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Update on Signal Transduction

The Mechanism of Ethylene Perception[1]

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Almost one century ago, Anton Nicolyovitch Neljubov determined that the gaseous olefin ethylene was capable of altering the development of higher plants in a variety of ways. In the ensuing decades, the involvement of endogenously produced ethylene in plant growth and development was firmly established (reviewed by Abeles et al., 1992). Ethylene has been implicated as a factor that controls the timing of seed germination, the rate and dimensions of etiolated seedling growth and leaf expansion, the initiation and progression of abscission and fruit ripening, and the expression of a number of stress-related responses in plants (Abeles et al., 1992). More recently, the biochemical and molecular characterization of the biosynthetic pathway for ethylene has provided insights into the mechanisms by which plants control the internal concentrations of this hormone (reviewed by Kende, 1993).

Understanding the mechanisms by which plant cells perceive and transduce the ethylene signal has been a more daunting problem. Physiochemical considerations have prompted a number of researchers to postulate that ethylene might interact with a receptor through a protein-bound transition metal such as Cu(I) (Burg and Burg, 1967; Kovacic et al., 1991; Sisler, 1991). High-affinity, saturable binding sites for ethylene have been detected in plant tissues and extracts (reviewed by Sisler, 1991). However, the physiological relevance of these sites with respect to ethylene responses has never been demonstrated, nor have the biochemical agents responsible for binding been isolated and characterized from plants.

Recent advances in understanding ethylene signal transduction have come from pursuing a genetic approach with *Arabidopsis thaliana*. A number of mutants affecting ethylene responses in Arabidopsis have been identified (reviewed by Ecker, 1995), and these define a common initial pathway for all recognized ethylene-induced changes in the plant. In this *Update*, we will describe the initial pathway for ethylene signal transduction as it is currently understood and emphasize, in particular, evidence that the *ETR1* gene codes for an ethylene receptor. We will also consider possible mechanisms by which the ETR1 protein perceives and transduces the ethylene signal and how dominant mutations in *ETR1* and related isoforms confer ethylene insensitivity on plant tissues.

THE PATHWAY FOR ETHYLENE SIGNAL TRANSDUCTION

As first reported by Neljubov, applied ethylene inhibits elongation of both shoots and roots of dark-grown seedlings. This effect appears to be the result of a decrease in cell proliferation and a reorientation of the direction of cell expansion in the stem from the vertical to isodiametric, resulting in a shorter, thicker stem (Eisinger, 1983). The seedling bioassay has been used to isolate mutants in Arabidopsis that are either insensitive to applied ethylene (Bleecker et al., 1988; Guzman and Ecker, 1990; Van der Straeten et al., 1993; Roman et al., 1995) or express constitutively the ethylene-induced phenotype (Kieber et al., 1993). Several classes of mutants obtained using the seedling bioassay as a screen affect a broad range of ethylene responses throughout the life cycle of the plant, indicating that these responses share a primary signal transduction pathway. In particular, multiple mutant alleles at the *ETR1* and *EIN2* loci confer global insensitivity to ethylene (Guzman and Ecker, 1990; Chang et al., 1993). In contrast, mutations at the *CTR1* locus lead to constitutive activation of ethylene-regulated pathways (Kieber et al., 1993), indicating that *CTR1* is a negative regulator of the ethylene response. Double-mutant analysis indicates that *CTR1* is epistatic to *ETR1* and that *EIN2* is epistatic to *CTR1* (Kieber et al., 1993). Based on these findings, a model for ethylene signal transduction has been proposed (Ecker, 1995) in which the product of the *ETR1* gene acts earliest in the signal cascade (Fig. 1).

Both *CTR1* and *ETR1* have recently been cloned. Their deduced amino acid sequences reveal that plants have incorporated two evolutionarily distinct components, one typically associated with prokaryotic and the other with eukaryotic signal transduction systems, into a single signal transduction pathway. The amino acid sequence of ETR1 is closely related to members of the two-component signal transduction systems from bacteria (Chang et al., 1993) and contains all of the conserved residues required for His kinase activity. CTR1 is related to the RAF-type Ser/Thr

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Abbreviations: MAP, mitogen-activated protein; MAPKKK, MAP kinase kinase kinase; nr, never-ripe.

