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Molecular Basis of the Ethylene Signaling and Response Pathway in *Arabidopsis*

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Abstract

The gaseous phytohormone ethylene is a key regulator in plant growth and developmental process as well as biotic and abiotic stress response. This review focuses on the recent advances in the ethylene-signaling pathway in *Arabidopsis*, with particular emphasis on the latest information about the downstream events of the ethylene-response pathway. Notable new findings include identification of a specific regulator of the ethylene receptor ETR1, discovery of protein degradation and RNA turnover processes in modulating EIN3-dependent transcriptional regulation, demonstration of the involvement of auxin biosynthesis in ethylene-mediated inhibition of root growth, and determination of possible integration points between ethylene and other hormonal and environmental signals (gibberellin, jasmonic acid, light, and sugar) in various plant processes. The elucidation of the molecular mechanisms of the ethylene-signaling and ethylene-response pathway in *Arabidopsis* might provide a framework for understanding how other plant species sense and respond to ethylene.

Key words: Ethylene; Signaling pathway; Hormone crosstalk; Transcriptional regulation; *Arabidopsis*

Introduction

Despite its structural simplicity, ethylene is a gaseous hormone that participates in many aspects of plant developmental processes, including seed germination, cell elongation, fruit ripening, organ senescence, root nodulation, programmed cell death, abscission and response to environmental stress, and pathogen attack (Bleecker and Kende 2000; Johnson and Ecker 1998). When applied exogenously, ethylene or its metabolic precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), can invoke a specific morphological response, known as the ''triple response'' of dark-grown (etiolated) seedlings. In the model plant *Arabidopsis thaliana*, the triple response is characterized by inhibition of hypocotyl and root cell elongation, radial swelling of the hypocotyl, and exaggerated curvature of the apical hook (Ecker 1995; Roman and Ecker 1995). Based on this highly reproducible and specific response phenotype at the early stage of plant development, more than a dozen mutants that display an aberrant triple response phenotype have

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been identified in *Arabidopsis* over the past decades. These mutants can be classified into five distinct categories: (1) ethylene overproduced mutants, such as *eto1* (*ethylene overproduction1*), *eto2*, *eto3* (Kieber and others 1993); (2) constitutive ethylene-signaling mutants, including *ctr1* (*constitutive triple response1*) and *ran1* (*responsive to antagonist1*)/*ctr2* (Kieber and others 1993; Hirayama and others 1999); (3) ethylene-insensitive or ethylene-resistant mutants that show a partial or complete defect in all aspects of the triple response phenotype, for example, *etr1* (*ethylene receptor1/ethylene resistant1*), *etr2*, *ein2* (*ethylene insensitive2*), *ein3*, *ein4*, *ein5*, *ein6*, *eil1* (Roman and others 1995; also see review by Guo and Ecker 2004); (4) mutants that display tissue-specific ethylene insensitivity, including *hls1* (*hookless1*), *eir1* (*ethylene insensitive root1*), and several auxin-resistant mutants (Lehman and others 1996; see review by Stepanova and Alonso 2005); (5) mutants hypersensitive to exogenous ethylene or ACC, like *ebf1* (*ein3-binding F-box protein1*), *ebf2* (Guo and Ecker 2003; Potuschak and others 2003; Gagne and others 2004), *eer1* (*enhanced ethylene response1*) (Larsen and Chang 2001), and *rte1* (*reversion to ethylene sensitivity1*) (Resnick and others 2006).

A largely linear ethylene signal transduction pathway from hormone perception at the endoplasmic reticulum (ER) to transcriptional regulation in the nucleus has been defined through the combination of genetic and molecular analysis of those mutants (see reviews by Nehring and Ecker 2004; Chen and others 2005). In *Arabidopsis*, ethylene is perceived by a family of five membrane-associated receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) that possess sequence similarity with bacterial two-component His kinases (Bleecker 1999; Schaller and Kieber 2002). Ethylene binds to the receptors via a copper co-factor, and a copper transporter RAN1 (a homolog of the human Menkes Wilson P-type ATPase) is likely involved in copper delivery to the receptors (Hirayama and others 1999; Woeste and Kieber 2000). Two recent studies reported that *RTE1*, as well as the tomato homolog *Gr*, is a negative regulator of ethylene responses, and it was shown that RTE1 is an important regulator of ETR1 function (Resnick and others 2006; Barry and Giovannoni 2006). Genetic studies predict that the receptors remain active in the absence of ethylene gas, and ethylene binding leads to functional inactivation of the receptors. The ethylene-free receptors can somehow activate CTR1, a Raf-like Ser/Thr kinase, which is also a negative regulator of the pathway (Kieber and others 1993). In the presence of ethylene, CTR1 loses its ability to repress a positive component of the pathway, the membrane protein ETHYLENE INSENSITIVE2 (EIN2). EIN2 is a crucial molecule in transmitting the ethylene signal, because *ein2* loss-of-function mutations result in complete ethylene insensitivity in most if not all ethylene-related responses (Alonso and others 1999).

Despite its extreme importance in ethylene signal transduction, the subcellular localization and biochemical function of EIN2 remain a mystery (Alonso and others 1999). EIN3, EIL1 (EIN3-Like1), EIN5, and EIN6 are also positive regulators that act further downstream in the signaling pathway. EIN3 and EIL1 are plant specific-transcription factors (Chao and others 1997), and the protein level of EIN3 is down-regulated by the ubiquitin/proteasome pathway specifically mediated by SCF complexes containing F-box proteins EBF1/2 (EIN3 binding F-box protein1/2) (Guo and Ecker 2003; Potuschak and others 2003; Gagne and others 2004). Both EIN3 and EIL1 are found to induce the expression of other transcription factors such as ERFs (ethylene-response factors) and EDFs (ethylene-responsive DNA-binding factors) (Solano and others 1998; Alonso and others 2003a), which represents a transcriptional cascade in the ethylene-response pathway. EIN5, a 5′ → 3′ exoribonuclease, has been recently shown to decrease the level of *EBF1* and *EBF2* mRNAs through a yet unknown mechanism, and consequently stabilize EIN3 protein (Guo and Ecker 2003; Olmedo and others 2006; Potuschak and others 2006). Although EIN6 is also shown to act as a positive regulator of EIN3 protein stability (Guo and Ecker 2003), it has not yet been characterized at the molecular level.

