The Ethylene Signaling Pathway

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Plants use a structurally very simple gas molecule, the hydrocarbon ethylene, to modulate various developmental programs and coordinate responses to a multitude of external stress factors. How this simple molecule generates such a diverse array of effects has been the subject of intense research for the past two decades. A fascinating signaling pathway, with classical as well as novel plant-specific signaling elements, is emerging from these studies. We describe the four main modules that constitute this signaling pathway: a phosphotransfer relay, an EIN2-based unit, a ubiquitin-mediated protein degradation component, and a transcriptional cascade. The canonical and *Arabidopsis* ethylene signaling pathways in the Signal Transduction Knowledge Environment Connections Maps provide a complete panoramic view of these signaling events in plants.

Several signaling molecules with hormone-like functions have been identified in plants, the simplest of all being ethylene. Despite its structural simplicity, this gaseous hormone plays a critical role in the regulation of developmental programs throughout the plant life cycle and serves as a major response mediator to various environmental signals (*1*–*3*). Seed germination, cell elongation, fertilization, fruit ripening, seed dispersal, defense against pathogens, and response to external stress factors are among the essential processes regulated by ethylene (*2*, *4*). A combination of genetic, biochemical, and molecular approaches is uncovering this remarkable signaling pathway in plants (*5*). Although the initial hunt for the major elements of the ethylene pathway was performed in the model plant *Arabidopsis thaliana*, identification and functional analysis of the corresponding genes in other plant species uncovered a high degree of conservation of this signaling cascade in the plant kingdom (*6*). The general components of the pathway are described in (*7*); a more detailed view focusing on *Arabidopsis* is presented in (*8*).

The first prerequisite for any signaling molecule to be functional is the existence of a detection system to precisely monitor its levels. The specific recognition of ethylene by a receptor protein presents uncommon challenges because of the extreme structural simplicity of this hormone and the consequent small number of possible interaction points between the signal molecule and its receptor. In plants, this challenge is met by a family of endoplasmic reticulum (ER)–localized ethylene receptors that share sequence similarity with the bacterial two-component histidine kinases. The particular physicochemical properties of the ethylene gas allow it to freely diffuse through the membranes and the cytoplasm, eliminating the need for an active transporter system to deliver the ligand to its receptors in the ER. The required high binding affinity and specificity of the ethylene receptors is achieved with the help of a copper cofactor associated with the hydrophobic ligand-binding pocket of the receptor molecule (*9*–*11*). Mutations in the hydrophobic domain of any of the five *Arabidopsis* receptors—ETR1, ETR2, EIN4, ERS1, and ERS2—result in dominant ethylene insensitivity of the corresponding mutant plants (*12*, *13*). Interestingly, some of these mutations abolish ethylene binding, which suggests that in the absence of the hormone, the receptors actively repress the ethylene response (*14*). Conclusive evidence for the negative regulatory role of the receptors was obtained from the analysis of loss-of-function mutants (*15*). Lack of phenotypes in the single loss-of-function receptor mutants indicated a high degree of functional redundancy among the receptors, whereas the constitutive activation of the ethylene response in double, triple, and quadruple loss-of-function mutants confirmed the role of the receptors as repressors of this signaling pathway (*15*).

In contrast with the ethylene-binding domain, the histidine and receiver domains of the receptors are not conserved across the five family members. Only ETR1 and ERS1 contain a canonical histidine kinase moiety, and the receiver domain is completely absent in ERS1 and ERS2 (*12*). The role of these two domains in ethylene signal transduction remains controversial. Analysis of the gain- and loss-of-function mutants suggests that although all of the receptors can sense ethylene, only the histidine kinase domain of ETR1 and ERS1 can transduce the signal to the downstream components. On the other hand, the essential role of the histidine kinase is by itself questionable, because the expression of a kinase-dead version of ETR1 in the double hypomorphic mutant *etr1 ers1* is capable of restoring the normal ethylene response (*16*). Furthermore, ERS1 and all of the type II receptors (ERS2, ETR2, and EIN4) have recently been shown to possess a serine kinase activity in vitro, thus suggesting an alternative, histidine kinase–independent mechanism to transduce the phosphorylation signal from the receptors to the downstream components (*17*). The in vivo roles of both the histidine and the serine kinase activities need to be tested in sensitive assays using new receptor mutants harboring mutations in the kinase domain.