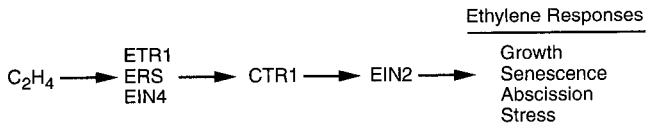

Figure 1. Proposed linear sequence of gene action in the ethylene signal transduction pathway. The sequence is based on the finding that double mutants of *ctr1* with *ein2* show the ethylene-insensitive phenotype of *ein2*, whereas double mutants of *etr1*, *ers*, or *ein4* with *ctr1* show the constitutive response phenotype of *ctr1* (Kieber et al., 1993; Chen and Bleecker, 1995; Ecker, 1995). The *etr1*, *ers*, and *ein4* mutants all show similar dominant ethylene-insensitive phenotypes and may all code for isoforms of the ethylene receptor.

protein kinases from mammals (Kieber et al., 1993), indicating that ethylene signal transduction could feed into a MAP kinase cascade, with CTR1 representing a MAPKKK. Thus, based strictly on sequence homology, phosphorylation appears to play a key role in the pathway for ethylene signal transduction. This is consistent with biochemical data from plants in which ethylene-induced changes in protein phosphorylation are observed (Raz and Fluhr, 1993).

This pathway for ethylene signal transduction in plants shows many similarities to an osmosensing, signal transduction pathway currently being elucidated in yeast. This yeast osmosensing pathway also contains elements related to the two-component signal transduction systems of bacteria, as well as elements of a MAP kinase-like cascade. In yeast, changes in osmolarity affect the activity of SLN1, which, like ETR1 in Arabidopsis, is a putative His kinase and represents the first of the two components in the prototypic two-component system (Ota and Varshavsky, 1993; Maeda et al., 1994). Activity of the SLN1 protein is then thought to regulate the phosphorylation state of SSK1, a response regulator that corresponds to the second of the two components (Maeda et al., 1994). From here, the yeast osmosensing pathway feeds directly into a MAP kinase cascade (Brewster et al., 1993; Maeda et al., 1994, 1995) in which SSK1 regulates the activity of SSK2 and SSK22, both CTR1-like MAPKKKs (Maeda et al., 1995).

STRUCTURE OF THE *ETR1* GENE PRODUCT AND PHYSIOLOGY OF *ETR1* MUTANTS

The basic structural elements of the protein encoded by the *ETR1* gene are depicted in Figure 2. The protein contains a hydrophobic domain within the N-terminal third of the protein, followed by a stretch of amino acids that show no significant homology to sequences in the database. The C-terminal portion of the protein contains sequences with striking homology to the His kinases and response regulators of bacterial two-component systems (Chang et al., 1993).

Hydropathy analysis of ETR1 identifies three potential transmembrane segments at the N terminus. Although ETR1 does not contain a canonical signal sequence for membrane insertion, topological analysis using a model recognition approach (Jones et al., 1994) predicts that the N terminus would lie on the extracytoplasmic side of the membrane, followed by the three transmembrane segments, with the large C-terminal domain on the cytoplasmic side of the membrane (Schaller et al., 1995).

Four mutant alleles of the *ETR1* gene have been identified to date. As indicated in Figure 2, all four mutations involve single amino acid changes within the hydrophobic N-terminal domain of the encoded protein, with mutational changes distributed across all three putative transmembrane segments. Dose-response curves for stem and root growth in wild-type and mutant seedlings indicate that the *etr1–1* and *etr1–4* alleles completely eliminate responsiveness to ethylene, whereas the *etr1–2* and *etr1–3* alleles show reduced responsiveness to ethylene. (Note that *etr1–3* and *etr1–4* were originally named *ein1–1* and *ein1–2*, respectively [Guzman and Ecker, 1990].) Further details of the physiology of ethylene responses in the *etr1–1* and *etr1–2* mutant lines may be found in Chen and Bleecker (1995). All four mutant alleles are genetically dominant over the wild-type allele (Bleecker et al., 1988; Guzman and Ecker, 1990; Chang et al., 1993). Plants heterozygous for either *etr1–1* or *etr1–2* show the same degree of insensitivity as the homozygous mutants.