In this review, we provide an update on the molecular basis of the ethylene-signaling pathway in *Arabidopsis* (Figure 1), with particular focus on the new findings of the downstream response pathway achieved in the past few years. These findings include identification of a novel regulator RTE1 in modulating the function of the ethylene receptor ETR1; discovery of protein degradation and mRNA turnover pathways in regulating EIN3; characterization of two auxin biosynthetic enzymes WEI2/WEI7 that are responsible for ethylene-induced root cell inhibition, and determination of the underlying molecular mechanisms of cross-talks between ethylene and other signals (such as GA, JA, light, and sugar) in plant growth and defense response (Figure 2).

Early Events of Ethylene Perception and Signaling

The Ethylene Receptors

Ethylene is perceived by a family of five membrane-bound proteins (ETR1, ETR2, ERS1, ERS2, and

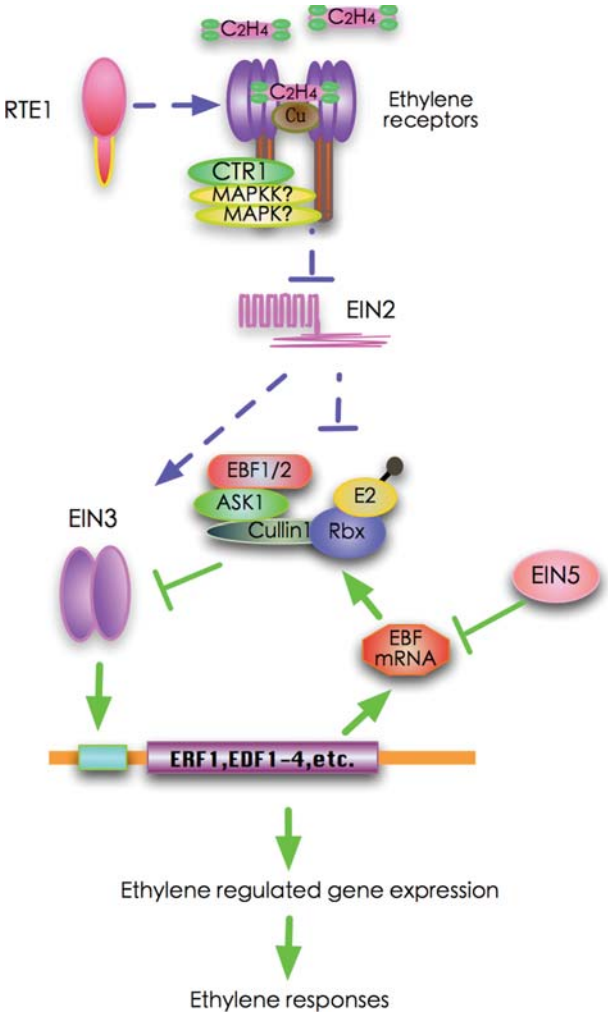

Figure 1. A model of the ethylene signal transduction pathway in *Arabidopsis*. Ethylene binding leads to the inactivation of ER-localized receptors by an unknown mechanism. A novel membrane protein, RTE1, might specifically enhance the function of the ETR1 receptor. An inactive receptor is incapable of recruiting the negative regulator CTR1 to the ER membrane, which in turn shuts off its activity. EIN2 is then free from inhibition by CTR1 and increases the nuclear accumulation of EIN3 protein by repressing its turnover, which is mediated by SCF complexes containing the F-box proteins EBF1/2. There are two possibilities for how EIN2 stabilizes EIN3: EIN2-derived signal modulates EIN3 directly or inhibits the SCF^{EBF} complex. One of the *EBF* genes, *EBF2*, is induced by ethylene in an EIN3-dependent manner. Thus, a negative feedback loop is formed between EIN3 and EBF. EIN5, an exoribonuclease, seems to downregulate the level of *EBF1* and *EBF2* mRNAs without affecting their half-life. The nuclear accumulation of EIN3 induces a large amount of gene expression, and ultimately triggers various ethylene responses. Arrows and t-bars represent positive and negative regulations, respectively. Solid arrows and t-bars correspond to direct interactions and dotted lines indicate the likely existence of unidentified elements between upstream and downstream components.

EIN4) in *Arabidopsis* (see reviews by Chang and Stadler 2001; Chang and Bleecker 2004; Hall and others, in this issue). ETR1 has been localized to the endoplasmic reticulum (ER), and the other four receptors possibly reside at the ER as well. On the basis of structural similarities, the receptor family can be divided into two types: type-I receptors (ETR1 and ERS1) contain three transmembrane segments in the amino terminus and a carboxyl-terminal conserved histidine kinase domain; type-II receptors (ETR2, ERS2, and EIN4) contain four hydrophobic extensions at the amino terminus and a degenerate histidine kinase domain that is presumed to lack catalytic activity in the carboxyl terminus. In addition to the transmembrane domain and His kinase domain, ETR1, ETR2, and EIN4 also contain a receiver domain at their carboxyl end. The amino-terminal transmembrane domains are responsible for formation of disulfide-linked dimerization as well as ethylene binding, and thus they function as the sensor domains of all five receptor proteins (Schaller and Bleecker 1995; O'Malley and others 2005). In addition, the sensor domain contains a copper co-factor that is required for high-affinity ethylene binding (Schaller and Bleecker 1995; Rodriguez and others 1999). Copper ions are delivered to the receptors by RAN1 (Responsive to Antagonist1), a putative copper-transporting P-type ATPase homologous to the yeast Ccc2p and human Menkes Wilson disease proteins (Hirayama and others 1999). In contrast to the amino-terminal transmembrane domains, the biochemical functions of the carboxyl-terminal His kinase domains and receiver domains in the ethylene sensing and signaling are poorly understood. However, many recent studies on the functional analysis of ETR1 and its derivative forms have begun to unravel the detailed roles of these domains in ethylene perception and signaling, although more conclusive data are still to be obtained to clarify the exact functions of these domains (Wang and others 2003; also see reviews by Chen and others 2005, and Hall and others, 2007, in this issue).

