Journal of Plant Biology, April 2007, 50(2) : 109-116 **REVIEW**

A Gaseous Plant Hormone Ethylene: The Signaling Pathway

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Phytohormone ethylene has profound effects on growth and development in plants. Molecular genetic studies using *Arabidopsis* have defined a linear pathway for ethylene signal transduction leading from initial hormone perception to changes in gene expression. Ethylene is perceived by a family of ethylene receptor complex at endoplasmic reticulum (ER), which negatively regulates the ethylene response. Ethylene binding inactivates the receptors and represses the Raf-like kinase CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) that actively represses ethylene response pathway in the absence of ethylene. Consequently, the ETHYLENE INSENSITIVE2 (EIN2), a membrane protein with similarities to Nramp metal ion transporter becomes activated and positively regulates the ethylene signaling pathway by transmitting the signal into the nucleus. Finally, the nuclear signal initiates the transcriptional cascade via the transcription factors ETHYLENE INSENSITIV3/ETHYLENE INSENSITIVE3-LIKE proteins (EIN3/EILs). This review will summarize the up-to-date understanding of ethylene signal transduction, in aiming to illustrate how challenges in hormone biology have been resolved through the power of molecular genetics and to provide references for interested readers searching for further information.

Keywords: Arabidopsis, ethylene, hormone, signaling

HISTORY OF ETHYLENE RESEARCH

To survive in a challenging environment, every organism needs the ability to sense and react in an appropriate manner against every changing condition. Being a sessile organism, plants extremely rely on properly integrating all those internal and external signals so that they can make their adequate responses. One of the main ways plants can disseminate a reaction is through the use of phytohormones. Indeed, one of the biggest challenges in plant biology is to understand the mechanisms of hormone action, that is, the perception, signal transduction and cascade which leads to the response.

Ethylene (C_2H_4) is a gaseous plant hormone. Ethylene triggers fruit ripening in climacteric fruits, influences senescence and abscission of plant organs (Abeles et al., 1992). Ethylene has also been implicated in developmental processes such as seed germination, cell elongation, root formation, sex determination, pollination and flowering, and regulates plant responses to biotic and abiotic stresses as well (Abeles et al., 1992).

In 1998, Nobel Prize in Physiology or Medicine was awarded for the discovery of nitric oxide as a signaling molecule in animals. The Nobel press released the announcement that "Nitric oxide is a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell which represents an entirely new principle for signaling in biological systems". In contrary to what the Nobel assembly declared, ethylene was identified as the first gaseous hormone by a Russian graduate student Dimitry Neljubow in 1901, more than a hundred years ago before the Nobel Prize was awarded (Kende, 1998). As a graduate student, Neljubow discovered that illumination gas (coal gas) was responsible for reduced stem elongation, increased lateral growth and abnormal horizontal growth in etiolated pea seedlings and identified ethylene as a biologically active gas which causes this response. Later in 1910, H. H. Cousins recognized that oranges stored near bananas ripened faster than the oranges stored elsewhere. Even though orange is presently known as a nonclimacteric fruit which does not produce as much ethylene as climacteric fruit, this was the first indication that ethylene is naturally produced from plant tissues. It was R. Gane who first isolated ethylene from plant (Gane, 1934). He analyzed volatiles emitted by 60 lbs of ripening apples, showing direct chemical proof that plant tissues endogenously synthesize ethylene, thus settling the stage to investigate the function of ethylene as a plant hormone. A breakthrough in the ethylene research came with the invention of gas chromatography (Burg and Stolwijk, 1959). The ability to analyze trace amount of ethylene opened the way to rediscover the significance of this growth regulator in various physiological responses. By 1980s, tremendous progresses were made in discovering the biosynthetic pathway. The whole pathway was biochemically dissected, genes encoding the biosynthetic enzymes have been cloned in numerous species, and their regulations are extensively studied (Yang and Hoffman, 1984; Kende, 1993; Lee et al., 1999; Park et al., 2001). However, little was known about the perception and signal transduction pathway of ethylene.