The C-terminal portion of ETR1 is homologous to the His kinases and response regulators of environmental sensory

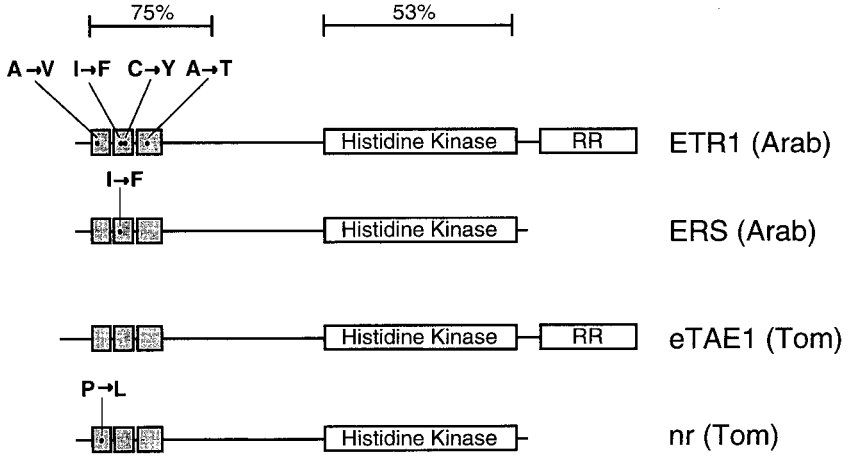

Figure 2. Schematic representations of four putative isoforms of the ethylene receptor from Arabidopsis (Arab; ETR1 and ERS) and tomato (Tom; eTAE1 and nr). The shaded boxes represent hydrophobic sequences. The open boxes labeled Histidine Kinase and RR represent sequences homologous to the kinase and response regulator domains, respectively, of bacterial two-component environmental sensor systems. The percentages at the top of the figure are the amino acid sequence identities for all four isoforms in the region indicated. Specific amino acid alterations that result in dominant ethylene insensitivity are indicated.

systems in bacteria (Chang et al., 1993). These systems allow the bacteria to sense and respond to diverse stimuli and are involved in chemotaxis, osmotic sensing, host recognition, sporulation, and changes in response to various metabolites such as nitrogen and phosphate (reviewed by Stock et al., 1990; Parkinson, 1993; Swanson et al., 1994). These sensory systems contain two conserved motifs that are frequently found on separate proteins, termed the sensor and the response regulator, and for this reason they are referred to as two-component systems. Because of its role in directly sensing environmental stimuli, the sensor is typically membrane localized. The intracellular portion of the sensor contains a transmitter region with His kinase activity, which, in response to an environmental stimulus, autophosphorylates on a conserved His residue. This phosphate is then transferred to an Asp residue of a response regulator. There are bacteria in which His kinase and response regulator domains are present in the same polypeptide, as with ETR1, and in these cases the response regulator domain may serve to modulate activity of the His kinase (Parkinson, 1993; Swanson et al., 1994). However, in all known examples of two-component systems, even in cases with a His kinase fused to a response regulator domain, signal transduction is effected through a separate response regulator. A separate response regulator has not yet been found in the ethylene signal transduction pathway.

IDENTIFICATION OF FUNCTIONAL ISOFORMS OF ETR1

Models that account for the ethylene-insensitive phenotypes of *etr1* mutants must take into account the finding that only dominant mutations have been isolated. One possibility is that functionally redundant isoforms of the receptor are present and a null mutation in one isoform does not result in a detectable ethylene-insensitive phenotype. In this regard, at least two additional genes with high degrees of sequence similarity to *ETR1* have been identified in Arabidopsis (Chang and Meyerowitz, 1995). The *ERS* gene product shows homology to ETR1 throughout but lacks a response regulator domain (Hua et al., 1995). When a mutation equivalent to the *etr1–4* mutation (Ile69Phe) was introduced into a genomic clone of *ERS* and the mutated *ers* gene was transferred into wild-type Arabidopsis, insensitivity to ethylene was conferred on transformed plants (Hua et al., 1995), providing evidence that ERS is functionally related to ETR1. Since mutations in both ETR1 and ERS affect responses in the same range of tissues, some functional redundancy between these two isoforms is implied.

The *ETR1* gene has also been used as a heterologous probe to identify related sequences in tomato. Mapping studies indicated the presence of at least five distinct cross-hybridizing sequences in the tomato genome (Yen et al., 1995). Two of these DNA fragments have been cloned and sequenced (Wilkinson et al., 1995; Zhou et al., 1996). Structural features of the derived amino acid sequences are provided in Figure 2. The *eTAE1* gene product contains all of the structural elements found in the Arabidopsis *ETR1* gene product and has an overall amino acid sequence identity to ETR1 of 81% (Zhou et al., 1996). On the other hand, the *nr* gene product lacks a response regulator domain and is structurally similar to the *ERS* gene product from Arabidopsis (Wilkinson et al., 1995).

