THE ROYAL SOCIETY

# The ethylene-receptor family from *Arabidopsis*: structure and function

**Anthony B. Bleecker, Jeffrey J. Esch, Anne E. Hall, Fernando I. Rodríguez and Brad M. Binder**

*Department of Botany, 430 Lincoln Drive, University of Wisconsin-Madison, Madison, WI 53706, USA*

The gaseous hormone ethylene regulates many aspects of plant growth and development. Ethylene is perceived by a family of high-affinity receptors typified by the ETR1 protein from *Arabidopsis*. The *ETR1* gene codes for a protein which contains a hydrophobic N-terminal domain that binds ethylene and a C-terminal domain that is related in sequence to histidine kinase–response regulator two-component signal transducers found in bacteria. A structural model for the ethylene-binding domain is presented in which a Cu(I) ion is coordinated within membrane-spanning α-helices of the hydrophobic domain. It is proposed that binding of ethylene to the transition metal would induce a conformational change in the sensor domain that would be propagated to the cytoplasmic transmitter domain of the protein. A total of four additional genes that are related in sequence to *ETR1* have been identified in *Arabidopsis*. Specific missense mutations in any one of the five genes leads to ethylene insensitivity *in planta*. Models for signal transduction that can account for the genetic dominance of these mutations are discussed.

**Keywords:** ethylene; receptor; signal transduction; plant hormone

## 1. INTRODUCTION

While the concept of small gas molecules acting as biological signals may be something of a novelty in animal systems, almost a century has passed since Neljubov (Abeles *et al.* 1992) demonstrated that nanomolar concentrations of ethylene could elicit dramatic effects on plant growth and development. In addition to the commonly recognized role in fruit ripening, ethylene influences a range of developmental processes throughout the life cycles of higher plants. The stimulation of seed germination, the adjustment of seedling growth to varying soil conditions, the rate and extent of leaf expansion, and the timing of vegetative senescence and abscision are just a few of the plant processes that are regulated by ethylene. Ethylene may also mediate responses to environmental challenges such as wounding, pathogen invasion, and water stress (Abeles *et al.* 1992; Ecker 1995; Bleecker & Schaller 1996; Keiber 1997).

Despite a century of scientific investigation, the mechanisms by which this small gas molecule works at such low concentrations to influence so many different processes in plants remained a mystery. Burg & Burg were the first to hypothesize that ethylene might interact with a transition metal cofactor coordinated in the presumptive receptor for ethylene (Burg & Burg 1965, 1967). In the 1970s and 1980s, investigators in the USA (Sisler 1979) and the UK (Sanders *et al.* 1989, 1991) identified and characterized saturable ethylene binding sites in a variety of plant tissues using $^{14}$C-labelled ethylene. While these sites showed sufficiently high affinity for ethylene to account for biological activity, no direct connection could be made between this ethylene binding and *in vivo* responses to ethylene. Early events in the transduction of the ethylene signal were also a complete mystery. The study of these processes has come of age in the past decade through the use of mutational analysis in *Arabidopsis* to identify the genes coding for early components in ethylene signal transduction (Bleecker *et al.* 1988; Guzman & Ecker 1990; Chang *et al.* 1993; Kieber *et al.* 1993; Roman *et al.* 1995).

The genetic approach to identify the biochemical components involved in ethylene signal transduction is a deceptively simple one, and it illustrates the power of the method. Ethylene inhibits the elongation growth of etiolated (dark-grown) seedlings. Ethylene-insensitive mutants were readily identified in dark-grown seedlings incubated in ethylene (Bleecker *et al.* 1988; Guzman & Ecker 1990; Kieber *et al.* 1993; Roman *et al.* 1995; Chao *et al.* 1997). Insensitive mutants obtained in these screens have been extensively characterized and many of the represented genes have been cloned. This pioneering work by a number of research groups has been the subject of a number of recent reviews (Ecker 1995; Bleecker & Schaller 1996; Kieber 1997; Fluhr 1998) and will not be covered in detail here. Briefly, the framework of the early signal transduction pathway can be proposed from the characteristics of the identified genes. The perception of ethylene is apparently achieved by catalytic receptors coded for by the *ETR1*-like gene family (Chang *et al.* 1993; Hua *et al.* 1995, 1998; Sakai *et al.* 1998). ETR1 is related in structure and sequence to the two-component regulators from bacteria which transduce signals via phosphotransfer reactions (Parkinson 1993). This receptor system signals downstream via an RAF kinase-like negative regulator, designated CTR1,