In the middle of 1980s, *Arabidopsis* was introduced as a model system in plant biology and the power of the new genetic system opened another chapter for ethylene research. The primary way in which the ethylene response mutants have been isolated is using the 'triple response' phenotype, which includes the three effects of ethylene on etiolated seedlings: shortened/thickened hypocotyl, inhibition of root elongation, and exaggerated apical hook (Guzman and Ecker, 1990). Numerous mutants which render insensitivity to ethylene or display constitutive ethylene response have been identified from genetic screenings uti-

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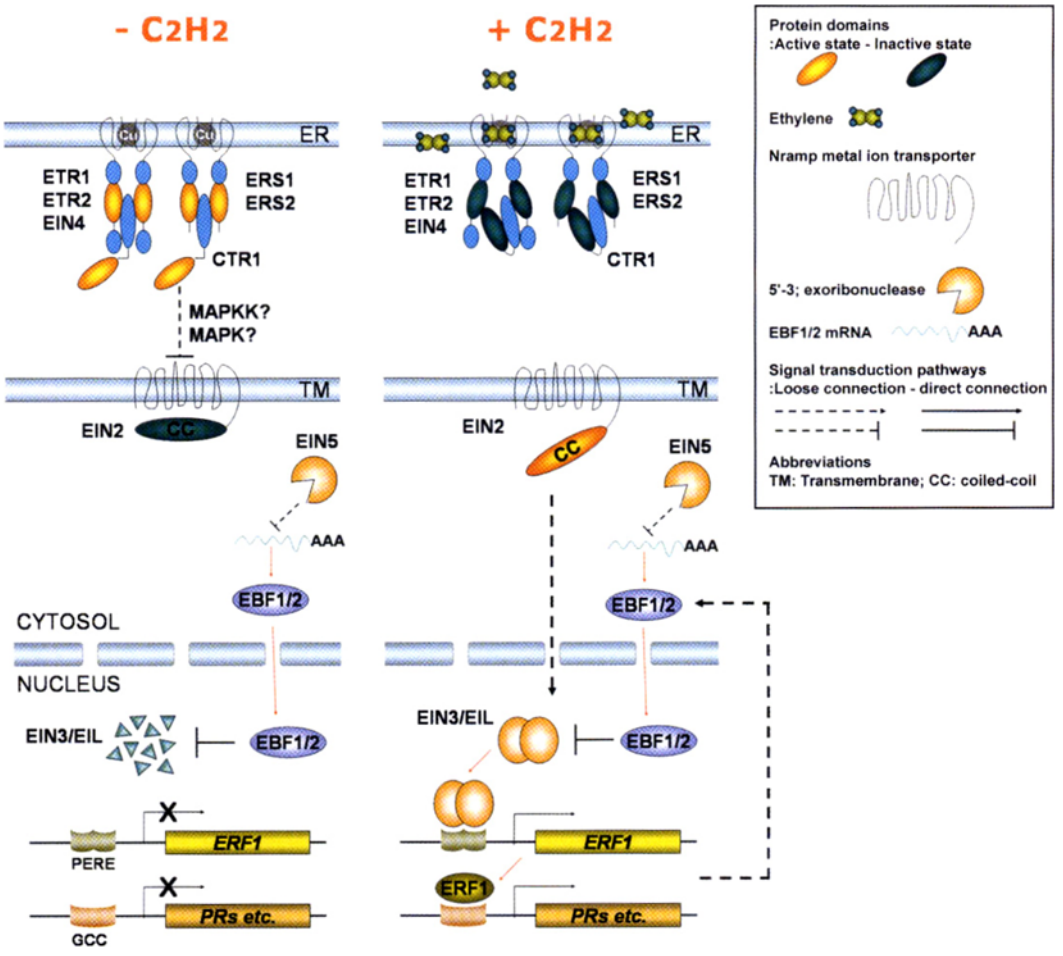