A dominant mutation has been identified in the *nr* gene that results in ethylene insensitivity in the mutant tomato plant, establishing the significance of *nr* with respect to ethylene signal transduction (Wilkinson et al., 1995; Yen et al., 1995). Tomato plants carrying the *Nr* mutation show reduced sensitivity to ethylene with respect to seedling growth responses, flower abscission and senescence, and fruit ripening (Lanahan et al., 1994). Sequence analysis indicates that the *Nr* mutant gene product has a single amino acid change located in the first hydrophobic domain of the protein (Fig. 2). It is interesting that wild-type *nr* mRNA levels become elevated in developing tomato fruits at the time when the fruits attain sensitivity to ethylene with respect to the ripening process (Wilkinson et al., 1995). In contrast, *ETR1* and *eTAE1* appear to be expressed constitutively at the mRNA level in most tissues (Zhou et al., 1996).

THE ETR1 PROTEIN FUNCTIONS AS A MEMBRANE-ASSOCIATED DIMER

Biochemical characterization of the ETR1 protein has taken place both in its native Arabidopsis and in a transgenic yeast system (Schaller et al., 1995). When expressed in transgenic yeast, the ETR1 protein is present at about a 100-fold greater level than in its native Arabidopsis. In both transgenic yeast and Arabidopsis, antibodies generated against ETR1 recognize a 79-kD polypeptide, consistent with the molecular mass of 83 kD predicted from the amino acid sequence. Cellular fractionation studies indicate that the protein from both Arabidopsis and transgenic yeast is associated with membranes, as expected based on the hydrophobic domain present at the N terminus of ETR1.

When extracts from both Arabidopsis and transgenic yeast were analyzed by SDS-PAGE in the absence of reducing agent, the majority of immunodetectable ETR1 protein migrated as a 147-kD protein, indicating that the native protein exists as a disulfide-linked dimer (Schaller et al., 1995). Expression of truncated forms of the protein in yeast indicate that the N-terminal hydrophobic domain of the protein is necessary and sufficient for this dimerization. The requirements for formation of the disulfide-linked dimer were further resolved by expressing forms of ETR1 in yeast in which specific Cys residues had been altered. This analysis revealed that a pair of Cys residues at the amino terminus (Cys^4 and Cys^6) are required for formation of the disulfide-linked dimer. These Cys residues are conserved in all homologs of ETR1 identified to date (Chang et al., 1993; Hua et al., 1995; Wilkinson et al., 1995; Zhou et al., 1996), indicating that the ability to form a covalent dimer may be a general attribute of all of these proteins. The finding that the native ETR1 protein exists as a dimer is also of particular interest because the bacterial sensor proteins are known to operate as dimers, with the kinase of one monomer phosphorylating a conserved His residue in

trans on the other monomer (Parkinson, 1993). Although this dimeric form is apparently in place prior to perception of the signal in bacteria, in all known cases it is a noncovalent association. The ETR1 protein appears to be unique in that it is the first example in which monomers are covalently linked.

INTERACTION OF ETHYLENE WITH THE ETR1 PROTEIN

Based on a number of characteristics, it has been hypothesized that the *ETR1* gene could code for an ethylene receptor. Genetic analysis provided evidence that *ETR1* acts upstream of other loci that affect ethylene signal transduction (Kieber et al., 1993), and it was also determined that plants containing the mutant *etr1–1* allele display one-fifth the saturable ethylene binding of that found in wild-type plants (Bleecker et al., 1988). The similarity of ETR1 to sensor proteins in bacteria is consistent with ETR1 serving an ethylene sensor protein (Chang et al., 1993). However, the amino acid sequence of ETR1 does not contain any obvious features suggestive of ethylene-binding capability (e.g. a metal-binding motif). In addition, the fact that only dominant mutations have been isolated at the *ETR1* locus has made an unambiguous assignment of the wild-type function of the protein impossible (Chang et al., 1993; Ecker, 1995).