 

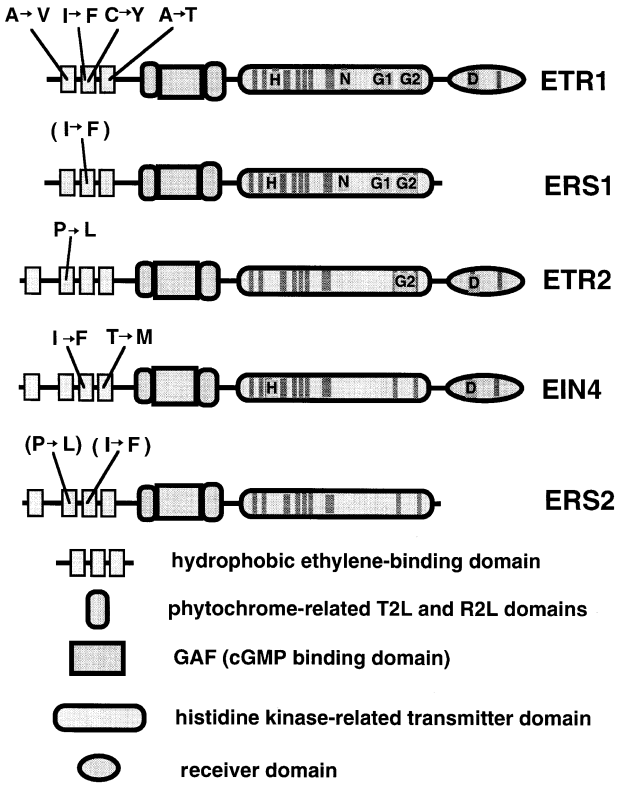


Figure 1. Structural features of the *ETR1*-like gene family. The ethylene binding domain is composed of three putative membrane-spanning domains. The *ETR2*-like subfamily is characterized by a hydrophobic N-terminal extension. The hydrophobic domain is followed by a domain that shows homology to the chromophore-binding domain of phytochrome (Kehoe & Grossman 1996) and contains a cGMP binding motif (Aravind & Ponting 1998). The histidine kinase domains of ETR1 and ERS1 contain all the subdomains indicative of functional kinase activity. The ETR2-like family lacks some of these subdomains (Hua *et al.* 1997, 1998). Receiver domains of ETR1, ETR2 and EIN4 contain all the sequences thought to be essential for phosphotransfer. Amino-acid conversions that lead to dominant ethylene insensitivity are indicated. Amino-acid conversions shown in parentheses were introduced *in vitro* and the mutated genes were transferred back into plants (data from Chang *et al.* 1993; Hua *et al.* 1995, 1998; Sakai *et al.* 1998).

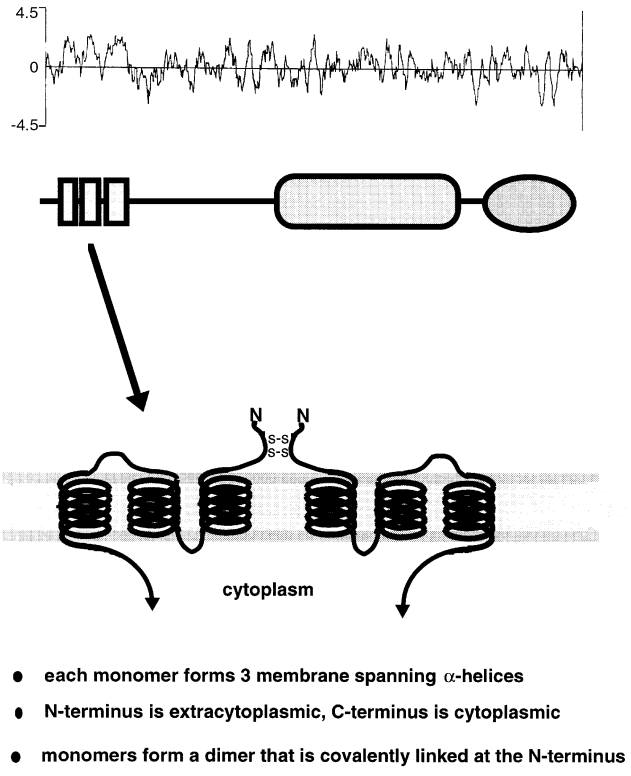


Figure 2. Proposed membrane topology of the ethylene-binding domain of ETR1. Hydropathy analysis indicates three potential membrane-spanning hydrophobic regions near the N-terminus. Computer modelling indicates a likely topology by which these three hydrophobic subdomains are arranged in the membrane as α-helices. Monomers are thought to be linked as a dimer by disulphide bridges at the N-terminus (data from Schaller *et al.* 1995).