RTE1/GR, a Novel Regulator of the ETR1 Receptor

Genetic studies indicate that the ethylene receptors serve as negative regulators of the ethylene-signaling pathway (Hua and Meyerowitz 1998). However,

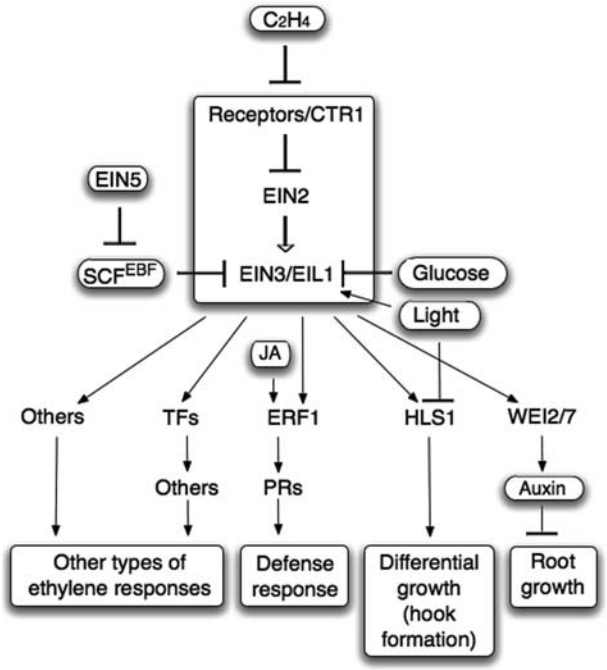

Figure 2. Molecular basis of cross-talk between the ethylene-response pathway and other signals. Ethylene signaling leads to the stabilization of EIN3 and EIL1 transcription factors and, subsequently, the activation of gene expression. Many integration points of ethylene with other signals are represented by EIN3/EIL1-regulated genes, such as ERF1, which converges the JA and ethylene signals in the defense response; HLS1, which combines the light and ethylene effect to modulate the auxin response in apical hook formation; WEI2/WEI7, which mediate ethylene-induced auxin synthesis in inhibition of root growth. Other interaction nodes could be EIN3/EIL1. For instance, glucose, light, and ethylene all regulate EIN3 stability in seedling development. T-bars and arrows represent negative and positive regulation, respectively.

it is not clear how ethylene binding turns off the receptors' activity. Recently, a newly identified regulator of ethylene responses, *RTE1* (*reversion to ethylene sensitivity1*) is likely to provide some hints on how the biological function of the receptors is regulated (Resnick and others 2006). *RTE1* was identified by a genetic screen for suppressors of the dominant gain-of-function allele *etr1–2*. Loss-of-function *rte1* mutants show enhanced response to ethylene, similar to the *etr1* null mutant or the *rte1 etr1* double null mutant, suggesting that RTE1 is a negative regulator of the ethylene response, and that RTE1 and ETR1 act in the same pathway. Moreover, although loss of *rte1* function can suppress ethylene insensitivity of *etr1-2*, it fails to suppress a stronger allele *etr1-1*, or gain-of-function mutations in the four other ethylene-receptor genes, implying that RTE1 is required specifically for the *etr1-2* mutant receptor to repress the downstream ethylene pathway. No biochemical function has been assigned to this interesting protein yet, except that RTE1 appears to be a membrane protein based on sequence prediction (Resnick and others 2006). In a separate study, a dominant *Green-ripe* (*Gr*) mutant of tomato was recently characterized at the molecular level, and its corresponding gene encodes a homolog of *RTE1* (Barry and Giovannoni 2006). Ectopic expression of *Gr*, either by a gain-of-function mutation or by a transgenic overexpression approach, leads to reduction in a subset of (but not all) ethylene responses, including fruit ripening (Barry and Giovannoni 2006). Therefore, RTE1/GR is an evolutionarily conserved protein that plays a positive role in modulating the function of the ethylene receptor, and its biochemical function and the regulatory mechanism on the receptor would be of interest for further investigation.

CTR1, a Raf-like Kinase as a Negative Regulator

Genetic epistasis analysis has placed CTR1, a Raf-like Ser/Thr protein kinase, downstream of the ethylene receptors in the ethylene-signaling pathway (Kieber and others 1993). CTR1 has also been found to associate with ER membranes in *Arabidopsis*, although it has no obvious trans-membrane domain or membrane attachment motifs (Gao and others 2003).

Two lines of evidence suggest that CTR1 could be recruited to the ER membrane by interaction with the ER-associated ethylene receptors. First, co-immunoprecipitation shows that affinity purification of CTR1 from an *Arabidopsis* ER-membrane fraction can co-purify ETR1 (Gao and others 2003). Second, overexpression of an intact CTR1 amino-terminal domain leads to preventing endogenous CTR1 from associating with the receptors, thus causing a loss-of-function *ctr1* mutant phenotype (a dominant negative effect). In contrast, overexpression of a mutated CTR1 amino-terminal domain (CTR1-8) does not produce the *ctr1* phenotype, because this domain is incapable of interacting with the receptors (Huang and others 2003). The current belief is that, in the absence of ethylene, the active receptors can interact with and recruit CTR1 to the ER membrane, which in turn activates CTR1 and shuts down the ethylene pathway. Although CTR1 is a critical component of the ethylene signal transduction pathway, several lines of evidence implicate the existence of a CTR1-independent pathway operating in the ethylene-response pathway. For

instance, *ctr1* loss-of-function mutants are still responsive to ethylene treatment with regard to both triple response phenotype and EIN3 protein accumulation (Roman and others 1995; Larsen and Chang 2001; Guo and Ecker 2003); the quadruple ethylene receptor loss-of-function mutant, as well as the *etr1 ers1* double mutant, displays a more severe phenotype than *ctr1* loss-of-function mutations (Hua and Meyerowitz 1998; Hall and Bleecker 2003); and *EIN3* overexpression lines and *ebf1 ebf2* double mutants also show stronger ethylene-related growth response than the *ctr1* mutant (see below).

A MAP Kinase Cascade in Debate

For more than 10 years, ever since CTR1 was suggested to function as a putative MAPKKK (Kieber and others 1993), a MAPK kinase cascade in the ethylene-signaling pathway has been sought. In fact, a MAPK pathway involving SIMK (salt-stress–inducible MAPK) in *Medicago* or MPK6 in *Arabidopsis* was recently implicated in operating downstream of CTR1 as a positive regulator of the ethylene response (Ouaked and others 2003). Nonetheless, the reduction or elimination of *Arabidopsis MPK6* expression/function by RNA interference or the T-DNA insertion approach does not have an appreciable defect on ethylene responses (Ecker 2004; Menke and others 2004). Through a series of biochemical studies, another group of investigators failed to observe any significant difference of MPK6 activity upon ACC treatment in *Arabidopsis* between wild-type and mutant plants (Liu and Zhang 2004). In addition, the same group presented convincing evidence to indicate that MPK6 regulates ethylene biosynthesis rather than the signaling pathway (Liu and Zhang 2004; review by Benavente and Alonso 2006). Overall, other than the sequence similarity of CTR1 to MAPKKK, there is so far no conclusive evidence to support a MAPK kinase cascade operating in the ethylene signal transduction pathway.