To determine whether ETR1 is capable of interacting directly with ethylene, experiments were performed on yeast expressing the ETR1 protein (Schaller and Bleecker, 1995). Expression of the full-length ETR1 protein in yeast resulted in the creation of high-affinity binding sites for ethylene (Schaller and Bleecker, 1995) when assayed using a modification of the in vivo [^{14}C]ethylene-binding assay developed by Sisler (1979). These experiments were facilitated by the fact that control yeast showed no saturable binding sites for ethylene. Analysis of mutant forms of the ETR1 protein indicated that the *etr1–1* mutation eliminates the ethylene-binding potential from transgenic yeast. This provides an explanation for the ethylene-insensitive phenotype observed in plants carrying this mutation (Bleecker et al., 1988; Chang et al., 1993) and is also consistent with the decreased ability of *etr1–1* mutant plants to bind ethylene in vivo (Bleecker et al., 1988).

The kinetic analysis of ethylene binding in transgenic yeast (Schaller and Bleecker, 1995) indicated a K_d of 0.04 μL L^{-1} ethylene (gas phase). This value is close to the amount of ethylene required for a half-maximal response in the seedling growth assay (Chen and Bleecker, 1995). The release of bound ethylene from transgenic yeast showed a half-life of 12 h, a rate similar to that observed with one class of binding activity reported from several plant sources (Sanders et al., 1991; Sisler, 1991). The binding of [^{14}C]ethylene in yeast was also inhibited by *trans*-cyclooctene and norbornadiene, both competitive inhibitors of ethylene binding and action in plants (Sisler et al., 1990; Sisler, 1991). Thus, the characteristics of ethylene binding to the ETR1 protein expressed in yeast are quite similar to in vivo binding sites previously observed in plant tissues (Sanders et al., 1991; Sisler, 1991).

Based on two independent lines of evidence, the site of ethylene binding is the N-terminal hydrophobic domain of ETR1. First, only truncated forms of the ETR1 protein that contain the hydrophobic domain produce ethylene-binding sites in transgenic yeast (Schaller and Bleecker, 1995). Second, all four mutations in the *ETR1* gene are point mutations causing single amino acid changes within this hydrophobic domain (Chang et al., 1993), and it has been demonstrated that one of these mutations *(etr1–1)* eliminates ethylene binding by the protein (Schaller and Bleecker, 1995). It should also be noted that this N-terminal region shows the greatest degree of amino acid conservation among the various ETR1 homologs (Fig. 2), attesting to its functional significance.

These biochemical data, coupled with prior genetic data placing ETR1 early in the pathway of ethylene signal transduction (Kieber et al., 1993), serve as compelling evidence that ETR1 functions as an ethylene receptor in plants. Based on the capacity of ETR1 to bind ethylene (Schaller and Bleecker, 1995) and on its homology to the bacterial sensor proteins (Chang et al., 1993), it is also appropriate to refer to ETR1 as an ethylene sensor protein.

ETHYLENE RECEPTORS AND THEIR MECHANISM OF ACTION

The hypothesis that ethylene may bind to its receptor through a transition metal was first suggested in 1967 (Burg and Burg, 1967) and is based on the well-established interactions of olefins with transition metals (Collman et al., 1987; Kovacic et al., 1991). The idea that small, gaseous molecules can bind reversibly to protein-based receptors through direct interaction with a transition metal cofactor is well established; examples include hemocyanin and hemoglobin (da Silva and Williams, 1991), the oxygen-sensing FixL protein in bacteria (Gilles-Gonzalez et al., 1991), and the nitric oxide receptor in animals (Ignarro, 1991). Preference for an olefin-metal-binding site as opposed to a direct interaction of ethylene with the receptor protein is based on the consideration that reasonable models for the direct interactions of ethylene with the protein would involve enzymatic reactions such as hydrogenation or 1,2 addition. These reactions are unlikely to be reversible because of the strength of the σ bonds created. On the other hand, ethylene coordinates readily to metal ions—particularly electron-rich, low-valence ions—to form stable olefin complexes in which the double bond engages in both σ and π acceptor interactions. In contrast to C—C and C—H bonds (99 and 83 kcal/mol), the strength of metal-olefin interactions is significantly weaker (approximately 40 kcal/mol) (Collman et al., 1987), providing a stable ethylene-binding site from which ethylene can more easily dissociate. With the identification of the ETR1 protein as a protein capable of reversibly binding ethylene, the metal-olefin hypothesis is the most viable and reasonable explanation for this interaction.