perhaps by initiating a MAP kinase cascade (Kieber 1993). Further signal processing requires the product of the *EIN2* gene, which is purported to code for a membrane transporter-like protein. Finally, the system appears to operate through a family of transcription factors represented by the *EIN3*-like gene family (Chao *et al.* 1997).

While all of the mentioned steps in ethylene signal transduction are being actively investigated, we will focus on the ethylene receptor system in this review. After reviewing the basic structural elements of the receptor protein, we will discuss the structural and functional relationships between different members of this small gene family from *Arabidopsis*. We will then consider the structure, function, and evolutionary origin of the ethylene-sensing domain of the receptor. Finally, we will discuss the possible mechanisms by which this family of receptors may transmit signals to downstream effectors.

## 2. STRUCTURAL FEATURES OF THE ETR1 PROTEIN

The *ETR1* gene was the first member of a family of genes to be cloned from *Arabidopsis*. The coding sequence can be divided into three domains, based on structural features and sequence relationships.

As shown in figure 1, the N-terminal sensor domain consists of three hydrophobic subdomains. Studies of the ETR1 protein expressed in yeast indicated that this domain is membrane-associated (Schaller *et al.* 1995). ETR1 protein obtained from plants and from transgenic yeast exists as a covalently linked dimer (Schaller *et al.* 1995). There are two cysteines at the N-terminus (Cys4 and Cys6) which are the sites of disulphide cross-linkage between ETR1 monomers. This N-terminal sensor domain also contains all of the elements necessary and sufficient for binding ethylene with high affinity (Schaller & Bleecker 1995) when expressed in yeast. Significantly, mutations in ETR1 that confer dominant insensitivity to ethylene are all clustered in this hydrophobic region.

The C-terminal half of the ETR1 protein contains all of the conserved sequence elements found in the histidine kinase domains of bacterial two-component regulators. Two-component regulators are typically composed of a sensor protein with an input domain that receives signals and a catalytic transmitter domain that autophosphorylates on an internal histidine residue. The second component, a response regulator protein, is composed of a

receiver domain that receives phosphate from the transmitter and an output domain that mediates responses depending on the phosphorylation state of the receiver. The basic features of two-component regulators are provided in figure 2. The ETR1 protein has a receiver domain fused to the C-terminus of the histidine kinase domain. This modular arrangement is found in a number of bacterial sensors and in the osmosensing Sln1 protein from yeast (Parkinson 1993; Ota & Varshavsky 1993; Maeda *et al*. 1994).

The region located between the hydrophobic sensor domain and the histidine kinase transmitter domain is of unknown function. There are two blocks of sequence within this region which show homology to sequences that flank the chromophore-binding domain in the light-sensing phytochromes from higher plants and cyanobacteria (Kehoe & Grossman 1996). It has recently been reported that the region between these blocks of homology in ETR1 contains a GAF domain (Aravind & Ponting 1997). GAF domains are associated with cyclic GMP binding in a number of proteins. This opens up the rather intriguing possibility that ETR1 may mediate responses to more than one signal. However, the chromophore-binding domain of phytochrome was also identified as a GAF domain, indicating that this motif may have been recruited for a variety of functions over evolutionary time.

## 3. THE *ETR1*-LIKE FAMILY HAS FIVE MEMBERS IN *ARABIDOPSIS*

To date, five *ETR1*-like genes have been identified in *Arabidopsis* (see figure 1). These five genes can be divided into two main subfamilies based on sequence similarity and structural features of the proteins: the *ETR1*-like subfamily and the *ETR2*-like subfamily. ETR1 and ERS1 (Hua *et al*. 1995; Hua *et al*. 1998) are most closely related in sequence and have three hydrophobic subdomains at the N-terminus. ETR2 (Sakai *et al*. 1998), EIN4 and ERS2 (Hua *et al*. 1998) share close sequence similarity and all have four hydrophobic subdomains at the N-terminus.