Downstream Events of Ethylene Signal Transduction and Transcriptional Regulation

EIN3 and EIL1, Two Crucial Transcriptional Factors

EIN3 is a plant-specific transcription factor mediating ethylene-regulated gene expression (Chao and others 1997). It belongs to a multigene family in *Arabidopsis*, including EIN3, EIN3-like 1 (EIL1), EIL2, EIL3, EIL4, and EIL5, in which EIN3 and EIL1 are the most closely related homologs. Overexpression of *EIN3* or *EIL1* results in constitutive activation of the ethylene-response pathway, whereas the loss-of-function *ein3* or *eil1* mutants show partial ethylene insensitivity (Chao and others 1997). The weaker ethylene insensitivity phenotype of the *eil1* mutant can be explained by the lower expression level of *EIL1* compared with that of *EIN3* (Alonso and others 2003b). Although overexpression of *EIL1* or *EIL2* in the background of *ein3* can recover the sensitivity of *ein3* to ethylene (Chao and others 1997), *ein3 eil1* double mutants show complete ethylene insensitivity in etiolated seedlings and adult plants (Alonso and others 2003b). *EIN3* and *EIL1* have also been found in many other plant species, for instance, three tomato *LeEILs*, five tobacco *NtEILs*, two mung bean *VrEILs*, three carnation *DcEILs*, and several rice *OsEILs* have been identified and functionally characterized in recent years (Kosugi and Ohashi 2000; Tieman and others 2001; Lee and Kim 2003; Rieu and others 2003; Iordachescu and Verlinden 2005; Mao and others 2006). Interestingly, all these EIL proteins are more closely related to *Arabidopsis* EIN3 and EIL1 than to EIL2–5. Together with these observations, it is thus presumed that EIN3 and EIL1 are the major transcription factors in mediating ethylene responses, whereas EIL2 through EIL5 might regulate ethylene responses in specific tissue types or certain developmental stages, or instead, function in ethylene-unrelated pathways.

Biochemical studies showed that EIN3 and EIL1 can directly bind to the promoter of *ERF1* (ethylene-response factor 1), which belongs to the EREBP (ethylene-response element binding protein) family of transcription factors (Solano and others 1998). Overexpression of *ERF1* can rescue only a subset of *ein3* phenotypes, suggesting that EIN3 regulates additional target genes in mediating distinct ethylene responses (Solano and others 1998). Consistent with this notion, four novel transcription factors, EDF1–4 (ethylene-responsive DNA binding factors), could also be potential target genes of EIN3, because their mRNA levels are rapidly accumulated upon ethylene treatment, and their knockout mutants result in partial ethylene insensitivity (Alonso and others 2003a). Collectively, a transcriptional cascade from EIN3/EIL1 to ERF1 and EDF1–4 is involved in the ethylene-response pathway.

EBF1 and EBF2, Two F-box Proteins Mediating EIN3 Protein Turnover

Because the level of *EIN3* mRNA is unaffected by ethylene treatment in either wild-type plants or

ethylene-related mutants, a post-transcriptional regulatory mechanism for EIN3 action had been proposed (Chao and others 1997). Recently, a ubiquitin/26S proteasome-mediated protein degradation pathway was demonstrated in controlling EIN3 activity (Guo and Ecker 2003; Potuschak and others 2003; Yanagisawa and others 2003; Gagne and others 2004). In the absence of ethylene, EIN3 is continuously degraded, whereas ethylene treatment quickly stabilizes EIN3 protein (Guo and Ecker 2003; Yanagisawa and others 2003). EIN3 degradation is mediated by two F-box proteins called EBF1 and EBF2 (EIN3 binding F-box), which can form the SCF-type (Skp1, Cullin, F-box, and Rbx1) E3 ligase (Deshaies 1999). Loss-of-function mutations in either *EBF1* or *EBF2* lead to increased EIN3 accumulation and show enhanced ethylene response, whereas *ebf1 ebf2* double mutants show constitutive ethylene responses or even seedling lethality (Guo and Ecker 2003; Potuschak and others 2003; Gagne and others 2004). Moreover, overexpression of either *EBF1* or *EBF2* leads to reduced EIN3 accumulation and a decrease in ethylene sensitivity. Together, these results demonstrate that EBF1 and EBF2 play a negative role in ethylene signaling by targeting EIN3 for degradation.

Interestingly, ethylene treatment results in increasing the transcription level of *EBF2*, suggesting that there exists a negative feedback mechanism in ethylene signaling (Guo and Ecker 2003; Potuschak and others 2003). Although EBF1 and EBF2 proteins appear to work redundantly in controlling the EIN3 protein level, they might play different roles in regulating EIN3 function. It is conceivable that EBF1 works at low ethylene concentrations, possibly to keep a relatively low basal level of EIN3 protein in the nucleus. When the ethylene signal is enhanced, the EIN3 protein becomes stabilized, which in turn induces the expression of *EBF2*. The accumulation of EBF2 is likely to suppress the high level of EIN3 protein to its basal level, thus restoring plant responsiveness to ethylene again (Guo and Ecker 2003; Gagne and others 2004). Until now, the mechanism for ethylene-mediated EIN3 stabilization remains unclear. Two distinctive but not mutually exclusive possibilities could exist: one is that ethylene may induce a post-translational modification on EIN3, such as phosphorylation or dephosphorylation, which consequently affects the interaction between EBF1/2 and EIN3; the other is that ethylene may regulate EBF1/EBF2 stability or other aspects of their function, which consequently affects their interaction with EIN3 (see Figure 1).

EIN2, EIN5, and EIN6, Three Positive Regulators of EIN3 Action

Genetic studies have demonstrated that EIN2, EIN5, and EIN6 are positive components of the ethylene-signaling pathway (review by Stepanova and Ecker 2000). When compared with wild-type seedlings, *ein5* and *ein6* mutants are impaired in ethylene-induced EIN3 accumulation, whereas EIN3 accumulation is completely blocked in *ein2* (Guo and Ecker 2003). Therefore, EIN2, EIN5, and EIN6 are all required for EIN3 accumulation, suggesting that these components function upstream of EIN3. Moreover, these results imply that the ethylene insensitivity observed in *ein2*, *ein5*, and *ein6* might be the result of reduced EIN3 abundance.

EIN2 functions as a pivotal positive regulator of the ethylene-response pathway, because loss-of-function *ein2* mutations result in complete ethylene insensitivity in all ethylene-related responses examined. EIN2 is an integral membrane protein but its biochemical function is still unknown. The amino-end of EIN2 sequence possesses sequence and structural similarity to the Nramp family of metal ion transporters, but no transport activity has been demonstrated so far. Overexpression of the EIN2 carboxyl terminus can constitutively activate a subset of ethylene-response phenotypes (but not the triple response), and induce ethylene-regulated gene expression, but it cannot restore ethylene sensitivity in an *ein2* null mutant (Alonso and others 1999). It is thus hypothesized that the amino-terminus of EIN2 represents an input domain in sensing upstream signaling, whereas the carboxyl terminus represents an output domain interacting with downstream components (Alonso and others 1999).

