Ethylene gas: perception, signaling and response
Roberto Solano and Joseph R Ecker

During the last decade a genetic approach based on the Arabidopsis ‘triple response’ to the hormone ethylene has allowed the identification of numerous components of the signal transduction pathway. Cloning of the genes and biochemical analysis of the proteins that they encode are uncovering the molecular mechanisms that allow a plant cell to perceive and respond to this gaseous regulator of plant growth/stress responses.

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Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AIN</td>
<td>ACC(1-aminocyclopropane-1-carboxilic acid) insensitive</td>
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<td>CTR</td>
<td>constitutive triple response</td>
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<td>EIL</td>
<td>Ein3-like</td>
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<td>EIN</td>
<td>ethylene insensitive</td>
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<tr>
<td>EREBP</td>
<td>ethylene response element binding protein</td>
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<td>ERS</td>
<td>ethylene response sensor</td>
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<td>ETI</td>
<td>ethylene insensitive</td>
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<td>ETO</td>
<td>ethylene overproducer</td>
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<td>ETR</td>
<td>ethylene resistant/ethylene receptor</td>
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<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<td>MEK</td>
<td>MAPK kinase</td>
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Introduction

Ethylene is a phytohormone affecting virtually all stages of plant development. During the course of a plant’s life cycle, ethylene affects seed germination, cell elongation, cell fate, sex determination, fruit ripening, senescence and abscission. Ethylene is also a key regulator in the response to biotic and abiotic stresses [1–4,5•–8•]. The regulation of cell fate, sex determination, fruit ripening, senescence and abscission. Ethylene is also a key regulator in the response to the hormone perception, signaling events and ethylene-mediated transcription.

In the current view, ethylene is perceived at the plasma membrane by a family of ethylene receptors that includes five members: ethylene resistant/ethylene receptor (ETR)1, ETR2, ethylene response sensor (ERS)1, ERS2 and ethylene insensitive (EIN)4 [14•,16,17,18•]. From the membrane, the signal is transduced to the nucleus through a series of proteins that include constitutive response (CTR)1, EIN2, EIN5, EIN6 and EIN7. Among these, only the cloning of ctr1 has been reported to date [19]. In the nucleus, EIN3 and most likely other members of the EIN3/EIN3-like (EIL) family of positive regulatory proteins, initiate the events that will lead to the expression of effector genes involved in the diversity of responses to the hormone.

In this review we will briefly summarize our current view of the ethylene pathway, focusing on the recent findings on the hormone perception, signaling events and ethylene-mediated transcription.

Ethylene perception: the family of receptors

The ethylene receptor family in Arabidopsis consists of five members: ETR1, ETR2, EIN4, ERS1 and ERS2 [14•,16,17,18•]. All of them have been implicated in ethylene signaling by dominant missense mutations that confer ethylene insensitivity to the plant. ctr1 and ein4 were the first members of the family isolated by the ‘triple response’ screen [15,20]. Recently, dominant mutations in the ETR2 and ERS1 genes have also been obtained from the same type of screen [14•]; JM Alonso, JR Ecker, Abstract 385, 9th International Conference on Arabidopsis Research, 24-28 June 1998, Madison WI). In addition, mutations that confer dominant ethylene insensitivity to ETR1 have been introduced into ERS1 and ERS2, and the resulting transgenic plants also show an ethylene insensitive phenotype [17,18•]. ctr1 is epistatic to all mutants in members of this family and thus, prior to their...

**ETR1** was the first member cloned, using a positional approach [16]. All other members of the family were isolated due to their sequence homology to **ETR1** (**ERS1** and **ETR2**; [14•,17]) or to **ETR2** (**EIN4** and **ERS2**; [18•]). Sequence analysis of the **ETR1** gene uncovered its similarity to two-component histidine-kinase regulators that are sensors and transducers of environmental signals in bacteria [21]. Since then, other two-component sensors have been found in eukaryotes, including Arabidopsis [22–28]. Two component receptors consist of a sensor protein with a histidine autokinase domain and a response regulator protein. Activation of the histidine-kinase in the sensor protein promotes autophosphorylation of the histidine residue and a subsequent transfer of the phosphoryl group to an aspartate residue in the receiver domain of the response regulator [21]. In **ETR1**, as well as in **ETR2** and **EIN4**, the sensor and the response regulator components are present in the same protein. **ERS1** and **ERS2**, however, lack the response regulator, suggesting that, as in the case of bacteria, this second component may also exist in plants as an independent protein. In fact, five different response regulators have been recently cloned in Arabidopsis by sequence homology to bacterial response regulators [29•]. Whereas in vivo and in vitro evidence has been provided that they can function in His-Asp phospho-transfer signaling in E. coli, interactions among any of these proteins and **ERS1**, **ERS2** or the other ethylene receptors have not been reported.

