in both root and shoot [85,88]. The observation that *etr1-3*, unlike *ein2*, *Nr* and *epi*, exhibits a normal gravity response is likely to be linked to the presence of a low level of residual ethylene signaling in *etr1-3*. Another mutant that has been isolated for reduction in ethylene-dependent gravitropism (*egy1*) [113] has a defect in a gene encoding a plastid-targeted metalloprotease that may affect the formation of endodermal plastids, which are required for an ethylene-dependent gravity response in light-grown hypocotyls [114]. Findings from recent studies support a model for auxin–ethylene crosstalk in regulating the root gravitropic response (Figure 4), but the role of ethylene in regulating the shoot gravitropic response is more complex.

Root hair initiation and elongation

Another developmental process where auxin and ethylene may act synergistically is the initiation and elongation of root hairs. Root hairs are essential for the uptake of water and nutrients, facilitate interactions with soil microorganisms, and help anchor roots [115]. Numerous pharmacological and genetic studies have revealed that auxin and ethylene promote the processes of root hair initiation [116,117]. In addition, auxin-insensitive mutants that also show ethylene insensitivity, such as *axr2* [102], *axr3* [59] and *aux1* [23], exhibit reduced root hair initiation. Similarly, application of ACC or IAA to the root hair-deficient mutant *root hair defective 6* (*rhd6*) restores root hair initiation [118]. Although auxin and ethylene enhance root hair initiation, the intrinsic nature of the crosstalk between these two hormones makes it difficult to dissect their independent roles in regulating this process. A combinatorial approach using genetics and pharmacology indicates that loss of ethylene signaling in the *ein2-1* mutant does not affect the ability of the roots to initiate root hairs [117]. However, when the intracellular auxin level was reduced by the application of auxin influx inhibitors [47,119], *ein2-1* showed a twofold greater inhibition of root hair initiation than untreated seedlings, and root hair initiation in wild-type was reduced to the levels observed in untreated *aux1*

mutants. Similarly, the reduction in root hair initiation in the *aux1 ein2* double mutant is substantially greater than that in either single mutant [117]. Taken together, these results lead to the model shown in Figure 5, in which root hair initiation is directly linked to the amount of auxin and auxin signaling, but indicate that the effect of ethylene is less direct and likely to occur through intracellular auxin levels.

After root hairs are initiated, these structures grow by elongation of tip cells in a process that is also regulated by auxin and ethylene. Constitutive activation of ethylene signaling or treatment with exogenous ethylene or ACC promotes root hair elongation [81,120,121]. By contrast, the roots hairs of ethylene-insensitive mutants [117,118,121] and of seedlings treated with inhibitors of ethylene synthesis or signaling are significantly shorter [120,122]. Root hair elongation is enhanced by auxin treatment [117] and in mutants that have elevated auxin synthesis, including *sur1* and a gain-of-function *yuc* allele [57,123]. Like ethylene-signaling mutants, auxin-signaling mutants including the AUX/IAA mutants *axr2* (IAA7 [102]), *axr3* (IAA17 [59]), *slr1* (IAA14 [61]) and *iaa28* (IAA 28 [124] develop fewer and shorter root hairs. Given that all these mutants show strong resistance to root growth inhibition by exogenous ethylene, it is difficult to determine whether their observed root hair phenotypes are linked to auxin and/or ethylene responses. Collectively, these results indicate that ethylene and auxin responses are both required for maximal root hair elongation and that these two hormones act in concert on this process (Figure 5). This is further substantiated by the finding that, compared with *aux1*, 10-fold more auxin is required to restore the root hair length to the wild-type level in *ein2-1*, and the *aux1 ein2* double mutant exhibits an extreme short root hair phenotype compared with the single mutants [117].