Recently, EIN5 has been characterized at the molecular level and it encodes a previously described 5′ → 3′ exoribonuclease, XRN4, the *Arabidopsis* homolog of yeast XRN1 (Olmedo and others 2006; Potuschak and others 2006; Kastenmayer and Green 2000). The XRN4 exoribonuclease is responsible for degrading many unstable mRNAs, particularly the 3′ fragments of miRNA-mediated cleavage products (Souret and others 2004). In addition, XRN4 has been reported to play a role in RNA silencing of certain transgenes (Gazzani and others 2004). The *ein5* mutant partially suppresses the constitutive ethylene-response phenotype of *ctr1*, suggesting *EIN5* acts downstream of *CTR1* (Olmedo and others 2006; Potuschak and others 2006). To establish the connection between this mRNA decay enzyme and the ethylene-response pathway, global gene expression profiling

was performed for wild-type and *ein5* mutant seedlings. Among hundreds of *EIN5*-regulated genes, *EBF1* and *EBF2* mRNAs were found to significantly accumulate in the *ein5* mutant, implicating *EBF1* and *EBF2* as the possible missing link (Olmedo and others 2006). Genetic analysis revealed that the *ebf2* (but not *ebf1*) single mutant and the *ebf1 ebf2* double mutant can significantly suppress the phenotype of *ein5* (Olmedo and others 2006; Potuschak and others 2006). Consistently, ethylene-induced EIN3 accumulation can be largely restored in the *ein5 ebf2* (but not *ein5 ebf1*) mutant. Therefore, in the ethylene signal transduction pathway, EIN5 is likely to increase the accumulation of EIN3 protein by decreasing *EBF2* mRNA accumulation (and probably that of *EBF1* as well) (Olmedo and others 2006). Given that EIN5/XRN4 is involved in mRNA decay, the downregulation of *EBF1/2* mRNA level by EIN5 might be the consequence of a direct RNA turnover by EIN5. However, careful examination of the half-life of *EBF1* and *EBF2* mRNAs in the *ein5* mutant seems to rule out this possibility (Potuschak and others 2006). Furthermore, even though EIN5/XRN4 is implicated in an RNA interference process, none of the other known mutants in the miRNA and siRNA pathways show an appreciable ethylene-related defect, suggesting that EIN5 regulates ethylene signaling not via a RISC-based RNA silencing mechanism (Potuschak and others 2006). Although the molecular details on how EIN5/XRN4 regulates the level of *EBF1* and *EBF2* mRNAs remain unclear, the identification of EIN5/XRN4 as a new ethylene-signaling component adds RNA degradation as another post-transcriptional process that modulates the plant's response to ethylene gas.

Transcriptional Profiling, a Genome-wide Study of Ethylene Response

The microarray data gathered to date allow a global analysis of transcriptional regulation in ethylene responses. Several groups have performed such high throughput analyses by using a microarray approach. For instance, Affymetrix gene expression arrays have been used to examine the RNA levels of more than 22,000 genes in response to exogenous ethylene treatment or in various ethylene-response mutants in *Arabidopsis*. In one such study, the expression levels of 628 genes were significantly altered by ethylene treatment, among which, 244 were induced and 384 were repressed (Alonso and others 2003a). Meanwhile, an EST-based microarray containing about 6,000 unique *Arabidopsis* genes has been examined, and about 7% of the genes have been identified as ethylene-regulated genes (Zhong and Burns 2003). In another study, a kinetic analysis of the early response to ethylene using a cDNA microarray uncovered significant differences in gene expression among wild-type, *ctr1-1*, and *ein2-1* mutants (De Paepe and others 2004). Not surprisingly, ethylene-regulated genes identified from these microarray data are found to participate in many biological processes, from metabolism, cell wall regulation, protein turnover, and transcriptional regulation to defense responses. These studies also revealed overlaps of genes regulated by ethylene and other signals, including JA, auxin, ABA, and sugar, suggesting that many hormonal and signaling interactions might lie in the coordinated regulation of gene expression, and ultimately will form a complex regulatory network (Schenk and others 2000; De Paepe and others 2004).

Interactions between the Ethylene response Pathway and Other Signals

Previous physiological and genetic studies, together with the large-scale analyses of microarray data, indicated that ethylene has a wide range of cross-talk with auxin, light, gibberellin, jasmonic acid, salicylic acid, cytokinin, and sugar, among other signals. The combination of these signals controls plant growth, development, and response to myriad biotic and abiotic stresses. For example, ethylene and auxin are involved in a number of the same processes including root elongation, root hair formation, hook formation, leaf epinasty, and abscission (see review by Stepanova and Alonso 2005). However, the molecular details of many cross-talks between different hormones and signals remain poorly understood. In this final section of the review, we highlight the latest advances in a selected list of interplays involving ethylene and other signals, such as auxin, GA, JA, light, and sugar.

WEI2/WEI7: Auxin and Ethylene in Inhibition of Root Growth

Inhibition of root growth is one of the characteristic ethylene responses in *Arabidopsis* seedlings. Interestingly, many auxin response or transport mutants also show clear defects in ethylene-mediated root inhibition, leading to the hypothesis that ethylene-induced inhibition of root growth is mediated by accumulation of auxin in root tissues (see review by Stepanova and Alonso 2005). Experimental proof of this hypothesis came from the recent identification

of two root-specific ethylene-insensitive mutants *wei2* (*weak ethylene insensitive 2*) and *wei7* (Stepanova and others 2005). *WEI2* and *WEI7* encode α and β subunits of anthranilate synthase, respectively, which is a rate-limiting enzyme in tryptophan biosynthesis, and subsequently the auxin synthesis pathway (Bartel 1997). Ethylene treatment can induce the expression of *WEI2* and *WEI7* specifically in root tips, and simultaneously increase auxin accumulation, manifested with auxin-driven reporter gene expression. Loss-of-function *wei2* and *wei7* mutants prevent auxin accumulation upon ethylene treatment (Stepanova and others 2005). Moreover, *wei2* and *wei7* were able to suppress the phenotypes of two auxin-overproduced mutants *sur1* (*superroot1*) and *sur2*, further confirming the defect of auxin biosynthesis in *wei2* and *wei7* mutants (Stepanova and others 2005). So a simple explanation for ethylene-triggered inhibition of root growth is that ethylene induces *WEI2/7* expression specifically in root tips, which in turn accelerates auxin biosynthesis, and consequently inhibits root elongation.