Like ETR1, ERS1 binds ethylene when expressed in yeast (Schaller *et al*. 1998) and its transmitter domain contains all the conserved subdomains thought to be required for histidine kinase activity. ERS1 differs from ETR1 in that it lacks the fused receiver domain. By contrast, members of the *ETR2*-like subfamily appear to be degenerate in the histidine kinase domain. Each member of this subfamily lacks one or more elements thought to be essential for histidine kinase activity, so it is unlikely that they are functional kinases. It is not yet known whether this subfamily is capable of binding ethylene when expressed in yeast, although all of the residues thought to be essential for ethylene binding in ETR1 are conserved in the *ETR2*-like subfamily.

## 4. DOMINANT ETHYLENE INSENSITIVITY IS CONFERRED BY MUTATIONS IN ANY FAMILY MEMBER

Aside from the structural homology that ties the *ETR1*-like family members together, these genes also share some functional relationship, as evidenced by the observation that mutations in any one of the five genes lead to dominant insensitivity to ethylene (Bleecker *et al*. 1988; Hua *et al*. 1995, 1998; Sakai *et al*. 1998). As indicated in figure 2, mutagen-induced alleles of *ETR1*, *ETR2* and *EIN4* were originally identified by their ethylene-insensitive phenotype (Bleecker *et al*. 1988; Sakai *et al*. 1998; Hua *et al*. 1998). For *ERS1* and *ERS2* there are no dominant mutant alleles of the native genes. However, *in vitro* mutagenesis of genomic clones followed by transfer of the mutated sequences into wild-type plants led to dominant insensitivity (Hua *et al*. 1995, 1998).

For each family member, dominant mutations confer ethylene insensitivity throughout the plant. This is consistent with mRNA expression patterns for each family member: all members were expressed at some level in most tissues (Hua *et al*. 1998). On the other hand, there were differences in the levels of expression of different family members in different tissue types. It is also of interest that two members of the family, *ERS1* and *ETR2*, showed elevations in mRNA abundance in response to ethylene, while the other members did not (Hua *et al*. 1998). Interestingly, the *Never-ripe* (*nr*) gene from tomato, most closely related to *ERS1* in structure, was also transcriptionally regulated by ethylene. Induction of nr message is associated with changes in ethylene sensitivity associated with ripening and abscision (Wilkinson *et al*. 1995; Yen *et al*. 1995).

One common feature of all mutations that confer dominant ethylene insensitivity is that all of these amino-acid substitutions are clustered in the hydrophobic regions of the N-terminal ethylene sensor domain. The fact that *etr2-1* and *ein4-1* mutations affect the equivalent residue as the *nr* and *etr1-4* mutants, respectively, indicates that there may be a very limited number of possible mutations in these genes that lead to dominant ethylene insensitivity. No recessive mutants were obtained in mutant screens, implying that some functional redundancy must exist between the various isoforms of the ETR1-like proteins. Loss of functional mutants in any one isoform apparently does not produce a recognizable phenotype.

## 5. PROPOSED MEMBRANE TOPOLOGY OF THE HYDROPHOBIC DOMAIN OF ETR1

Based on the primary sequence of the hydrophobic domain of ETR1, a model for the topology of the protein in a membrane environment has been proposed (Chang *et al*. 1993; Schaller *et al*. 1995). Assuming that the three hydrophobic regions form membrane-spanning α-helices, the proposed topology (shown in figure 2) consists of an extracytoplasmic N-terminal sequence, while the C-terminal histidine kinase domain is cytoplasmic. Biochemical evidence consistent with this model was provided by studies of the native protein extracted from *Arabidopsis* and the recombinant protein expressed in yeast. In both cases the protein behaved as an intrinsic membrane protein that ran at the predicted molecular weight on SDS-PAGE in the presence of a reducing agent, but at twice the predicted size in the absence of a reducing agent, indicating that the protein was present in extracts as a disulphide-linked dimer. Mutated forms of the protein expressed in yeast indicated that cysteines at the N-terminus of the protein were responsible for the