**ERS1** shares a high degree of sequence similarity with **ETR1** (72% in the amino-terminus and 64% in the histidine-kinase domain), including all canonical motifs of bacterial histidine protein kinases [17]. **EIN4**, **ETR2** and **ERS2**, however, comprise an independent, more divergent class of receptors, in which the hallmarks of bacterial histidine kinases are barely detectable [14•,18•]. Whether or not all of these proteins can function as histidine kinases is not clear, although this activity has been demonstrated in vitro for **ETR1** [30••]. As the introduction into plants of a mutant version of **etr1** containing two kind of mutations (mutations that confers dominant ethylene insensitivity, and mutations in an amino acid residue essential for histidine-kinase activity in prototypical ‘two-component’ regulators), still conferred dominant ethylene insensitivity to transgenic plants, the in vivo significance of the histidine kinase domain is unclear ([31], J Hua, E Meyerowitz, Abstract 405, 9th International Conference on Arabidopsis Research, 24–28 June 1998, Madison WI). Homo- or hetero-dimerization of these receptors, however, may provide, in trans, kinase activity to the ‘killed-kinase’ mutant. Further proof of the in vivo significance of the kinase activity may be obtained by the introduction of various mutant transgenes into the loss-of-function mutant backgrounds (single, double, triple and quadruple) recently obtained by Hua and Meyerowitz [32••].

As mentioned earlier, genetic evidence that these proteins act upstream of **CTR1** in the ethylene pathway and their similarity to two-component bacterial sensors suggested their roles as ethylene receptors. Supporting biochemical evidence has been provided by ethylene binding experiments. It has been found that **etr1** mutants bind significantly less ethylene than wild-type plants [20]. Furthermore, heterologous expression of **ETR1** and **ERS1** in yeast cells allows them to reversibly bind the hormone ([33]; AE Hall, JL Findell, E Schaller, AB Bleecker, abstract 402, 9th International Conference on Arabidopsis Research, 24–28 June 1998, Madison WI).

The amino terminus of **ETR1** contains three predicted transmembrane domains that may anchor the protein to
the plasma membrane [16]. In fact, ETR1 homodimers have been found to be associated with membranes in transgenic yeast and in Arabidopsis [34]. The unique nature of this region and the fact that all mutations in the dominant alleles of all receptor family members (except for ERS1; JM Alonso, JR Ecker, Abstract 385, 9th International Conference on Arabidopsis Research, 24–28 June 1998, Madison WI) map to this region, suggest a role in ethylene binding. Consistent with this hypothesis, the amino terminus is the most conserved region among the putative receptor proteins; although a fourth transmembrane segment can be predicted in EIN4, ETR2 and ERS2 [14,18]. Moreover, mutations in specific Cys residues that are predicted to lie within the transmembrane domain prevent ethylene binding [33]. It has been previously proposed that a transition metal is needed to coordinate the binding of ethylene to the receptors [33]. It has been recently shown that the expression of Ps-ACO1 (1-aminocyclopropane-1-carboxylate oxidase) in the apical hook of etiolated pea seedlings is higher in the inner than in the outer side of the hook [38]. Analysis of the asymmetric distribution of other components of the pathway will provide new clues to understand the effect of the hormone on differential growth responses in particular tissues.

Signal transduction

On the basis of epistasis analysis, CTR1 is the initial component of the signaling pathway to act downstream of the receptors. As stated above, CTR1 is considered a negative regulator of ethylene signaling since recessive loss-of-function mutations confer constitutive ethylene responses to the plant throughout development [19]. The deduced amino acid sequence of CTR1 shares significant similarity with the Raf family of protein kinases, and in fact, its kinase activity has been shown using Arabidopsis thaliana MAPK kinase (AtMEK) as a substrate ([19,39]; H Li, Y Huang, J Kieber, abstract 409, 9th International Conference on Arabidopsis Research, 24-28 June 1998, Madison WI). In animals, Raf kinases are Ser-Thr protein kinases that phosphorylate MEKs (MAPK kinases), which in turn phosphorylate MAPKs [40]. In yeast, the osmolarity response pathway represents a paradigm in which a MAPK cascade is linked to a two-component receptor, SLN1 [23]. Because of the similarity between CTR1 and Raf, and by analogy with the yeast osmolarity response pathway, a MAPK cascade has been proposed to participate in the repression of ethylene responses [19]. In Arabidopsis, nine MAPks and several MEks have been identified [41–44], although their roles in ethylene signaling have yet to be demonstrated.