DELLA Proteins: Gibberellin and Ethylene in Salt Tolerance

Gibberellins (GA) are a group of phytohormones regulating cell elongation and seed germination. Although ethylene and GA work antagonistically in many cellular processes, such as stem elongation (Achard and other 2003), the requirement of GA signaling in ethylene-induced exaggeration of apical hook indicates that interactions between these two hormones are much more complex (Vriezen and others 2004). Moreover, the ethylene-GA cross-talks in these processes seem to occur at the level of the DELLA proteins. DELLA proteins are negative regulators of plant growth. Upon GA treatment, DELLA proteins can be degraded through the 26S proteasome-mediated protein degradation pathway (Harberd 2003). Five DELLA protein members have been characterized in *Arabidopsis*: GAI, RGA, RGL1, RGL2, and RGL3 (Cheng and others 2004). A recent study also integrated the effect of GA and ethylene in the salt stress response at the level of DELLA protein regulation (Achard and others 2006). While loss-of-function gai/rga/rgl1/rgl2 mutants leads to partial resistance to salt-induced vegetative growth inhibition, this mutant shows reduced tolerance to plant death caused by high concentration of salt, suggesting that the growth inhibiting factors DELLA proteins are also necessary for survival in response to adverse salt stress. Meanwhile, salt stress also induces ethylene production, and accordingly, *ctr1* and *ebf1 ebf2* double mutants are more tolerant than the wild type to salt stress, and *ein3* is less tolerant (Achard and others 2006). These results indicate that ethylene is involved in salt stress and that activation of the ethylene response pathway confers increased tolerance to salt stress. Interestingly, *ctr1-1/gai-t6/rga-24* triple mutants lose salt tolerance, suggesting that ethylene-induced salt tolerance is dependent on the function of DELLA proteins (Achard and others 2006). However, the exact mode of ethylene regulation of DELLA proteins needs further investigation by biochemical approaches.

ERF1: Jasmonic Acid and Ethylene in Defense Response

As described above, ERF1 is an EREBP family transcription factor acting directly downstream of EIN3 in the ethylene-signaling pathway. ERF1 binds to the GCC box in the promoter region of several pathogen-related genes (Ohme-Takagi and Shinshi 1995; Solano and others 1998). ERF1 was recently found to be the integration point of ethylene and the JA pathway in the plant defense response (Lorenzo and others 2003). *ERF1* expression is induced by both ethylene and JA, and such induction requires the presence of both pathways simultaneously, because mutations blocking either of the pathways prevent the expression of *ERF1*. Furthermore, ERF1 is necessary for both ethylene-mediated and JA-mediated defense-related gene expression. Conversely, overexpression of *ERF1* results in the constitutive activation of defense-related gene expression, even in the ethylene or JA signaling mutants (Solano and others 1998; Lorenzo and others 2003). Microarray analyses of ERF1-overexpression plants revealed that many ethylene-responsive and JA-responsive genes are constitutively induced (Berrocal-Lobo and Molina 2004). These studies unequivocally demonstrated that ERF1 is a key point in the integration of the ethylene and JA pathways in the plant-defense response, although the mechanism of a synergistic effect of the two hormones is not understood at this point.

HLS1: Light, Auxin, and Ethylene in Apical Hook Formation

Whereas ethylene treatment can exaggerate the curvature of the apical hook of dark-grown seedlings, light exposure can completely suppress the hook formation (Harpham and others 1991). *hls1 (hookless1)* was isolated as a special ethylene-insensitive mutant that does not display an apical hook,

but its hypocotyl and root respond to ethylene normally (Guzman and Ecker 1990; Roman and others 1995). The *hls1* mutant phenotype can be phenocopied by seedlings treated with auxin-transport inhibitors, suggesting that auxin plays a pivotal role in this ethylene-induced differential cell growth (Lehman and others 1996). Molecular genetic studies revealed that HLS1 encodes a putative N-acetyltransferase (Lehman and others 1996). The expression level of *HLS1* can be induced by ethylene, whereas light causes a decrease in the HLS1 protein level, providing a regulatory mechanism of ethylene and light on the formation of the apical hook (Li and others 2004). A suppressor screen of the *hls1* mutant has identified an auxin-response factor, *ARF2*, whose mutation can reverse the *hls1* phenotype in many developmental processes. Further biochemical analysis indicated that the level of ARF2 protein was decreased by ethylene in a *HLS1*-dependent manner, while light exposure decreased HLS1 protein levels and caused a concomitant increase in ARF2 accumulation (Li and others 2004). Therefore, ethylene and light signals affect apical hook formation by acting through HLS1 to modulate the auxin response. Identification of target proteins of HLS1, a putative *N*-acetyltransferase, would provide valuable information allowing us to more fully understand the interplay among ethylene, auxin, and light in controlling hook bending.

EIN3: Glucose, Light, and Ethylene in Seedling Development

Sugars modulate many essential processes during plant growth and development (Sheen and others 1999). Genetic screens of sugar-signaling mutants in *Arabidopsis* have uncovered the involvement of ethylene and ABA in sugar responses (Leon and Sheen 2003). Whereas the ethylene overproducer mutant, *eto1*, and constitutive ethylene-response mutant, *ctr1*, are insensitive to exogenous glucose (Cheng and others 2002), several ethylene-insensitive mutants display hypersensitivity to glucose (Zhou and others 1998; Leon and Sheen 2003). Similarly, the *ein3* mutant has a *glo* (*glucose-oversensitive*) phenotype, and overexpression of *EIN3* decreases glucose sensitivity (Yanagisawa and others 2003). Furthermore, glucose was found to enhance the degradation of EIN3 protein through the plant glucose sensor HXK1, suggesting that regulation of EIN3 protein stability could possibly contribute to the mechanism of glucose–ethylene cross-talk (Yanagisawa and others 2003; Moore and others 2003).

A recent study reported a possible role of light in the maintenance of EIN3/EIL1 protein levels (Lee and others 2006). It was found that ethylene induces the expression of an ACC oxidase gene through EIN3/EIL1 transcription factors, and this induction is enhanced under light conditions. An *in vitro* degradation assay showed that the stability of EIN3/EIL1 proteins is maintained by light, as well as by ethylene (Lee and others 2006). Thus, ethylene might interplay with light, sugar, and various hormones in many different ways, and EIN3 could be a common regulatory node among ethylene and other signaling pathways.