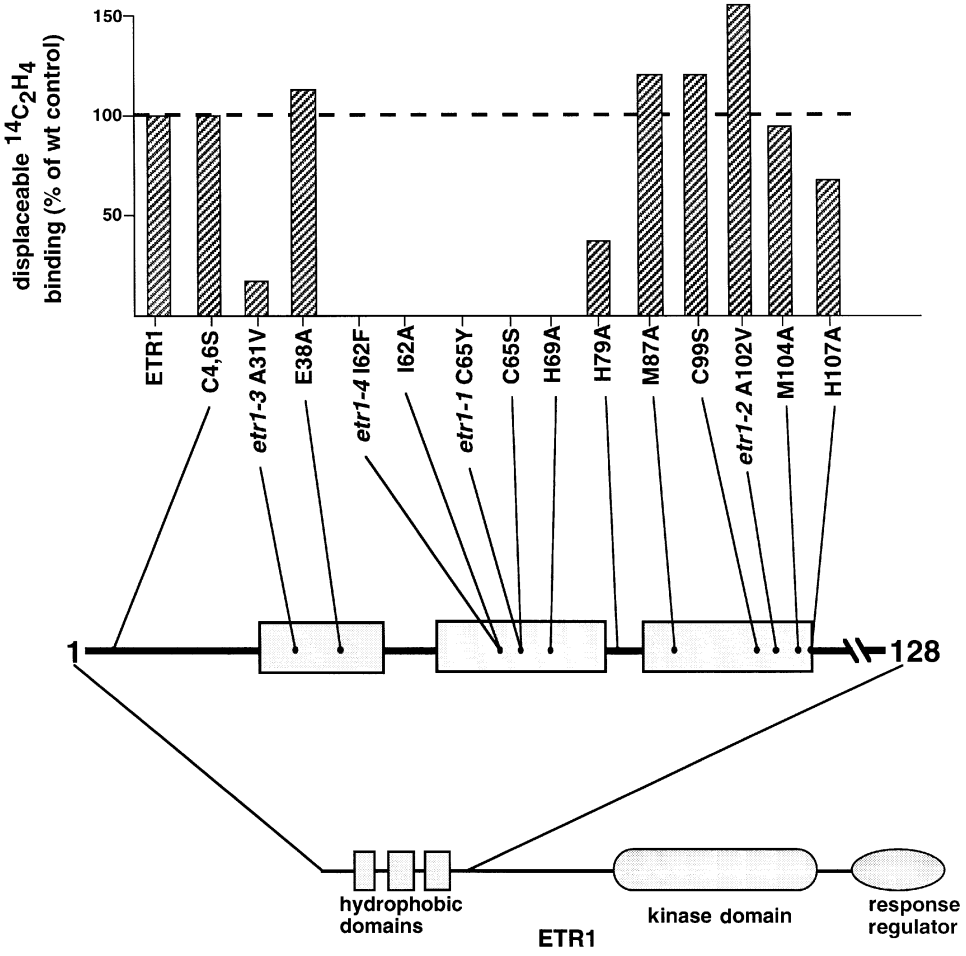


Figure 3. Effects of point mutations on the ethylene-binding activity of *ETR1* expressed in yeast. Mutations were introduced into the full-length cDNA of *ETR1* by *in vitro* mutagenesis. Ethylene-binding activity in yeast is expressed as a percentage of wild-type activity after normalization for protein expression level. Ethylene-binding assays were performed as previously described (Schaller & Bleecker 1995). Ethylene-binding activity was completely eliminated by mutations in the second hydrophobic region. Cys65 and His69 are considered to be candidate ligands for the Cu(I) cofactor. Mutations in other candidate ligands did not eliminate binding. Of the mutations that confer ethylene insensitivity *in planta*, *etr1-1*, *etr1-3*, and *etr1-4* are associated with loss of ethylene-binding activity. The exception is *etr1-2*, an Ala-to-Val conversion in the third hydrophobic region that actually binds more ethylene per unit of expressed protein than wild-type (data from Schaller & Bleecker 1995; Schaller *et al.* 1998; Hall *et al.* 1998).

disulphide linkage, consistent with an extracytoplasmic location for the N-terminus (Schaller *et al.* 1995).

This structural model for ETR1 conforms very well to the basic features of many bacterial two-component regulators which have intrinsic membrane sensor domains that operate as dimers (Milligan & Koshland 1988; Pan *et al.* 1993; Cochran & Kim 1996). According to the bacterial paradigm, the interaction of the ethylene signal with the hydrophobic sensor domain would induce a conformational change in the membrane-spanning α-helices. This conformational change would be propagated to the linked histidine kinase transmitter domain where signal output would be modulated.