Figure 1. Current model of the ethylene signal transduction pathway. In the absence of ethylene, formation of the ER-localized signaling complex, mediated by direct interaction of the histidine kinase domain of the receptors and N-terminal domain of CTR1, actively suppresses the downstream signal through CTR1 Ser/Thr kinase activity. With the active signaling complex, the signaling activity of EIN2 is repressed and the constitutively expressed EIN3 transcription factor is targeted for degradation through a ubiquitin-mediated proteasome pathway via the recognition of two F-box proteins, EBF1 and EBF2. When exposed to ethylene, the ligand-receptor binding leads to a conformational change in the receptor-CTR1 complex, resulting in the inactivation of CTR1. Without the ER membrane-bound CTR1 activity, EIN2 is derepressed and transmits the signal into the nucleus, which prevents EIN3 from degradation. The accumulated EIN3 directly binds to the PEREs (Primary Ethylene Responsive Element) located on the promoters of ERF1 and other primary target genes. In cascade, ERF1 turns on the secondary targets through binding to the GCC box elements. Ethylene signal leads to EBF1/2 accumulation, providing negative feedback loop on EIN3 activity. EIN5 is likely antagonizing the negative feedback regulation on EIN3 by promoting EBF1/2 mRNA decay, which consequently sets back to a ground state that allows the accumulation of EIN3 protein in response to ethylene. The dotted lines represent regulatory steps in which a direct physical link between upstream and downstream components has yet to be demonstrated.

lizing triple responses (Bleecker et al., 1988; Guzman and Ecker, 1990; van der Straeten et al., 1993; Roman et al., 1995). Further genetic and molecular analyses of these mutants and combined biochemical studies on isolated molecules have uncovered the current linear pathway for ethylene signal transduction that is defined from the initial hormone perception at the endoplasmic reticulum (ER) membrane to the changes in gene expressions via transcriptional cascades (Fig. 1).

ETHYLENE PERCEPTION: THE ETHYLENE RECEPTOR FAMILY

Ethylene is perceived by a family of five membrane-bound receptors, ETR1, ETR2, ERS1, ERS2 and EIN4, that share similarity with bacterial two-component histidine kinases (Chang et al., 1993; Hua et al., 1995, 1998; Sakai et al., 1998; Alonso et al., 2003). The most conserved portion among the ethylene receptor family members is the hydrophobic transmembrane domain at the amino-terminal end. This domain, when expressed in yeast, was found to be necessary and sufficient for high-affinity binding to ethylene (Schaller and Bleecker, 1995). In the case of ETR1, the functional unit for ethylene perception was found to be a dimer mediated in part by a cysteine residue at the amino-terminus that is capable of forming disulfide bonds (Schaller et al., 1995). In addition to the ethylene binding feature, the amino-terminal region, which includes the transmembrane region and the GAF-related domain with unknown function, is shown to play a role in targeting and retention of the receptor in ER membrane system (Chen et al., 2002). The

carboxy-terminus of the ethylene receptor family members seems more likely involved in signal output. It confers strong homology with histidine kinase domain, and in ETR1, ETR2 and EIN4, it is followed by a receiver domain of response regulator (Hua et al., 1998).

The five members of ethylene receptor can be divided into two subfamilies based on the sequence and structural similarities (Hua et al., 1998). The first subfamily includes ETR1 and ERS1, and the second subfamily includes ETR2, EIN4 and ERS2. All five proteins contain hydrophobic transmembrane domains in the amino-terminus, where the ethylene binding site has been localized, followed by the histidine kinase domain. However, in contrast to the members of subfamily I in which the essential residues for histidine kinase activity are completely conserved, the members of subfamily II lack several or all of the canonical functional motifs of histidine kinases, and possess an extended amino-terminus containing a predicted transmembrane domain (Hua et al., 1998).