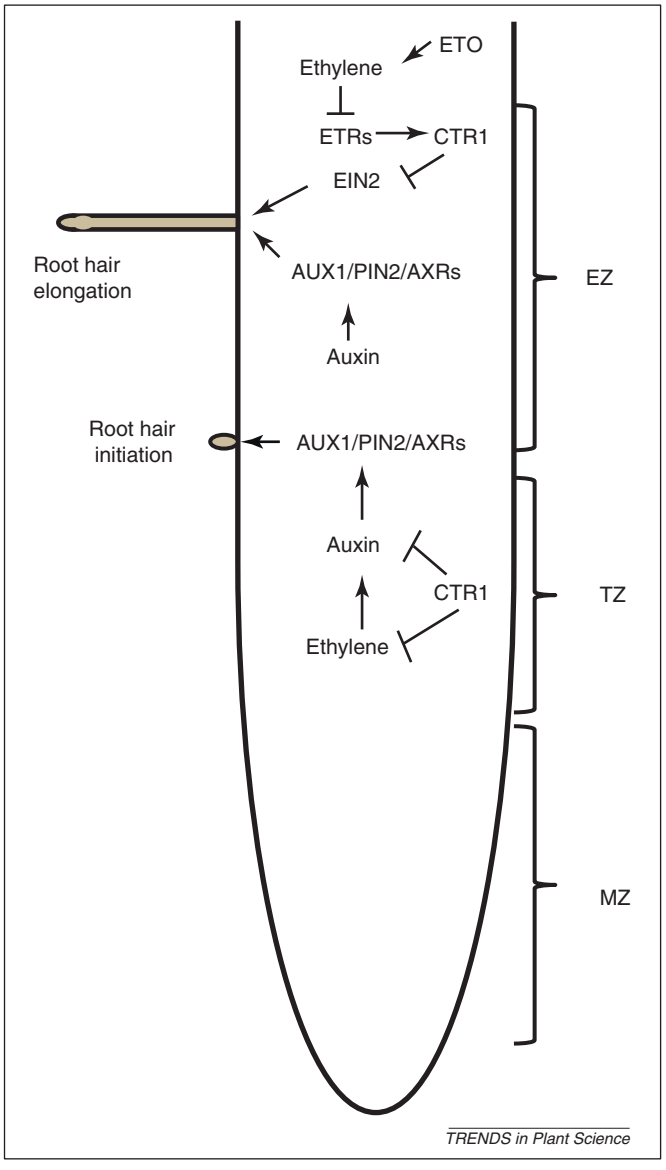

Figure 5. Auxin and ethylene synergistically regulate root hair initiation and elongation. Both auxin and ethylene stimulate root hair elongation through their canonical signaling pathways with a synergistic effect. Although both hormones stimulate root hair initiation, genetic and pharmacologic experiments suggest that the action of ethylene on this process is through enhancing auxin synthesis and signaling. The zones of the root are the meristematic zone (MZ), transition zone (TZ) and elongation zone (EZ), where root hairs begin to initiate.

Studies suggest that an intracellular threshold of auxin, controlled by auxin influx and efflux processes, is required for optimal elongation of root hairs. The auxin influx mutant *aux1* develops shorter root hairs [117,121,125]. The restoration of the root hair length of *aux1* to wild-type level with a minute amount of NAA that does not affect the intracellular ethylene response further confirms that, besides ethylene, auxin plays a major role in regulating the root hair elongation process [117]. The auxin efflux mutant *eir1/pin2* also shows a short root hair phenotype, which has been attributed to a reduced auxin supply from the root tip to the hair differentiation zone [126]. The requirement of auxin transport for the regulation of root hair elongation has been confirmed in several studies [127–129], including a report that the long root hair phenotype of the ethylene overproduction mutant *eto1* can be reversed by specifically blocking auxin influx in an *aux1 eto1* double mutant [81].

The cellular positioning of root hairs has been shown to be regulated by both ethylene and auxin. Compared with wild type, the *etr1*, *ein2* and *aux1* mutants show an apical shift in root hair positioning that is accentuated in the *aux1 ein2* double mutant [130–132]. Application of ACC or ethylene partially reverses root hair positioning in the *rhd6* mutant, where root hairs form more apically [118]. Planar polarity relies on the formation of an auxin gradient generated by local auxin biosynthesis and redistribution by auxin influx and efflux carriers in the root tip [68]. Interestingly, local auxin biosynthesis has been shown to be regulated by the ethylene signaling protein CTR1, which acts as a concentration-dependent repressor of auxin biosynthesis [68].

Taken together, these results suggest that auxin and ethylene act both synergistically and independently in controlling the initiation, elongation, and cellular positioning of root hairs. Although both auxin and ethylene positively regulate root hair formation, auxin is required for maximal root hair initiation and can overcome the reduced formation in the absence of ethylene. However, in root hair elongation and positioning, auxin and ethylene act synergistically, most likely through modulating the cellular auxin concentration, with the two hormones having equivalent positive regulatory roles.