## 6. ETHYLENE SENSING IS MEDIATED THROUGH A TRANSITION METAL COFACTOR

Expression of *ETR1* in yeast generates high-affinity binding sites for ethylene (Schaller & Bleecker 1995). Dose-binding curves using $^{14}$C-ethylene on whole yeast cells indicated a $K_D$ for binding of less than one nanomolar (Schaller & Bleecker 1995). Recent experiments in our laboratory indicate that the region of ETR1 that is necessary and sufficient for ethylene binding is contained within the first 128 amino acids of the protein (A. B. Bleecker, unpublished data). Membrane extracts from yeast expressing ETR1 showed a small amount of ethylene binding. This activity could be greatly enhanced by addition of copper sulphate to the extraction medium. With the exception of silver ions, no other transition metal ions had this effect on binding activity in membrane preparations. Significantly, membranes containing the *etr1-1* mutant form of the protein show no saturable ethylene binding activity even in the presence of added copper, indicating that the copper enhancement of binding is mediated by functional ETR1. We have recently solubilized and purified the ethylene-binding domain of ETR1. Our initial results indicate that stoichiometric amounts of copper copurify with the binding domain (A. B. Bleecker, unpublished results).

The identification of a copper ion associated with the ethylene-binding domain of ETR1 fits with theoretical considerations dating back to Stanley Burg's original hypothesis that ethylene binding would be mediated by a transition metal cofactor (Burg & Burg 1967). This hypothesis was based on the known interactions of olefins like ethylene with transition metals. The donation of π-electrons from ethylene to the *d*-orbitals of a transition metal result in a resonance structure that contains about half the binding energy of a carbon–carbon double bond (Muhs & Weiss 1962). This would provide the stability needed for high-affinity binding while still affording the reversibility of the interaction that can be demonstrated for the receptor.

## 7. A MECHANISTIC MODEL FOR THE ETHYLENE SENSOR DOMAIN

The topological model for the ETR1 hydrophobic domain can be coupled with the requirement for metal

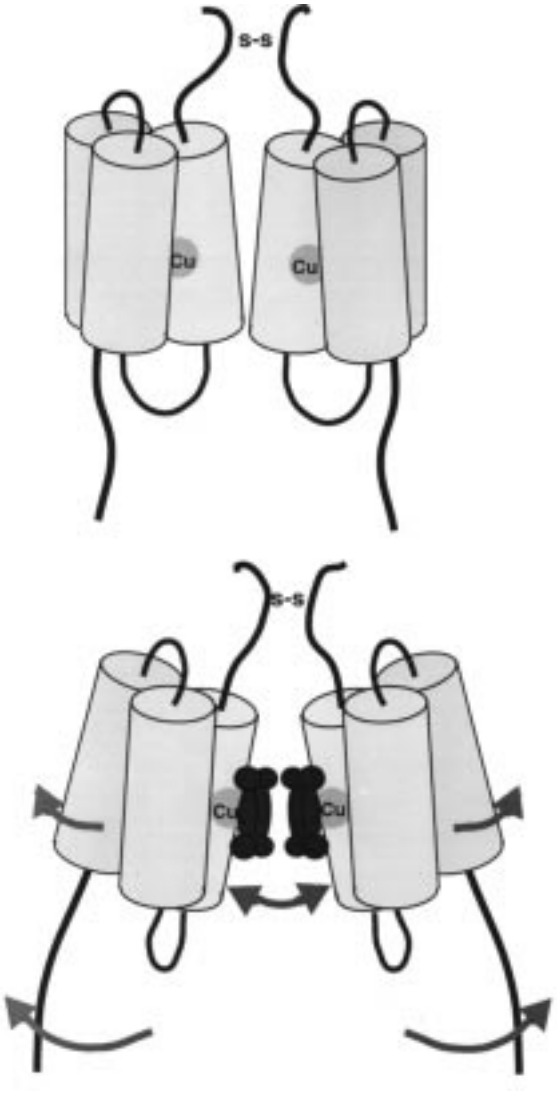


Figure 4. Proposed mechanism by which binding of ethylene to a Cu(I) cofactor could induce a conformational change in the ethylene-binding domain of ETR1. Movement of membrane-spanning α-helices would be propagated through the third helix to the histidine kinase transmitter domain. Mutations that disrupt ethylene binding (*etr1-1*, *etr1-3*, and *etr1-4*) could lock the receptor in one signalling mode. Mutations in the third helix (e.g. *etr1-2*) could alter signal propagation without disrupting ethylene binding.