Almost nothing is known about the role of the receiver domain in the receptor function. Along with the kinase domain, it participates in the protein-protein interaction between the receptor and yet another negative regulator of the pathway: CTR1, a Raf-like serine-threonine kinase (*18*). This interaction is important not only for the activation of CTR1 by the receptors, but also for its proper subcellular localization in the ER (*19*). Both null and kinase-dead CTR1 mutant alleles result in the constitutive activation of ethylene responses, indicating the negative regulatory role of CTR1 and suggesting that the kinase activity is important for the function of this protein in plants (*20*, *21*). It is not yet understood how the ETR1-CTR1 complex inactivates EIN2, the positive component of the pathway that genetically works downstream of CTR1. Studies in both *Arabidopsis* and *Medicago* suggested that a mitogen-activated protein kinase (MAPK) cascade might be involved in this step of the pathway (*22*). Considering all of the data available to date, a mechanistic model has been proposed (Fig. 1) in which the receptors are active in the absence of ethylene and stimulate the negative regulator CTR1, which in turn shuts off the ethylene pathway. Binding of ethylene to the receptors relieves this CTR1-mediated blockage by rendering the receptors (and hence CTR1) inactive (*15*). Awaiting final confirmation is the possibility that CTR1 regulates the activity of EIN2 by inactivating a MAPK cascade comprising SIMKK and MPK6.

The complete ethylene insensitivity of loss-of-function *ein2* mutants indicates the critical role this protein plays in the ethylene response. Despite the sequence similarity of EIN2 to the NRAMP family of metal ion transporters, no biochemical function has been assigned to EIN2 (*23*). The ability of high levels of the EIN2 C terminus to constitutively activate the ethylene response suggests that this unique domain of the protein participates in the transduction of the signal to the downstream components, whereas the NRAMP-like domain may sense the upstream signals (*23*). The mechanism by which the ethylene signal is transduced from CTR1 to EIN2, and the

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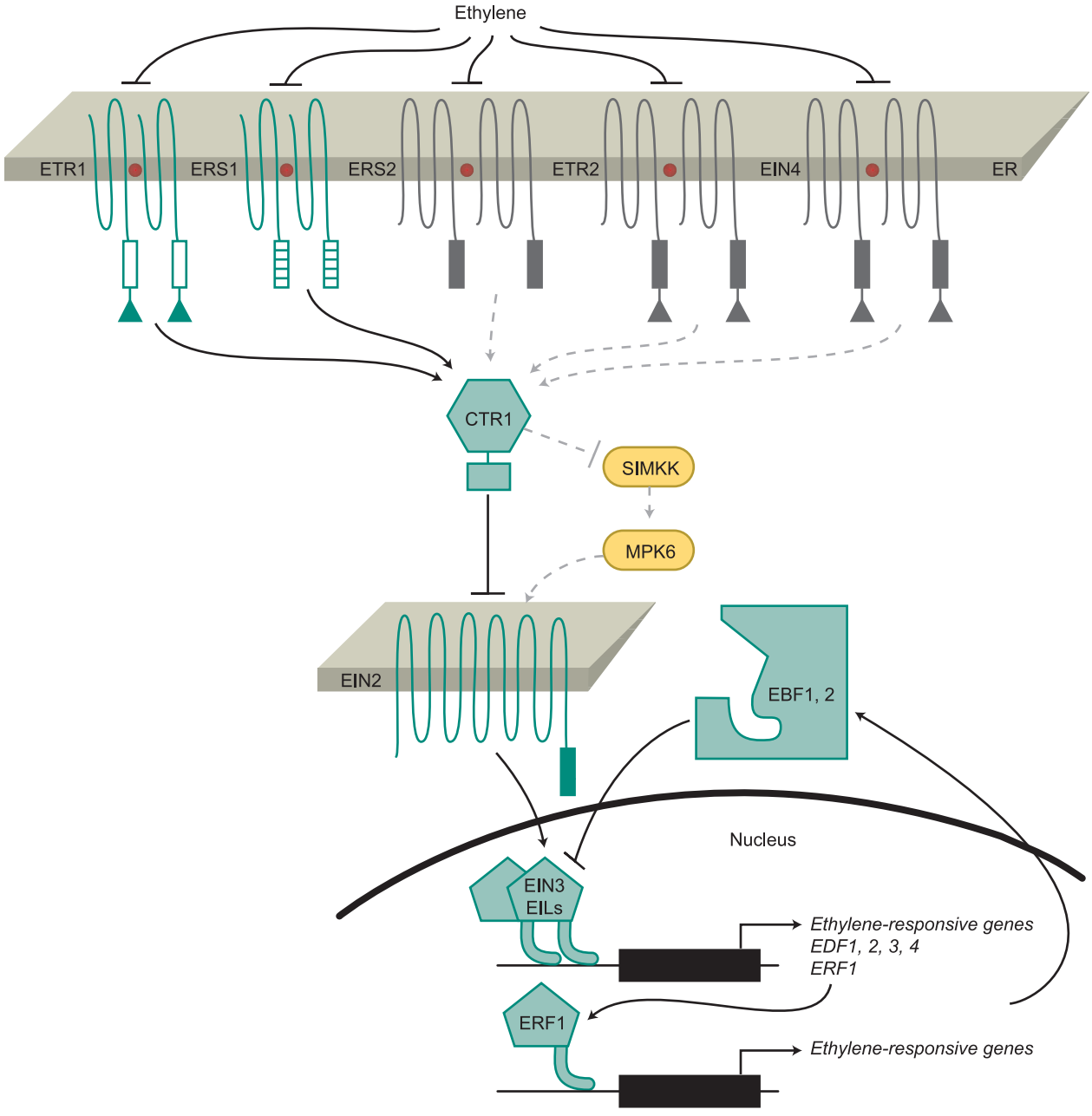