The mechanism of activation of CTR1 by the ethylene receptors is still unclear. As in the case of Raf, however, it may require recruitment to the plasma membrane. In fact, interactions between the amino terminus of CTR1, which is predicted to be a regulatory domain on the basis of findings with animal Rafs, and the carboxyl termini of ETR1 and ERS1 have been demonstrated in vitro and using the yeast two-hybrid system [45]. In addition, five different 14-3-3 isoforms — ubiquitously expressed proteins that interact with signaling molecules and cell cycle regulators [46] — have been also found to interact with the amino-terminal portion of CTR1 in two-hybrid assays, and one of...
them also interacts with ETR1 (W Ding, C Chang, Abstract 392, 9th International Conference on Arabidopsis Research, 24–28 June 1998, Madison WI). This suggests that additional factors may be required for the formation of the CTR1-receptor(s) complex in vivo.

Downstream of the putative MAPK cascade, several genes necessary for ethylene signaling have been described and include EIN2, EIN5, EIN6 and EIN7 (reviewed in [11,13,39]). EIN2 and EIN5 have been already cloned, and EIN6 will be soon (G Roman; S Nourizadeh; R McGrath; personal communications). Molecular characterization of these components will likely provide us with a better understanding of the mechanisms that transduce the ethylene signal from the cytoplasm to the nucleus.

**Nuclear members of the signaling pathway: the EIN3/EIL family**

Mutations in EIN3 impair a plant’s response to the hormone through all stages of development. Cloning of the EIN3 gene revealed that it encodes a novel protein that shares amino acid sequence similarity, conserved structural features and genetic function with four EIN3-LIKE (EIL) proteins ([47••]; R Solano, JR Ecker, unpublished data). Transgenic analysis has shown that EIL1 and EIL2 are able to functionally complement the ein3 mutation, suggesting their participation in ethylene signaling. High level expression of EIN3 or EIL1 in transgenic wild-type or ein2 mutant plants conferred constitutive ethylene response phenotypes in all stages of development, indicating their sufficiency for activation of the pathway in the absence of ethylene. The members of this family do not share significant sequence homology with other proteins in databases. Yet sequence analysis indicates that they contain features also found in families of transcription factors, such as acidic and basic domains (including two with predicted α-helical structure) that may represent activation and DNA-binding domains, respectively [47••]. In fact, it has been demonstrated that EIN3 contains signals sufficient for nuclear localization in transient assays, regardless of plant species or cell type, indicating that this family of proteins may function in the nucleus [47••]. On the basis of these observations, it has been proposed that EIN3 and EILs proteins may represent a new class of transcriptional regulators involved in the activation (or repression) of ethylene-regulated genes [47••].

Likely targets of EIN3/EILs are the ethylene-response-element-binding-protein (EREBP) family of transcription factors. These proteins were originally identified in tobacco by their ability to recognize a conserved ethylene response element (GCC box), and they possess a
DNA-binding domain homologous to that found in the floral homeotic protein APETALA2 (i.e. the AP2 domain; [11,48,49]).

In Arabidopsis, over thirty EREBP s have been identified by several research groups and by the Arabidopsis genome sequencing project, and thus they constitute one of the largest families of DNA-binding proteins in plants [50,51*,52*], R Solano, JR Ecker, unpublished data). This suggests a putative functional redundancy among members of this family that might explain the lack of a loss-of-function mutant in any of these genes. Gain-of-function experiments will be required to demonstrate the role of this family in ethylene signaling.

Conclusions

During the last few years the identification and cloning of genes involved in ethylene signaling has considerably improved our understanding of the pathway. It is clear now that the ethylene signal is perceived and transduced through a largely linear pathway that begins at the membrane and proceeds to the nucleus. Acting through a putative CTR1-MEK-MAP cascade of protein kinases, a family of transmembrane receptors (ETR1, EIN4, ETR2, ERS1, ERS2) functions as negative regulators of ethylene signaling events. In the nucleus, a family of positive regulatory proteins (EIN3/EILs) serves to activate transcriptional responses to the hormone upon repression of receptor function by the binding of ethylene.

The identification of new mutants using novel genetic screens and the characterization of additional ethylene signaling genes such as ein2, ein5 and ein6 should clarify our view of the ethylene action pathway. Still, our knowledge is quite limited on the biochemical function of most of the components of the pathway and how they are regulated, providing a significant future challenge.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


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