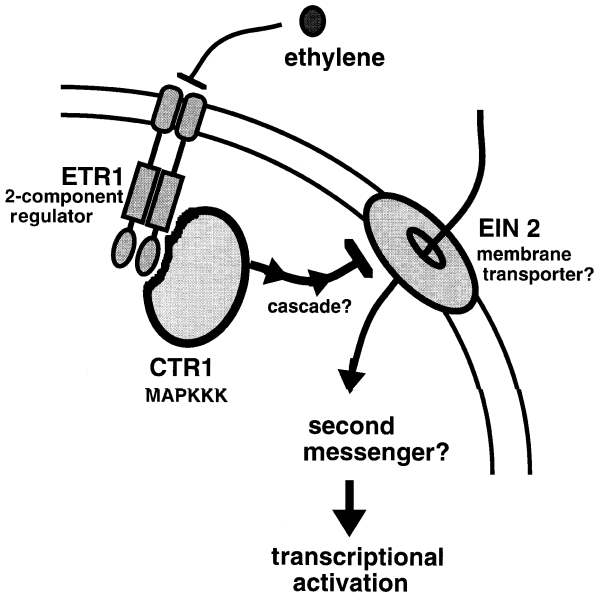


Figure 5. A model for the transduction of the ethylene signal. The ethylene receptor (ETR1) may interact directly with an Raf-type protein kinase (CTR1) that in turn regulates a membrane transporter (EIN2). The transporter could release a second messenger in the cell that would regulate transcription via transcription factors (EIN3).

coordination to formulate structural models of the ethylene binding site. A reasonable model would involve a copper ion embedded in the membrane, coordinated by amino-acid residues in the membrane-spanning α-helices. Candidate liganding side chains could be provided by cysteines, histidines or methionines. We have tested a number of such residues located in the ethylene-binding domain by using *in vitro* mutagenesis and assaying for ethylene-binding activity of mutagenized receptors expressed in yeast. The results are summarized in figure 3.

Of particular interest is the fact that, of all candidate metal-liganding residues tested, only mutations in cysteine 65 or histidine 69 completely eliminated binding activity (Schaller & Bleecker 1995; Schaller *et al.* 1998). When the hydrophobic domain in which these two residues are located is modelled as an α-helix, the side chains align on the same face of the helix. We suggest that these two residues may act to coordinate a copper ion in a hydrophobic pocket formed by the membrane-spanning helices of ETR1. The hydrophobic pocket would provide a favourable environment to stabilize a Cu(I) ion which, in turn, could act as a site for ethylene binding (Thompson *et al.* 1983).

It is imagined that the interaction of ethylene with the Cu(I) complex would displace or alter the coordination chemistry and thereby induce a conformational change in the membrane-spanning helices of the receptor. This change would be propagated by translational or rotational changes of the third transmembrane domain to the linked histidine kinase transmitter domains of the dimer pair (figure 4). This model is consistent with what is known about signal propagation through the aspartate chemotaxis receptor system in *E. coli* (Falke & Koshland 1987; Milligan & Koshland 1988; Cochran & Kim 1996).

## 8. TRANSDUCTION OF THE ETHYLENE SIGNAL TO DOWNSTREAM EFFECTORS

The homology of the ETR1 cytoplasmic domain to transmitter and receiver domains of bacterial two-component regulators provides a basic paradigm for signal transduction that could apply to the ETR1-like receptors. In these systems, input signals alter the histidine kinase activity of transmitter domains. These domains act as dimer pairs and autophosphorylate in *trans* on a specific internal histidine residue. This phosphate is then transferred to a response regulator protein. It is the phosphorylation state of the response regulator that mediates downstream responses (Parkinson 1993; Swanson *et al.* 1994).

Recently, autophosphorylating histidine kinase activity has been demonstrated for the catalytic domain of ETR1