Fig. 1. Representation of the ethylene signal transduction pathway. Ethylene is perceived by a family of receptors located in the ER membrane. Binding of ethylene to the hydrophobic pocket of the receptors is mediated by a copper cofactor. The receptors physically interact with the Raf-like kinase CTR1. Binding of ethylene to the receptors results in the inactivation of both receptors and CTR1, causing derepression of a positive regulatory molecule, EIN2. A MAPK cascade (yellow) may be involved in the signal transduction between CTR1 and EIN2. By an unknown mechanism, a positive signal is then transmitted from EIN2 to the transcription factors EIN3/EILs, resulting in the stabilization and, consequently, accumulation of the EIN3/EIL proteins in the nucleus, where they induce transcription of *ERF1*, *EDF1*, *2*, *3*, *4*, and other ethylene-regulated genes as the first step in a transcriptional cascade that unleashes the downstream ethylene responses. The levels of EIN3 are regulated by two F-box proteins, EBF1 and 2, whose transcription is inducible by ethylene.

question of how EIN2 activates the EIN3 family of transcription factors, remain two of the most intriguing aspects of ethylene signaling.

The ethylene signal arrives at the nucleus through EIN3, a plant-specific transcription factor that belongs to a small gene family (*24*). Although there are five other *EIN3*-like genes (*EILs*) in *Arabidopsis*, the nearly complete ethylene insensitivity of the *ein3 eil1* double mutant suggests that the rest of the *EILs* may have a marginal role in the response to high levels of ethylene used in the typical ethylene response assays (*25*). The marked reduction in the ethylene sensitivity of plants harboring mutations in these transcription factors also highlights the critical role of transcription in ethylene responses. Modulation of the *EIN3* activity by ethylene is achieved, at least in part, through the control of EIN3 protein levels (*26*, *27*). The direct interaction between EIN3 and two F-box proteins (EBF1 and EBF2), the phenotypic analysis of loss- and gain-of-function *ebf* mutants, and the results of pharmacological studies clearly implicate an SCF (SKP1/cullin/F-box protein) E3 ubiquitin ligase complex and the proteasome in the regulation of EIN3 protein levels (*26*–*28*). The ethylene-mediated modification of EIN3, EBFs, or both that prevents EIN3 from being targeted for degradation remains unknown. The stabilization of EIN3 by ethylene results in the transcriptional activation of hundreds of genes (*29*). The search for direct targets of