Auxin–ethylene crosstalk and hypocotyl growth

Although most studies on auxin–ethylene crosstalk have focused on roots, the role of this crosstalk has also been examined in the maintenance of the apical hook and control of hypocotyl elongation. Generally, ethylene inhibits hypocotyl growth as part of the 'triple response', whereas auxin is best known for its ability to enhance growth [82,133]. The effects of these hormones on shoot growth also depend on environmental conditions and species. In the dark, ethylene inhibits the growth of *Arabidopsis* hypocotyls [1,2], whereas in the light it stimulates growth [134]. At high concentrations, auxin is more effective in stimulating growth in light-grown pea seedlings than in dark-grown plants [135–137]. Auxin can also stimulate or inhibit *Arabidopsis* hypocotyl growth depending on assay conditions [134,138]. Application of 1-naphthylphthalamic acid (NPA) inhibits the hypocotyl growth of light-grown *Arabidopsis* seedlings [139], but has a minimal effect on that of dark-grown seedlings [139–141], suggesting that auxin transport is important for hypocotyl elongation in the light but not in the dark. Consistent with this, auxin transport in the hypocotyls of *Arabidopsis* [142] and tomato [143] is lower in dark-grown seedlings than in light-grown seedlings. Ethylene inhibits auxin transport in excised pea (*Pisum sativum*) stems [82,133], and high concentrations of IAA increase ethylene production that, in turn, diminishes growth. Thus, each hormone can influence the other in apical tissues. For example, ethylene-stimulated hypocotyl nutational bending of dark-grown *Arabidopsis* seedlings is blocked by NPA, suggesting that altered auxin transport can have subtle growth effects on dark-grown hypocotyls [144].

Several genes that might be important in auxin–ethylene crosstalk in hypocotyls have been identified. One is the *Arabidopsis POLARIS* (*PLS*) gene, which is rapidly downregulated by exogenous ethylene [145] and upregulated by the application of auxin [146]. Dark-grown *pls* seedlings show reduced auxin responsiveness in roots and a constitutive hypocotyl triple response, whereas *PLS* overexpressing plants are taller and have a diminished hook angle [145,146]. The shorter stature of *pls* mutants cannot be because of increased levels of ethylene biosynthesis, because mutants have unaltered ethylene levels [145]. However, this trait might be caused by defective auxin transport and accumulation. Auxin levels and transport are reduced in the roots of *pls* mutants [146]. Thus, PLS influences responses to both auxin and ethylene. There is evidence that PLS can act at several levels in the ethylene signaling pathway [145]; however, it is unclear exactly how PLS exerts these effects to influence both auxin and ethylene responses.

RCN1 protein may also mediate auxin–ethylene crosstalk. *RCN1* encodes a protein phosphatase 2A regulatory subunit A that was found in a screen for mutants with altered responses to NPA [140]. In addition to a root-curling phenotype, *rcn1* mutants are shorter than wild-type seedlings and have diminished apical hook curvature in air. The *rcn1* mutant has increased rootward transport of IAA through the hypocotyl and enhanced ethylene biosynthesis [88,147,148]. The alterations in auxin transport may be due to alterations in the localization of specific PIN proteins, because PP2A phosphatase is involved in PIN localization within root cells [149], although this relationship has not been reported for hypocotyls. The overproduction of ethylene in *rcn1* mutants requires ACS2 and ACS6, and the turnover of ACS6 protein is reduced when protein phosphatase 2A activity is reduced [148]. When treated with ethylene, *rcn1* mutants exhibit a phenotype of shorter hypocotyls and less severe hooks [140,147]. ACC has little effect on IAA transport in wild-type or *rcn1* hypocotyls, indicating that the altered responses to ethylene are independent of large changes in auxin transport [88]. Together, these observations suggest that reduced growth of *rcn1* mutants in air is due to increased ethylene biosynthesis. However, other traits such as reduced apical hook curvature may result from altered auxin transport, given that increased ethylene production in the mutant is predicted to enhance hook curvature.