Direct evidence that ETR1 proteins contain a coordinated transition metal is not yet available. The amino acid sequence of the protein does not show any homology to recognized metal-binding motifs. Nevertheless, it is worth

considering how a transition metal could be coordinated within the hydrophobic domain of the ETR1 protein. Amino acid side chains that could be involved in coordinating a metal include His, Cys, Met, and possibly acidic residues (da Silva and Williams, 1991). Residue Cys^{65} is an excellent candidate as a metal ligand because, when mutated to either a Tyr (etr1–1) or a Ser, the resultant protein no longer binds ethylene when expressed in yeast (Schaller and Bleecker, 1995). On the other hand, conversion of Cys^{99} in the third transmembrane domain to a Ser does not disrupt ethylene binding (Schaller and Bleecker, 1995), indicating that it is not involved in metal coordination. The other known mutations in ETR1 that create dominant insensitivity to ethylene are not likely to be involved directly in metal coordination but may indirectly disrupt metal coordination, ethylene binding, or subsequent conformational changes needed to transduce the ethylene signal to the transmitter domain of the protein. The fact that mutations are found in all three putative membrane-spanning domains (Chang et al., 1993) indicates that the binding pocket for metal chelation and ethylene interaction may be formed by the interactions of these three hydrophobic domains or possibly by interactions between monomer domains within the native dimer of the protein. All of these data are consistent with a model in which a metal is coordinated by amino acid residues in membrane-spanning α helices of the hydrophobic domain of ETR1.

The evidence that the ethylene-binding site is located in the N-terminal hydrophobic domain of the ETR1 protein provides us with a reasonable hypothesis as to how the receptor transduces the ethylene signal. We predict that ethylene binding induces a conformational change within or between the subunits of the ETR1 dimer, consequently altering the rate of *trans*-phosphorylation between the His kinase domains. This basic mechanism is consistent with current models for signal transduction by the related two-component sensory systems in bacteria in which conformational changes in membrane-spanning α helices are thought to transduce signals (Lynch and Koshland, 1991; Milligan and Koshland, 1991). Our proposal that the metal involved in ethylene binding is chelated by residues within α helices in the hydrophobic N terminus of ETR1 is consistent with the mechanism by which conformational changes induced by oxygen binding in hemoglobin are propagated through α helical ligands (da Silva and Williams, 1991) and, perhaps more directly applicable, the mechanism by which the chelation of Cu_B by membrane-spanning α helices in Cyt oxidase functions to induce conformational changes associated with proton pumping (Wikstrom and Babcock, 1990; Tsukihara et al., 1995).

MECHANISMS BY WHICH ETHYLENE RECEPTORS MAY SIGNAL DOWNSTREAM COMPONENTS

Although there is currently no direct biochemical evidence that the kinase domain of the ETR1 protein transmits the ethylene signal by the same mechanism used by the related bacterial two-component systems, the sequence homologies of ETR1 to these systems (Chang et al., 1993) are entirely consistent with this possibility. If the bacterial paradigm applies, we would expect to find a response regulator protein that would act as a substrate for the His kinase of ETR1. However, as yet, the genetic analysis of signal transduction in Arabidopsis has revealed no such component acting between ETR1 and the next identified component of the pathway, CTR1. It is possible that no such protein exists and that the ethylene receptors transmit their signals by some alternative mechanism. In this respect, it should be noted that one class of protein kinases that operate in mitochondria shows strong sequence homology to bacterial His kinases but does not appear to autophosphorylate at the conserved His residue. Rather, these kinases appear to transfer phosphate from ATP directly to Ser residues on substrate proteins that show no homology to bacterial response regulators (Popov et al., 1992, 1993). On the other hand, it is possible that genetic screens for ethylene response mutants have missed an intermediate response regulator protein because of the presence of functionally redundant isoforms of this protein. Support for a response regulator-mediated pathway in eukaryotes is provided by the osmosensing pathway in yeast. In this case the ETR1-like sensor SLN1 interacts with a response regulator protein SSK1 (Maeda et al., 1994).

Despite our current lack of specific knowledge as to how the ethylene receptor transmits information to downstream components, it is instructive to assume that ethylene binding to the receptor alters the activity of the His kinase domain, which in turn influences downstream components CTR1 and EIN2. Two alternative models for the regulation of primary signal transduction are provided in Figure 3. The models are based on the genetic evidence that CTR1 negatively regulates EIN2 and that EIN2 may be responsible for generating some second messenger that drives ethylene responses (Guzman and Ecker, 1990; Kieber et al., 1993; Ecker, 1995).