While it was initially thought that the receptors were either redundant or had unique roles, the reality may be someplace in between. The receptors seem to have some amount of complete functional redundancy in that single knock-out mutant of any receptor was still able to respond to ethylene (Hua and Meyerowitz, 1998). The receptor genes are ubiquitously expressed with widely overlapping expression patterns (Hua et al., 1998) and the proteins are located in ER membranes (Chen et al., 2002; Gao et al., 2003). Likewise, when expressed in yeast, all receptor isoforms displayed high-affinity ethylene binding with similar binding activity per unit expressed protein (O'Malley et al., 2005). However, subfamily I seems to play a much larger role, as the *ETR1* and *ERS1* double null mutant had a more severe phenotype than the subfamily II triple null mutant (Zhao et al., 2002; Wang et al., 2003; Hall and Bleecker, 2003; Qu et al., 2007). Subfamily II receptors expressed under the control of a subfamily I promoter were unable to alleviate this phenotype, supporting the unique role of the subfamily I members (Wang et al., 2003).

The originally identified ethylene receptor mutants for *ETR1*, *ETR2* and *EIN4* were caused by an amino acid substitution within the ethylene binding amino-terminal domain which prevents ethylene binding (Chang et al., 1993; Schaller and Bleecker, 1995; Hua et al., 1998; Sakai et al., 1998). Because the found mutants were all genetically dominant alleles, these observations, raised the question weather the dominant ethylene insensitivity arose from constitutively active mutant ETR1 proteins (a gain-of-function) or from dominant negative effect. The answer came from the isolation and genetic studies of loss-of-function alleles of the receptors (Hua and Meyerowitz, 1998). Each loss-of-function mutants, isolated by intragenic reversion of dominant mutations or identification of a T-DNA insertion, was still able to respond to ethylene implying the functional redundancy among the receptors. Moreover, homozygous triple and quadruple loss-of-function mutants displayed constitutive ethylene phenotype rather than showing ethylene insensitivity, suggesting that the receptors are negative regulators. These results also tell us that the receptors are active in the absence of hormone to repress ethylene signaling whereas binding of ethylene inactivates the receptors, release the repression of the pathway, consequently leading to the ethylene response (Hua and Meyerowitz, 1998). Consistent with the hypothesis, based on these results, the triple and quadruple mutants, which have less number of ethylene receptors, could react with higher sensitivity to ethylene because less ethylene is needed to inactivate the receptors (Hua and Meyerowitz, 1998).

As first proposed by Burg and Burg (1967), high affinity binding of ethylene to the receptor requires a transition metal, the copper ion. Rodrigues et al. (1999) demonstrated the interaction of copper ion with yeast expressed ETR1 protein and furthermore, showed that this interaction is mediated by Cys65 that is located at the conserved hydrophobic amino-terminal transmembrane domain. The role of copper in ethylene perception was further confirmed in plant by the cloning of *RAN1* (*RESPONSE TO ANTAGONIST1*) gene (Hirayama et al., 1999). *ran1-1* and *ran1-2* was isolated by a screen for mutants showing ethylene response to an ethylene antagonist trans-cyclooctene. The RAN1 product has high similarity to copper transporting P-type ATPases, and was shown to rescue a copper transport defect in yeast (Hirayama et al., 1999). RAN1-1 and RAN1-2 proteins have residual copper transport function in yeast, suggesting that reduced copper levels can lead to alter the ligand specificity of ethylene binding domain, which is therefore no longer antagonized by cyclooctene. Co-suppression of *RAN1* and loss-of-function mutant of *RAN1* resulted in a constitutive ethylene response phenotype without ethylene (Hirayama et al., 1999; Woeste and Kieber, 2000), indicating that copper is not only required for proper conformation of ethylene binding domain but also for signaling output of the ethylene receptors.

Based on the structural similarity of the ethylene receptors to bacterial two-component signaling systems, it has been suggested that the ethylene receptors could function similar to these bacterial sensor proteins by modulating the histidine kinase activity after ethylene binding. Indeed, ETR1 autophosphorylates *in vitro* on its conserved histidine residue (Gamble et al., 1998). However, the involvement of histidine kinase activity with a phosphorelay event is still questionable. Mutations that eliminate histidine kinase activity *in vitro* as well as removal of the entire histidine kinase domain do not appear to disrupt *in vivo* functions of the ethylene receptors (Gamble et al., 2002). An independent line of evidence ruling out the requirement of a histidine kinase activity for the ETR1 signaling comes from the study using *etr1ers1* double loss-of-function mutant, in which the ethylene receptors with conserved active histidine kinase domain are depleted (Wang et al., 2003). Transformation of *etr1ers1* mutant with an inactive kinase domain containing ETR1 genomic clone restored a number of ethylene responsiveness, which again supports the idea that canonical histidine kinase activity is not absolutely required for ethylene receptor signaling.