Auxin–ethylene crosstalk has also been studied in the context of differential growth leading to hypocotyl curvature. Nutational bending of shoots has been linked to alterations in auxin transport and accumulation in the zone of bending [150]. Ethylene also stimulates nutations in the hypocotyls of etiolated *Arabidopsis* seedlings [144]. These nutations are eliminated by application of NPA, suggesting that ethylene-stimulated nutations require normal auxin transport. Interestingly, the ETR1 ethylene receptor is both necessary and sufficient for nutations but is not necessary for apical hook formation [144,151]. Thus, ETR1, unlike the other receptor isoforms, may be subtly influencing auxin transport or accumulation. Molecular links between ETR1 and auxin transporters or synthesis have yet to be identified in this tissue, although *PIN3*, *PIN7*, and *AUX1* transcripts show reduced abundance in ACC-treated *etr1* roots relative to wild-type roots [32].

Auxin–ethylene crosstalk and the apical hook

Most research examining auxin–ethylene crosstalk in the hypocotyl has focused on apical hook development. There are three phases to hook development – formation, maintenance and opening – with hook formation and maintenance involving asymmetrical growth caused by both altered cell division and elongation [152]. Evidence that auxin is involved in hook development comes from the observation that blocking auxin transport with NPA reduces the apical hook, and mutants with altered transport have a reduced apical hook [88,140,141,153,154]. Similarly, wild-type seedlings in the presence of high levels of exogenous auxin or mutants that over-accumulate auxin lack an apical hook [57,123,141,155], and auxin-resistant or auxin transport impaired mutants have a reduced hook or no hook [19,141,156]. Application of ethylene to wild-type plants or to the *eto1* and *ctr1* mutants leads to exaggerated apical hooks [2,4]. By contrast, ethylene-insensitive mutants fail to form an exaggerated apical hook in the presence of applied ethylene [24]. Time-lapse imaging of apical hooks shows that ethylene prolongs the formation phase in apical hook development, leading to an increased hook angle [157,158] during a limited developmental window [152].

Various studies indicate that ethylene affects auxin transport, synthesis, and, perhaps, signaling to regulate the differential growth leading to the apical hook. Figure 6