Conclusions and Perspectives

Although significant progress has been made using a combination of genetic and biochemical approaches to understanding the ethylene-signaling pathway in plants, many challenging questions still remain to be answered. Particularly, we are at the very beginning of understanding the biochemical features and the regulatory mechanisms of the known key components in the pathway, as well as the molecular basis of various interactions between ethylene and other signaling pathways. For instance,

1. In the case of signal perception, it is not clear how ethylene receptors perceive signals and how ethylene modulates the receptors' functions.
2. With respect to signal transduction, it remains unknown how the receptors regulate the action of CTR1, how CTR1 turns off downstream signaling, and what the biochemical functions of EIN2 are.
3. In terms of transcriptional regulation, whether other EILs participate in the ethylene-response pathway, how they carry out their functions, and what their direct target genes are need to be addressed.
4. It is intriguing to know how EIN5 regulates the level of *EBF1* and *EBF2* mRNAs; moreover, the molecular and biochemical characterization of EIN6 is yet to be completed.
5. Despite the observation of a wide range of cross-talks between ethylene and other hormones, our current knowledge of the mechanisms underlying each of them is far from complete.

In the upcoming years, we should see new biochemical mechanisms and complex interaction networks revealed through the identification of additional signaling components by genetic means, or through the isolation of proteins that interact with the known components. New genetic screens

such as activation tagging approaches to obtain gain-of-function mutants, and searching for suppressors or enhancers of existing mutants would be helpful for further dissection of the ethylene-signaling pathway. In addition, because transcriptional regulation seems to play an essential role in regulating ethylene responses, genome-wide studies of gene expression and proteomic profiles would provide new information to advance ethylene research.

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REFERENCES

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, and others. 2006. Integration of plant responses to environmentally activated phytohormonal signals. Science 311(5757):91–94.

Achard P, Vriezen WH, Van Der Straeten D, Harberd NP. 2003. Ethylene regulates arabidopsis development via the modulation of DELLA protein growth repressor function. Plant Cell 15:2816–2825.

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. 1999. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science 284(5423):2148–2152.

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, and others. 2003a. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 301:653–657.

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, and others. 2003b. Five components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in *Arabidopsis*. Proc Natl Acad Sci USA 100:2992–2997.

Barry CS, Giovannoni JJ. 2006. Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci USA 103:7923–7928.

Bartel B. 1997. Auxin biosynthesis. Annu Rev, Plant Physiol Plant Mol Biol 48:51–66.

Benavente LM, Alonso JM. 2006. Molecular mechanisms of ethylene signaling in *Arabidopsis*. Mol Biosyst 2:165–173.

Berrocal-Lobo M, Molina A. 2004. Ethylene response factor 1 mediates *Arabidopsis* resistance to the soilborne fungus *Fusarium oxysporum*. Mol Plant Microbe Interact 17:763–770.

Bleecker AB. 1999. Ethylene perception and signaling: an evolutionary perspective. Trends Plant Sci 4:269–274.

Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18.

Chang C, Bleecker AB. 2004. Ethylene biology. More than a gas. Plant Physiol 136:2895–2899.

Chang C, Stadler R. 2001. Ethylene hormone receptor action in *Arabidopsis*. Bioessays 23:619–627.

Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, and others. 1997. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell 89:1133–1144.

Chen YF, Etheridge N, Schaller GE. 2005. Ethylene signal transduction. Ann Bot (Lond) 95:901–915.

Cheng H, Qin L, Lee S, Fu X, Richards DE, and others. 2004. Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131:1055–1064.

Cheng WH, Endo A, Zhou L, Penney J, Chen HC, and others. 2002. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14:2723–2743.

De Paepe A, Vuylsteke M, Van Hummelen P, Zabeau M, Van Der Straeten D. 2004. Transcriptional profiling by cDNA-AFLP and microarray analysis reveals novel insights into the early response to ethylene in *Arabidopsis*. Plant J 39:537–559.

Deshaies RJ. 1999. SCF and Cullin/Ring H2-based ubiquitin ligases. Annu Rev Cell Dev Biol 15:435–467.

Ecker JR. 1995. The ethylene signal transduction pathway in plants. Science 268:667–675.

Ecker JR. 2004. Reentry of the ethylene MPK6 module. Plant Cell 16:3169–3173.

Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, and others. 2004. Arabidopsis EIN3-binding F-box 1 and 2 form ubiquitin–protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. Proc Natl Acad Sci USA 101:6803–6808.

Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, and others. 2003. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. J Biol Chem 278:34725–34732.

Gazzani S, Lawrenson T, Woodward C, Headon D, Sablowski R. 2004. A link between mRNA turnover and RNA interference in *Arabidopsis*. Science 306:1046–1048.

Guo H, Ecker JR. 2003. Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell 115:667–677.

Guo H, Ecker JR. 2004 The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7:40–49.

Guzman P, Ecker JR. 1990. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2:513–523.

Hall AE, Bleecker AB. 2003. Analysis of combinatorial loss-of-function mutants in the Arabidopsis ethylene receptors reveals that the ers1 etr1 double mutant has severe developmental defects that are EIN2 dependent. Plant Cell 15:2032–2041.

Hall BP, Qureshi SN, Schaller GE, 2007. Ethylene receptors: ethylene perception and signal transduction. J Plant Growth Regul (DOI: 10.1007/s00344-9000-0).

Harberd NP. 2003. Botany. Relieving DELLA restraint. Science 299:1853–1854.

Harpham NVJ, Berry AW, Knee EM, Rovedahoyos G, Raskin I, and others. 1991. The effect of ethylene on the growth and development of wide-type and mutant *Arabidopsis-thaliana* (L) Heynh. Ann Bot (Lond):55–61.

Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, and others. 1999. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis. Cell 97(3):383–393.

Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 94:261–271.

Huang Y, Li H, Hutchison CE, Laskey J, Kieber JJ. 2003. Biochemical and functional analysis of CTR1, a protein kinase that

negatively regulates ethylene signaling in *Arabidopsis*. Plant J 33:221–233.

Iordachescu M, Verlinden S. 2005. Transcriptional regulation of three EIN3-like genes of carnation (*Dianthus caryophyllus* L. cv. Improved White Sim) during flower development and upon wounding, pollination, and ethylene exposure. J Exp Bot 56:2011–2018.

Johnson PR, Ecker JR. 1998. The ethylene gas signal transduction pathway: a molecular perspective. Annu Rev Genet 32:227–254.