EIN3 resulted in the identification of *ERF1*, a gene encoding a member of the EREBP family of transcription factors whose expression is rapidly induced by ethylene in an EIN3-dependent manner and whose promoter harbors an EIN3-binding site (*30*). In addition to being regulated by EIN3, *ERF1* is also induced by jasmonate and is likely to represent a point of interaction between the response pathways of these two hormones (*31*). Additional putative targets of EIN3 have been identified among other members of the EREBP family of transcription factors. Four genes, *EDF1* to *EDF4*, that encode proteins with two distinct plant-specific DNA-binding domains, AP2 (a feature common to all EREBP family members) and B3, have been found to be rapidly induced by ethylene and to control a subset of ethylene responses (*29*). A transcriptional cascade acting downstream of EIN3 offers the opportunity not only to explain the diversity of ethylene effects but also to integrate ethylene with other signaling pathways.

Ethylene signal transduction and its cross-talk with other signals remain an exciting topic of current research in plants. It is foreseeable that the ethylene field will continue to expand and offer many interesting discoveries as the existing gaps in the pathway are filled in.

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VIEWPOINT

Keeping the Leaves Green Above Us

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The plant immune system relies to a great extent on the highly regulated expression of hundreds of defense genes encoding antimicrobial proteins, such as defensins, and antiherbivore proteins, such as lectins. The expression of many of these genes is controlled by a family of mediators known as jasmonates; these cyclic oxygenated fatty acid derivatives are reminiscent of prostaglandins. The roles of jasmonates also extend to the control of reproductive development. How are these complex events regulated? Nearly 20 members of the jasmonate family have been characterized. Some, like jasmonic acid, exist in unmodified forms, whereas others are conjugated to other lipids or to hydrophobic amino acids. Why do so many chemically different forms of these mediators exist, and do individual jasmonates have unique signaling properties or are they made to facilitate transport within and between cells? Key features of the jasmonate signal pathway have been identified and include the specific activation of E3-type ubiquitin ligases thought to target as-yet-undescribed transcriptional repressors for modification or destruction. Several classes of transcription factor are known to function in the jasmonate pathway, and, in some cases, these proteins provide nodes that integrate this network with other important defensive and developmental pathways. Progress in jasmonate research is now rapid, but large gaps in our knowledge exist. Aimed to keep pace with progress, the ensemble of jasmonate Connections Maps at the Signal Transduction Knowledge Environment describe (i) the canonical signaling pathway, (ii) the *Arabidopsis* signaling pathway, and (iii) the biogenesis and structures of the jasmonates themselves.

The importance of the jasmonate pathway, in a nutshell, is that it plays a central role in maintaining the balance between biomass in the green and red kingdoms, between plants and animals. Plants unable to synthesize or perceive jasmonates are highly susceptible to a wide range of herbivores and pathogens (*1*, *2*), because the pathway regulates the expression of a plethora of inducible defense-related genes. For example, within 3 to 5 hours of insects' feeding on leaves, the levels of hundreds of transcripts change, and of these, 67 to 84% are estimated to be under the control of the jasmonate pathway (*3*). In addition, and depending on the plant species in question, jasmonate signaling components can control the development and/or function of entire defensive structures, such as trichomes (*4*) or extrafloral nectaries (*5*). The biological roles of jasmonates extend to reproductive development. Here, differences between species are apparent. Unlike *Arabidopsis*, in which mutations that impair jasmonate perception have a particularly high impact on male fertility, similar mutations in tomato have a much greater effect on female fertility (*4*). The pathway even boasts its own novel organelle; the wound-stimulated biogenesis of endoplasmic reticulum (ER) bodies is jasmonate-dependent (*6*), although functions for this new structure have yet to be defined.

Jasmonates are small lipid derivatives. Discounting enantiomeric variants, about 20 naturally occurring jasmonates have been described. This growing family is presented at the Jasmonate Biochemical Pathway at STKE (*7*). Several members of the jasmonate family may have discrete roles as signals. In *Arabidopsis*, jasmonic acid (JA) is necessary for the expression of a number of genes, whereas cyclopentenone jasmonates may regulate others (*7*). Furthermore, the *Arabidopsis* JASMONIC ACID RESISTANT 1 (JAR1) protein is an ATP-dependent JA-amino synthetase that conjugates JA to hydrophobic amino acids, in particular, isoleucine (Ile) (*8*). The *jar1* mutation renders root growth less sensitive to exoge-

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