Recently, REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1), an evolutionarily conserved membrane protein with unknown biochemical activity was identified by a genetic screen for suppressors of the weak ethylene-insensitive mutant *etr1-2* (Resnick et al., 2006). Genetic experiment suggest that RTE1

act as a negative regulator in ethylene response and lies at or upstream of ETR1. Despite the involvement of modulating ETR1's activity, the connection between RTE1 and ETR, and the role in ethylene signaling needs to be further clarified.

EARLY SIGNALING MODULE: THE RAF-LIKE KINASE CTR1

A screen for *Arabidopsis* plants showing a constitutive response to ethylene yielded several mutants, all of which were overproducers of ethylene except for the *ctr1-1* (Kieber et al., 1993). Genetic analysis located CTR1 downstream of the ethylene receptors (Kieber et al., 1993). Because of the recessive nature of *ctr1-1* mutant, and the constitutive ethylene response phenotype, CTR1 was suggested to be a negative regulator for downstream signaling flow. When cloned, *CTR1* gene encoded a kinase with an amino-terminal domain of unknown function, and a carboxy-terminal kinase domain that possess high homology to the Raf-family of Ser/Thr kinases (MAPKKK) (Kieber et al., 1993).

At the time ethylene receptors were cloned, it was quite puzzling having a eukaryotic signaling system (a MAP kinase module) and a prokaryotic signaling system (two-component systems) in the same pathway. Several examples of pathways came out later in yeast where a histidine kinase mediated phospho-relay initiates the MAP kinase pathway through the interaction of a response regulator (Posas et al., 1996; Shieh et al., 1997; Buck et al., 2001), which led to speculate that the ethylene signaling pathway may be very similar to those pathways. However, no intermediate components have been identified yet, which connect the ethylene receptors and the CTR1 kinase. Instead, the physical interaction between ETR1, ERS1 and CTR1 was demonstrated using yeast-two hybrid assay and pull down experiment (Clark et al., 1998), suggesting that the ethylene receptor may modulate the activity of CTR1 directly. Fractionation study and co-purification of ETR1 with affinity purified CTR1 from the ER membrane fraction demonstrated that CTR1 localize to the ER membrane and also supported the *in vivo* association between ETR1 and CTR1 (Gao et al., 2003). The ER localization of CTR1 was dependent on ethylene receptor since double and triple loss-of-function receptor mutants resulted in reduced levels of ER-associated CTR1. But ethylene binding to the receptors did not affect the interaction between the receptor and CTR1 nor their subcellular localization. CTR1 interacts to the carboxy-terminal half of ETR1 through the amino-terminal domain. However, substantial loss of the ER-assoicated CTR1 in a triple mutant of subfamily II (*etr2/ers2/ein4*) which lack the canonical histidine kinase residues suggests that the histidine kinase activity is not required for the interaction (Gao et al., 2003).

So how does CTR1 repress the downstream ethylene signaling? Work with purified recombinant CTR1 protein has shown that CTR1 has intrinsic Ser/Thr protein kinase activity while CTR1-1 protein, in which the conserved residue within the kinase catalytic domain is altered, possesses no *in vitro* kinase activity (Huang et al., 2003). Together with the loss-of-function phenotype of *ctr1-1* mutant that carries mutation in the kinase domain (Kieber et al., 1993), these results imply that the importance of kinase activity in suppressing the ethylene responses. Besides the kinase activity, localization of CTR1 to the ER seems also required for the CTR1 function. A missense mutation in the amino-terminal of CTR1 (*ctr1-8*) completely disrupted the interaction with ETR1, which resulted in redistribution of CTR1 proteins from ER membrane to cytosol (Huang et al., 2003). Even with the intact kinase activity, *ctr1-8* mutant showed severe constitutive ethylene phenotype which suggests that the association with receptor is also required for CTR1 function. Taken together, current understanding tells us that the function of CTR1 depends on both its kinase activity and the association with ethylene receptors in the ER membrane. The CTR1 signaling complex is inactivated, possibly by a conformational change transmitted through the receptors and releases the downstream ethylene signaling (Huang et al., 2003).