Kastenmayer JP, Green PJ. 2000. Novel features of the XRN-family in *Arabidopsis*: evidence that AtXRN4, one of several orthologs of nuclear Xrn2p/Rat1p, functions in the cytoplasm. Proc Natl Acad Sci USA 97:13985–13990.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. 1993. *CTR1*, a negative regulator of the ethylene response pathway in arabidopsis, encodes a member of the raf family of protein kinases. Cell 72:427–441.

Kosugi S, Ohashi Y. 2000. Cloning and DNA-binding properties of a tobacco ethylene-insensitive3 (EIN3) homolog. Nucleic Acids Res 28:960–967.

Larsen PB, Chang C. 2001. The Arabidopsis *eer1* mutant has enhanced ethylene responses in the hypocotyl and stem. Plant Physiol 125:1061–1073.

Lee JH, Deng XW, Kim WT. 2006. Possible role of light in the maintenance of EIN3/EIL1 stability in *Arabidopsis* seedlings. Biochem Biophys Res Commun 35:484–491.

Lee JH, Kim WT. 2003. Molecular and biochemical characterization of VR-EILs encoding mung bean ETHYLENE INSENSITIVE3-LIKE proteins. Plant Physiol 132:1475–1488.

Lehman A, Black R, Ecker JR. 1996. HOOKLESS1, an ethylene response gene, is required for differential cell elongation in the *Arabidopsis* hypocotyl. Cell 85:183–194.

Leon P, Sheen J. 2003. Sugar and hormone connections. Trends Plant Sci 8:110–116.

Li H, Johnson P, Stepanova A, Alonso JM, Ecker JR. 2004. Convergence of signaling pathways in the control of differential cell growth in *Arabidopsis*. Dev Cell 7:193–204.

Liu Y, Zhang S. 2004. Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. Plant Cell 16:3386–3399.

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R. 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15:165–178.

Mao C, Wang S, Jia Q, Wu P. 2006. *OsEIL1*, a rice homolog of the *Arabidopsis EIN3* regulates the ethylene response as a positive component. Plant Mol Biol 61:141–152.

Menke FL, van Pelt JA, Pieterse CM, Klessig DF. 2004. Silencing of the mitogen-activated protein kinase MPK6 compromises disease resistance in *Arabidopsis*. Plant Cell 16:897–907.

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, and others. 2003. Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300:332–336.

Nehring R, Ecker JR. 2004. In: Ethylene signal transduction in stem elongation. Plant Hormones: Biosynthesis, Signal Transduction, Action! Edited by P. J. Davies. Dordrecht, the Netherlands, Kluwer, p. 350–368.

Ohme-Takagi M, Shinshi H. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7:173–182.

Olmedo G, Guo H, Gregory BD, Nourizadeh SD, Aguilar-Henonin L, and others. 2006. ETHYLENE-INSENSITIVE5 encodes a 5′ → 3′ exoribonuclease required for regulation of the EIN3-targeting F-box proteins EBF1/2. Proc Natl Acad Sci USA 103:13286–13293.

O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, and others. 2005. Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from *Arabidopsis* and tomato. Plant J 41:651–659.

Ouaked F, Rozhon W, Lecourieux D, Hirt H. 2003. A MAPK pathway mediates ethylene signaling in plants. Embo J 22:1282–1288.

Penninckx IA, Thomma BP, Buchala A, Metraux JP, Broekaert WF. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103–2113.

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, and others. 2003. EIN3-dependent regulation of plant ethylene hormone signaling by two Arabidopsis F box proteins: EBF1 and EBF2. Cell 115:679–689.

!Potuschak T, Vansiri A, Binder BM, Lechner E, Vierstra RD, and others. 2006. The exoribonuclease XRN4 is a component of the ethylene response pathway in *Arabidopsis*. Plant Cell 18:3047–3057.

Resnick JS, Wen CK, Shockey JA, Chang C. 2006. REVERSION-TO-ETHYLENE SENSITIVITY1, a conserved gene that regulates ethylene receptor function in *Arabidopsis*. Proc Natl Acad Sci USA 103:7917–7922.

Rieu I, Mariani C, Weterings K. 2003. Expression analysis of five tobacco EIN3 family members in relation to tissue-specific ethylene responses. J Exp Bot 54:2239–2244.

Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, and others. 1999. A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science 283:996–968.

Roman G, Ecker JR. 1995. Genetic analysis of a seedling stress response to ethylene in *Arabidopsis*. Phil Trans R Soc Lond B Biol Sci 350:75–81.

Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR. 1995. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics 139:1393–1409.

Schaller GE, Bleecker AB. 1995. Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. Science 270:1809–1811.

Schaller GE, Kieber JJ. 2002. Ethylene. In Somerville C, Meyerowitz E, editors. The Arabidopsis Book (TAB, online), ISSN: 1543–8120, Rockville, Maryland, USA, American Society of Plant Biologists [DOI/101199/tab0071].

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, and others. 2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci USA 97:11655–11660.

Sheen J, Zhou L, Jang JC. 1999. Sugars as signaling molecules. Curr Opin Plant Biol 2:410–418.

Solano R, Stepanova A, Chao Q, Ecker JR. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714.

Souret FF, Kastenmayer JP, Green PJ. 2004. AtXRN4 degrades mRNA in *Arabidopsis* and its substrates include selected miRNA targets. Mol Cell 15:173–183.

Stepanova AN, Alonso JM. 2005. *Arabidopsis* ethylene signaling pathway. Sci STKE 2005:cm4.

Stepanova AN, Ecker JR. 2000. Ethylene signaling: from mutants to molecules. Curr Opin Plant Biol 3:353–360.

Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM. 2005. A Link between ethylene and auxin uncovered by the characterization

of two root-specific ethylene-insensitive mutants in *Arabidopsis*. Plant Cell 17:2230–2242.

Tieman DM, Ciardi JA, Taylor MG, Klee HJ. 2001. Members of the tomato LeEIL (EIN3-like) gene family are functionally redundant and regulate ethylene responses throughout plant development. Plant J 26:47–58.

Vriezen WH, Achard P, Harberd NP, van Der Straeten D. 2004. Ethylene-mediated enhancement of apical hook formation in etiolated *Arabidopsis thaliana* seedlings is gibberellin dependent. Plant J 37:505–516.

Woeste KE, Kieber JJ. 2000. A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype. Plant Cell 12:443–455.

Yanagisawa S, Yoo SD, Sheen J. 2003. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature 425:521–525.

Zhong GV, Burns JK. 2003. Profiling ethylene-regulated gene expression in *Arabidopsis thaliana* by microarray analysis. Plant Mol Biol 53:117–131.

Zhou L, Jang JC, Jones TL, Sheen J. 1998. Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. Proc Natl Acad Sci USA 95:10294–10299.