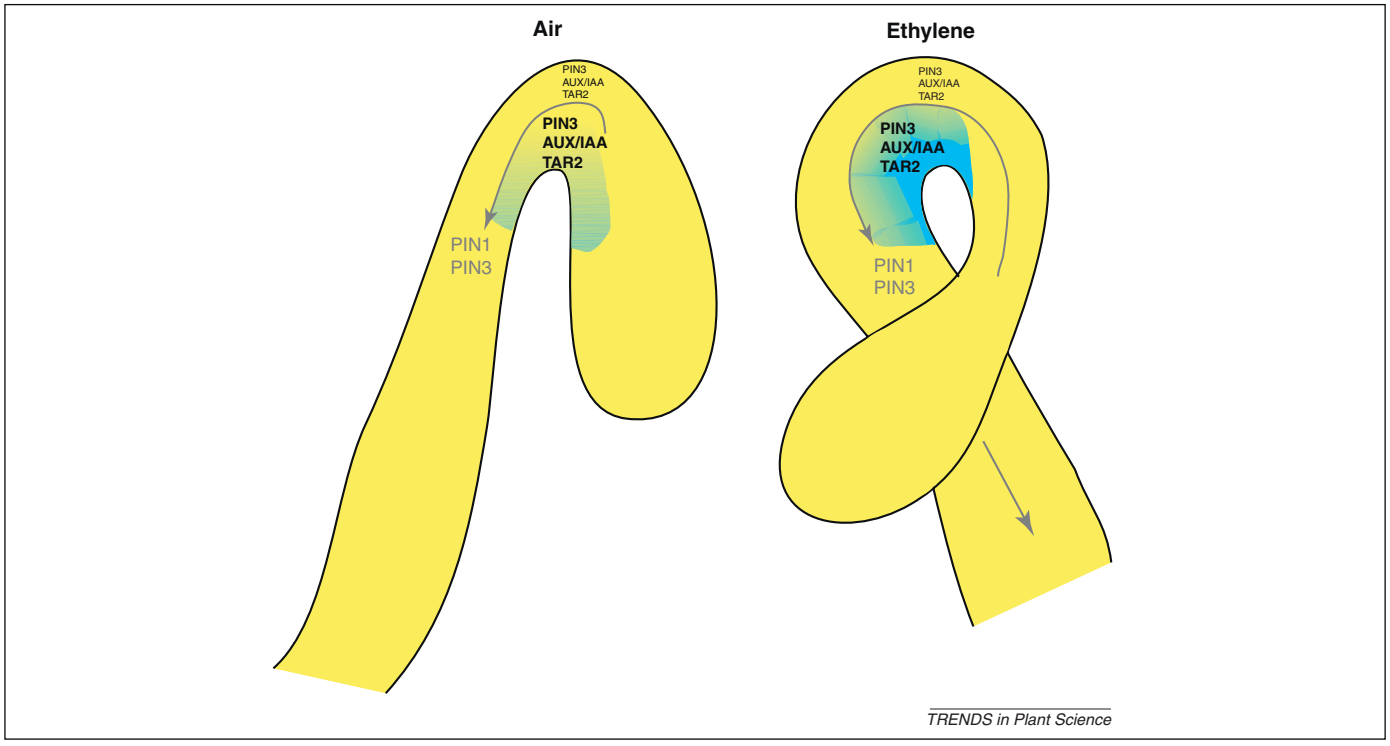

Figure 6. Model of the control of apical hook curvature by auxin and ethylene. Auxin is transported in at rootward direction from the cotyledons predominantly through the action of PIN1 and PIN3. The apical hook is formed because of the asymmetric distribution of auxin (shown in blue) that arises through differential auxin synthesis, auxin transport and auxin signaling. This is reflected by an asymmetrical distribution of proteins regulating these processes in the region of the apical hook. When ethylene is added (right-hand image), auxin levels rise on the concave side of the hook to cause an exaggerated curvature due to increases in PIN3, AUX1, IAA3, IAA12, IAA13 and TAR2 on the concave side of the hook. Based on information from [70,157,158].

illustrates a model of auxin–ethylene crosstalk in apical hook formation. Auxin transport is important for the effects of ethylene given that NPA blocks hook formation in *eto1* and *ctr1* mutants [141], whereas application of auxin restores hook formation in ethylene-insensitive mutants [157]. Further evidence for ethylene acting on auxin pathways comes from reporter gene and mutational experiments. *DR5:GUS* accumulates on the concave side of the apical hook and ethylene treatment results in greater accumulation [158,159]. When the apical hook opens, this expression of *DR5:GUS* becomes more diffuse [158]. Furthermore, application of ethylene enhances *PTAR2:GUS* levels on the concave side of the hook, and the apical hook is eliminated in *wei8 tar2* double mutants [70,157]. By contrast, ethylene reporter gene analysis shows that ethylene signaling is homogeneous across the hook in air [157], although application of exogenous ethylene leads to an asymmetrical distribution of transcripts of the ethylene biosynthesis gene *ACO2* (*ACC OXIDASE 2*) in the apical hook [152]. Thus, one likely factor underlying the development of the apical hook is the presence of higher levels of auxin on its concave side, with ethylene enhancing the curvature by altering auxin synthesis and transport to increase these levels (Figure 6).

Several auxin transporters have been found to play a role in normal hook development. Subtle effects on the apical hook have been found in *pin3*, *pin4*, *pin7*, *aux1* and *lax3* mutants [157,158]. Many of these transporters have overlapping roles in the apical hook, given that *pin4 pin7* and *aux1 lax3* double mutants have more severe apical hook phenotypes than the single mutants [157,158]. ACC increases *DR5:GUS*, *PIN3:GFP*, and *AUX1:GUS* expression on the concave side of the hook during the period of maximal curvature [157,158], suggesting that ethylene exerts at least some of its effects on hook curvature through both auxin influx and efflux carriers.

In addition to altering auxin levels across the apical hook, it is also likely that ethylene alters molecular components involved in auxin signaling. Expression of the promoter fusion of *IAA3-GUS*, *IAA12-GUS* and *IAA13-GUS* is asymmetrical, with higher levels of all three accumulating on the concave side of the hook as it forms [158]. The asymmetrical distribution of all three reporters is enhanced by the application of ACC (Figure 6), whereas NPA diminishes the asymmetrical distribution of *PIAA13-GUS* [158]. It has also been reported that *nonphototropic hypocotyl 4* (*nph4*) mutant alleles have a diminished auxin response and asymmetric growth resulting in a defect in apical hook formation [160,161]. However, these mutants show a normal triple response (including an exaggerated apical hook) when ethylene is added [160,161]. Thus, there appear to be complex interactions between ethylene and auxin that affect hook curvature.