While MAP kinase pathway is presumed to be downstream of CTR1, none of the MAP kinase components were identified in exhaustive mutant screens for ethylene response mutants. A full ten years after the cloning of *CTR1*, two MAP kinases, SIMK and MMK3, and one MAPKK, SIMKK were found to be activated in Medicago in response to ACC (Ouaked et al., 2003). SIMKK, when overexpressed, gave a constitutive activation of MPK6, an *Arabidopsis* homolog of SIMK, and also shows an apparent constitutive ethylene response phenotype in the absence of ethylene. This is somewhat surprising, and quite controversial, as CTR1 is a negative regulator of ethylene responses, and is inactivated by ethylene (Kieber et al., 1993), whereas SIMKK acts as a positive regulator and is activated by ethylene. In addition, a loss-of-function mutant of MPK6 does not show any ethylene related phenotype (Ecker, 2004; Menke et al., 2004). Moreover, MPK6 is demonstrated to play a role in stress induced ethylene production, in a separate study (Liu and Zhang, 2004). Therefore, the relationship between CTR1 and SIMKK needs to be re-examined.

Several observations indicate that the ethylene signal transduction pathway is not completely dependent on the activity of CTR1. Even completely null, *ctr1* loss-of-function mutants are still capable of displaying some ethylene responsiveness (Larsen and Chang, 2001). Moreover, loss-of-function mutation in four ethylene receptors shows more severe phenotype than the *ctr1* loss-of-function mutant (Hua and Meyerowitz, 1998) suggesting the possible partial redundancy with a CTR1-like protein or alternatively, the presence of an additional branch of ethylene signaling.

EIN2: AN NRAMP-LIKE MEMBRANE PROTEIN

Genetic analysis of ethylene signaling mutants has shown that EIN2 is required for propagating the signal from CTR1 to the nucleus. 24 out of 25 different alleles of *ein2* showed complete ethylene insensitivity suggesting that EIN2 is an essential positive regulator in ethylene signaling (Alonso et al., 1999). The fact that *ein2* mutants were also isolated in

screens for different hormone responses (Su and Howell, 1992; Fujita and Syono, 1996; Beaudoin et al., 2000; Ghassemian et al., 2000) and delayed senescence (Oh et al., 1997) implies that EIN2 mediates cross-talk of multiple hormone signaling and stress response pathways.

When cloned, *EIN2* was found to encode a novel integral membrane protein which shares homology with eukaryotic Nramp metal ion transporter (Alonso et al., 1999). While the transmembrane amino-terminal region exhibit strong similarity to a cation transporter, the extended carboxy-terminus consists of a novel sequence containing a coiled-coil motif, implying that this region may be a site for protein-protein interaction. In spite of the strong homology, EIN2 lacks detectable metal transport activity (Alonso et al., 1999). Overexpression of the carboxy-terminal domain in an *EIN2* null background provides constitutive activation of number of ethylene responses mostly in light grown plants, but not in dark grown seedlings. These results led to a hypothesis that the carboxy-terminal domain acts in transducing the signal to downstream components, while the amino-terminal domain is required for sensing the upstream ethylene signal. Although this implies the role of EIN2 in ethylene signal transduction, the mechanism by which EIN2 receives the signal and how it transduces to the downstream effectors remain unknown.