For model I, it is assumed that ethylene is a positive regulator of His kinase activity of the receptor. The active kinase would act directly or indirectly to decrease the activity of the CTR1 protein kinase. The result would be an increase in EIN2 activity and, consequently, an increase in ethylene responsiveness.

For model II, it is assumed that ethylene is a negative regulator of the His kinase activity of the receptor. In the unbound state, the active kinase would directly or indirectly act to keep CTR1 active. Binding of ethylene to the receptor would inhibit the kinase activity, resulting in decreased activity of CTR1 and consequently an increase in EIN2 activity, leading to increases in ethylene responsiveness. There is precedent for this type of mechanism in bacteria. The oxygen-sensing FixL receptor activates nitrogen fixation genes through its His kinase domain in the absence of oxygen. Oxygen binding to a heme moiety associated with FixL inhibits kinase activity, resulting in the down-regulation of nitrogen fixation genes (Gilles-Gonzalez et al., 1991; Monson et al., 1992).

To differentiate between models I and II, it will probably be necessary to determine biochemically whether ethylene binding increases or decreases kinase activity of the transmitter domain and whether there are indeed response reg-

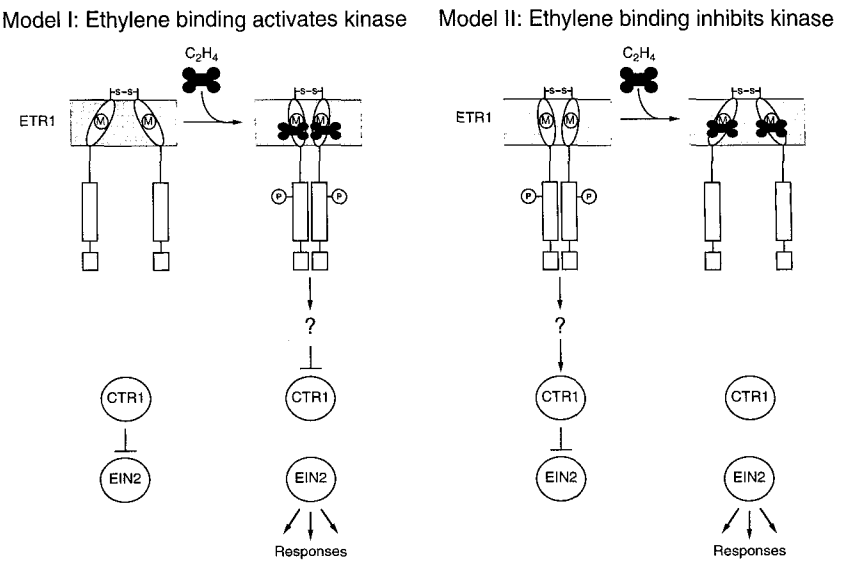

Figure 3. Alternative models for the mechanism of signal transduction from the ethylene receptor. Arrows represent activation steps, and the flat symbols represent inhibitory activities. For both models, it is assumed that ethylene binds to a transition metal (M) chelated in the hydrophobic domain of the receptor and that binding alters the His kinase activity through conformational changes in or between monomers. The question mark indicates the position in the pathway of a postulated response regulator protein. The proposed pathway is based on the assumption that CTR1 acts between ETR1 and EIN2 and functions as a negative regulator of EIN2 (Kieber et al., 1993). s-s, Disulfide bond; P, phosphohistidine.

ulator proteins that transduce the signal from receptor to downstream effectors. It should also be pointed out that models I and II are by no means the only possible mechanisms for signal transduction by the receptor—even if we assume that the bacterial two-component paradigm is correct. There is evidence that bacterial transmitters do not simply exist in a "kinase-on" or "kinase-off" state. In some cases, transmitters in the kinase-off state can act as phosphatases and actually facilitate dephosphorylation of cognate response regulators (Parkinson, 1993). Thus, ethylene may mediate conformational changes in the receptor that shift the transmitter domain between an active kinase state and an active phosphatase state. This consideration increases the number of possible models based on the bacterial paradigm.