The *HOOKLESS1* (*HLS1*) gene was identified in a screen in which apical hook formation was defective in the presence of exogenous ethylene [2] and has been the focus of research on auxin–ethylene crosstalk in the apical hook. Overexpression of *HLS1* causes an exaggerated apical hook [141]. Epistasis analysis placed *HLS1* downstream of *CTR1* [24,141]. *HLS1* mRNA levels increase when seedlings are treated with ethylene and are decreased in *ein2* mutants [141]. In the *hls1* mutant, both *SAUR-Ac1* transcripts and the *DR5:GUS* signal are reduced in the region where an apical hook should form [159],

suggesting that disrupted auxin distribution leads to failure to form an apical hook [141]. A *hls1* suppressor screen identified *ARF2* as a downstream component in this regulation in which *hls1 arf2* double mutants showed partially restored hook curvature, responsiveness to ethylene, and *DR5:GUS* expression [159]. Application of ethylene causes a decrease of ARF2 protein in wild-type plants, whereas *hls1* mutants over-accumulate ARF2 [159]. Thus, HLS1 appears to negatively regulate levels of ARF2 and may thereby lead to changes in auxin-induced gene expression.

Together, these studies suggest a model in which the asymmetrical distribution of auxin levels and signaling leads to hook formation. In this model, ethylene causes enhanced apical hook formation by both increasing the levels of components important for auxin signaling and increasing auxin levels on the concave side of the apical hook (Figure 6). The increased levels of auxin probably result from both increased synthesis and alterations in transport of auxin into or out of the hook. Although HLS1 is specifically involved in this ethylene response, the signaling pathway linking ethylene to altered auxin levels and distribution remains to be determined.

Concluding remarks

Ethylene influences many features of auxin-dependent seedling growth by altering auxin signaling, synthesis or transport, or in many cases all three. Ethylene mediates these effects on seedling growth by acting through the canonical ethylene signaling pathways. What is most fascinating about these interactions is that although ethylene and auxin synergistically affect many processes, such as root elongation and root hair formation, in other processes, such as lateral root formation and hypocotyl elongation, they act antagonistically. The interactions are even more complex for processes in which auxin is accumulated asymmetrically to drive differential growth, such as gravitropism or hook opening, where ethylene prevents this asymmetry by either lowering or raising auxin accumulation on both sides of these organs.

There are differences in our level of understanding regarding the modulation of auxin transport, synthesis, and signaling by ethylene. The evidence for ethylene altering auxin transport by altering the activity or synthesis of auxin transport proteins has now been clearly demonstrated, facilitated by the extensive understanding of the proteins that mediate auxin transport, and fluorescent reporter constructs that allow visualization of changes in the abundance and localization of these proteins. The interactions between ethylene and auxin synthesis are least clear because the complete pathways for auxin synthesis still elude identification. The area in which additional insights are likely to emerge in the near future is in identification of the signaling networks that regulate these synergistic and antagonist activities of ethylene and auxin in controlling tissue-specific growth and developmental responses.

A major research priority in improving our understanding of the molecular mechanisms of auxin–ethylene crosstalk in regulating growth and development is the identification of transcriptional networks that regulate the synthesis of proteins that act as developmental modulators. The growing numbers of high quality and publically available genome wide transcript data sets inform the exploration of signaling induced gene expression changes. Such data sets are available for auxin and ethylene individually, but the limited number of data sets that explore the response to both hormones limits understanding of the transcriptional crosstalk between these two hormones. Furthermore, these data sets were generated using diverse growth media, light conditions and hormone concentrations, making comparison between data sets almost impossible. As more data sets use mutants altered in transcription factors or signaling proteins to identify the downstream networks of transcription factors, consider the expression patterns of individual cell and tissue types, and explore expression changes with high temporal resolution, a clearer picture of hormone signaling will emerge. Finally, the inclusion of growth and developmental data, and localization of important protein products to parallel the transcriptional responses will inform the interpretation of the biological significance of these data sets. The increasing availability and decreasing costs of next generation sequencing approaches and developing collaborations between biologists and computer scientists are likely to lead to insights into the molecular basis of crosstalk between auxin and ethylene in both model and crop plants.

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