NUCLEAR EVENTS: TRANSCRIPTIONAL CASCADE

In many cases, ethylene responses involve changes in gene expression. The first direct evidence of transcriptional control came out with cloning of *EIN3* encoding a nuclear-localized DNA binding protein (Chao et al., 1997). Loss-of-function mutations in *EIN3* result in ethylene insensitive phenotype indicating that EIN3 is a positive regulator of ethylene pathway. In *Arabidopsis*, *EIN3* belongs to a multigene family that contains five additional EIN3-like proteins (EILs). Overexpression of *EIN3* and the most closely related protein *EIL1* in wild type or *ein2* mutant caused constitutive activation of ethylene response, suggesting their role as a positive regulator in ethylene signaling pathway (Chao et al., 1997). Isolation of *eil1* in a screen for weak ethylene insensitive mutant supports the role of EILs in ethylene signal transduction as well. Indeed, *ein3eil1* double mutant conferred almost complete ethylene insensitivity indistinguishable from *ein2*-5, indicating that EIN3 and EIL1 are the major contributors for ethylene signaling (Alonso et al., 2003).

A unique aspect of EIN3/EIL regulation seems to be at the protein level rather than RNA. None of the *EIN3/EIL* genes identified so far has been shown to response to ethylene at mRNA levels, but protein levels rapidly rise (Guo and Ecker, 2003; Potuschak et al., 2003; Yanagisawa et al., 2003; Gagne et al., 2004). As it turns out, EIN3 protein is constantly made, and then targeted for degradation through proteasome-mediated pathway by an interaction with two F-box proteins, EBF1 and EBF2 (Guo and Ecker, 2003; Potuschak et al., 2003; Gagne et al., 2004). Single knockouts of the *EBF1* or *EBF2* gene conferred a slight hypersensitivity to ethylene while overexpression resulted in plants insensitive to ethylene. The accumulation of EIN3 protein in double knockout mutant lacking EBF1 and EBF2 were above normal amounts, thus exhibiting constitutive ethylene response phenotype (Guo and Ecker, 2003; Potuschak et al., 2003) or showing severe growth inhibition in a different study (Gagne et al., 2004). Altogether, these results indicate that a ubiquitin-mediated proteasome pathway negatively regulates ethylene responses by targeting EIN3 for degradation. Although it is clear that EIN3 function is regulated by EBF1 and EBF2, how ethylene regulates these factors to prevent EIN3 from degradation is not known. The substrate recognition of EBFs can be altered by modification of EIN3 as it occurs in many other cullin-based E3 ligases (Deshaies, 1999; Cardozo and Pagano, 2004) or, alternatively, ethylene can modify the EBFs or associated proteins in the ubiquitination complex rather than the substrate as is the case in auxin signaling (Dharmasiri et al., 2005).

While EBFs negatively regulate EIN3 protein accumulation, the accumulated EIN3 protein conversely regulates *EBF1/2* mRNA level providing a feedback-loop to control the EIN3 protein level (Potuschak et al., 2003). Recently, the gene encoding *EIN5/AIN1* (for *ACC-INSENSITIVE1*), which was originally isolated in a screen for reduce ethylene responsiveness in the presence of ethylene (Roman et al., 1995), was identified. Genetic study revealed that EIN5/AIN1 is required for ethylene responses and was placed downstream of CTR1 but upstream of EBF1/2 (Olmedo et al., 2006). Interestingly, the mRNA levels for *EBF1/2* were increased in *ein5/ain1* mutant, which consequently resulted in instability of EIN3 protein and reduced ethylene sensitivity (Olmedo et al., 2006; Potuschak et al., 2006). These findings suggest that EIN5 play a role to antagonize the negative feedback regulation on EIN3 by accelerating the *EBF1/2* mRNA decay, thus resulting accumulation of EIN3 protein to trigger ethylene response (Olmedo et al., 2006). *EIN5/AIN1* was allelic to *XRN4*, previously identified as a gene encoding cytoplasmic 5′ → 3′ exoribonuclease. However, it seems more likely that XRN4 modulates EBF1/2 in an indirect way as the turnover rate of EBF1/2 mRNA was not affected in *ein5/ain1* mutant (Potuschack et al., 2006). Even though XRN4 has been found to play a role in miRNA-dependent mRNA decay and gene silencing (Souret et al., 2004), XRN4 more likely regulates *EBF1/2* mRNA levels by a distinct mechanism, since the level of EBF1/2 does not seem to be affected in other mutants of RISC-based RNA silencing (Potuschak et al., 2006). The exact mechanism how XRN4 regulates the negative feedback loop to equilibrate the EIN3/EIL proteins will be another important issue in the sense that slight changes in the levels of EIN3/EIL proteins can provide significant impact on the signal flux to downstream nuclear events.