THE DOMINANT NATURE OF MUTATIONS IN ETHYLENE RECEPTORS

The fact that only dominant mutations in *ETR1* and *nr* have been isolated from screens for ethylene-insensitive phenotypes is most easily explained by the concept of functional redundancy. Assuming the presence of multiple isoforms of the ethylene receptor, how is it that single amino acid changes in the ethylene sensor domain of one isoform of the protein can lead to complete insensitivity of the tissue to ethylene? In simplified terms, one of two possible mechanisms for genetic dominance is probably operating in these cases. Dominant insensitivity to ethylene is likely to occur by either a dominant negative-type mechanism or a gain-of-function-type mechanism.

As defined by Herskowitz (1987), dominant negative mechanisms often involve complexes of interacting proteins in which one mutant subunit of the complex poisons the entire system. In the case of *ETR1*, imagine a plant that is heterozygous for the *etr1–1* mutation and is thus producing an equal amount of wild-type and mutant monomers of ETR1. If we assume that mutant homodimers and mutant/wild-type heterodimers are functionally inactive, then it is possible that the number of functional receptors in the cell would be reduced to only one-fourth of the normal number. If this reduced number of receptors is below the threshold needed to elicit the seedling growth response under conditions of saturating ethylene concentration, then an ethylene-insensitive phenotype will be observed. Because the mutant forms of one isoform of the receptor appear to be dominant over other isoforms, we would also have to assume that monomers of different isoforms interact and that sufficient mutant monomers are present to reduce the total number of receptors below the threshold needed to elicit responses. This would be true only if the threshold for the seedling growth response required output from a large proportion of total receptors. Alternatively, mutant receptor subunits could inactivate a disproportionate number of wild-type receptor subunits if (a) oligomeric complexes of subunits were needed for signal transduction and (b) small numbers of mutant subunits poisoned the entire complex. Given that all of the dominant mutations are in the ethylene-sensing domain of the receptors and that at least one mutation, *etr1–1*, is known to eliminate ethylene binding (Schaller and Bleecker, 1995), the dominant negative mechanism is most consistent with model I from Figure 3.

A gain-of-function mechanism for dominance of mutants implies that the mutation increases the activity of the gene product or causes it to be active under conditions in which the wild-type protein is not active. If we consider model II from Figure 3, a gain-of-function mechanism would apply if mutations eliminated ethylene binding and locked the mutant receptors in an active state, which suppresses the ethylene response pathways. These mutant receptors would continue to keep response pathways shut off even when all wild-type receptor isoforms are saturated with ethylene. This mechanism would not require that mutant monomers poison wild-type subunits of the receptors. However, this mechanism for dominance and the mechanism for signal transduction illustrated in Figure 3 are difficult to reconcile with the observation that the *nr* gene

of tomato shows increases in mRNA level associated with increasing sensitivity to ethylene-induced fruit ripening (Wilkinson et al., 1995). One would predict that increased expression of a receptor isoform would result in decreased sensitivity to ethylene if model II is correct. Clearly, these issues can be resolved only when the relationships between isoforms are more clearly resolved at the biochemical level.

PROSPECTS FOR THE FUTURE

The identification of ETR1 as an ethylene receptor, coupled with the molecular characterization of additional components of the primary signal transduction pathway for ethylene responses, provides a framework for elucidating the mechanisms by which the ethylene signal is perceived and processed in plants. Many issues remain to be resolved concerning this mechanism of action. For example, we still do not know the mechanism of ethylene binding, much less how the binding of ethylene to the receptor is transduced through the protein and passed on to downstream components. Even this statement represents an oversimplification of a complex process. In Arabidopsis, mutations in *ETR1* block responses that occur over several orders of magnitude of ethylene concentration (Chen and Bleecker, 1995). However, when expressed in yeast, the ETR1 protein binds ethylene over a narrow range of ethylene concentrations. It may be that in Arabidopsis other isoforms of the receptor are required for sensing this broad range of hormone concentration, or perhaps an adaptation mechanism that is similar to the feedback methylation system used by the bacterial sensors operates in plants (Parkinson, 1993). We do not know what role differential expression of receptor isoforms might play in sensitivity changes to ethylene during developmental processes such as fruit ripening and abscission. We also do not know whether isoforms of the receptor interact to produce the observed spectrum of responses or how dominant mutations might disrupt these interactions. Clearly, answers to these and other questions will be forthcoming only by continuing to apply a combination of genetic, molecular, biochemical, and physiological approaches to the problem.

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