While it is clear that EIN3 and at least some of the EILs may act as transcriptional regulators of ethylene responses, the direct evidence for EIN3/EILs role as a transcription factor came out in further studies searching for target promoters of these proteins. Solano et al. (1998) discovered that EIN3 as well as EIL1and EIL2 bind as a homodimer to the specific *ERF1* upstream sequence that has similarity to a previously identified primary ethylene response-element (PERE). In addition, EIN3 is shown to be necessary and sufficient to

activate *ERF1* gene expression. ERF1 belongs to a large family of plant-specific transcription factors (Ohme-Takagi and Shinshi, 1995) referred to as ethylene-response element binding proteins (EREBPs) which are capable of binding to a secondary ethylene-response element known as GCC-box found in the promoters of several ethylene- and pathogen-induced genes (Solano et al., 1998). Consistently, overexpression of *ERF1* constitutively activates subsets of GCC-box-containing target genes and ethylene phenotypes suggesting that ERF1, in turn, is responsible for the modulation of secondary ethylene responsive genes and consequently regulates one branch of the ethylene response pathway downstream of EIN3 (Solano et al., 1998).

Considering the result that *ein3eil1* double mutant exhibits almost complete ethylene insensitivity, the transcriptional regulation seems to play much larger role in performing ethylene responses. Efforts have been made to discover the ethylene regulated gene expression in different processes and in different tissues (Zegzouti et al., 1999; Trentmann, 2000). The use of various techniques including cDNA-amplified fragment length polymorphism (AFLP), cDNA microarray and Affymetrix gene array uncovered broad range of ethylene regulated genes at the whole plant level (Alonso et al., 2003; de Paepe et al., 2004). Further genome-wide analyses utilizing genetic resources related to ethylene signaling and concentrating on specific tissue or cell types will provide us better understandings on unique ethylene responses as well as the ethylene's role in co-ordinated processes with other signaling pathways.

CONCLUDING REMARKS

Current research on ethylene signal transduction pathway exemplifies a linear signal transduction pathway, which consists of the four main modules: a phosphotransfer relay of bacterial two-component system and MAPKKK, an EIN2-based unit, a ubiquitin-mediated protein degradation component, and a transcriptional cascade. Although the basic framework for the ethylene signaling pathway is emerging, there are many questions that remain to be resolved. The biochemical properties of the known signaling components need to be further investigated and reconciled with the behavior of the ethylene signal transduction system in planta. Likewise, how the signal impacts a plant to a specific ethylene response needs to determined. Recent advances in the global genomics and proteomics area should allow for targeted studies that will provide new insights into molecular and biochemical responses of plants.

The notion that the configuration of ethylene signaling pathway is unique in comparison to other hormone signaling pathways identified in plants has led to a question on how ethylene signaling has been evolved. Interestingly, recent sequencing of *Physcomitrella patens* (Joint Genome Insitute, unpublished) reveals full array of ethylene signaling components, including ETRs, CTR1, EIN2, and EIN3, suggesting that the ethylene signal transduction pathway identified from *Arabidopsis* is conserved throughout plant kingdom. Since the roles of ethylene signaling in moss have been reported (Rohwer and Bopp, 1985; Fujiwara and Tohe, 2002), a comparative study on ethylene physiology and signal transduction will provide evolutionary perspectives on the first gaseous hormone ethylene.

ACKNOWLEDGEMENTS

We thank Jae-Hyeok Lee for critical reading and helpful comments on the manuscript. This work was supported by grants from the Korea Research Foundation (Basic Research Program Project No. 2005-C00129) and the Plant Metabolism Research Center at Kyung Hee University, Korea (Science Research Center Program from Korea Science and Engineering Foundation).

Received March 6, 2007; accepted March 14, 2007.

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