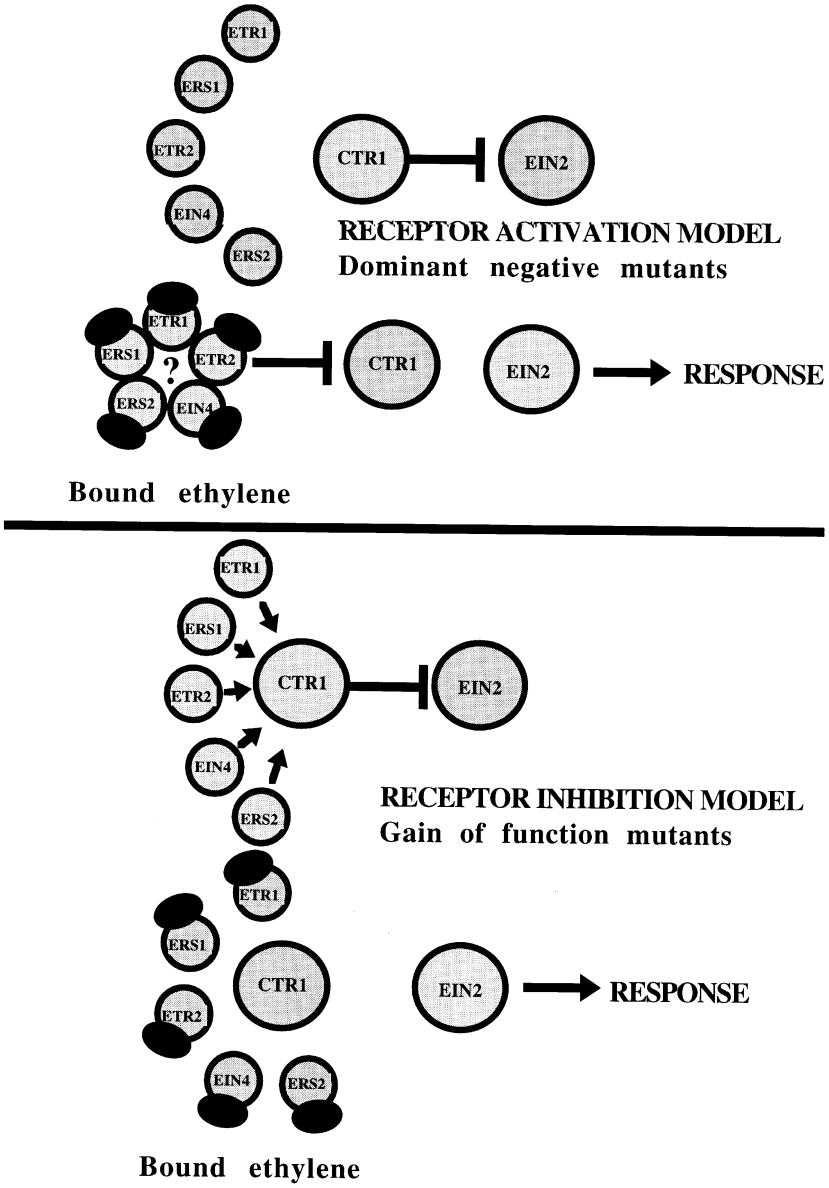


Figure 6. Alternative models for ethylene signalling that account for the dominance of mutations in receptor genes. (*a*) In the receptor activation model, binding of ethylene to receptor isoforms activates signalling to CTR1. Oligomeric interactions of receptor isoforms to produce a signal allows for dominant negative effects of mutant subunits. (*b*) In the receptor inhibition model, receptor isoforms signal to CTR1 in the absence of ethylene. Ethylene inhibits this signalling. Gain of function mutants would continue to activate CTR1 in the absence of ethylene and thus suppress response pathways.

(Gamble *et al.* 1998). However, no response regulator component has been identified genetically for ethylene signal transduction. In addition, it is very unlikely that the ETR2-like subfamily of proteins are functional histidine kinases given that they lack what are thought to be essential residues in their catalytic domains. Yet mutations in these isoforms confer ethylene insensitivity. If ethylene signal transduction does require phosphotransfer via a phosphohistidine intermediate, these degenerate members of the family must interact in some way with the catalytically active forms of the receptor.

Genetic analysis indicates that the RAF kinase-related CTR1 acts downstream of the receptors (Kieber *et al.* 1993; Roman *et al.* 1995; Sakai *et al.* 1998). CTR1 appears to be a MAP kinase kinase kinase and so is likely to initiate a MAP kinase cascade. This is in striking parallel to signalling by the osmosensor Slnl from yeast which is a two-component type receptor that signals via Ssk2 MAPKKK. In this case a response regulator Sskl accepts phosphate from the Slnl transmitter via a phospho-relay and regulates Ssk2 by direct protein–protein interactions (Posas *et al.* 1996). Interestingly, Clark *et al.* (1998) have obtained evidence, using two hybrid and *in vitro* binding experiments, that the regulatory domain of CTR1 directly interacts with the catalytic domain of ETR1. This may obviate, but does not preclude, the need for intermediate proteins in the signalling between ETR1 and CTR1.

The presumptive MAP kinase cascade that is initiated by CTR1 should have the EIN2 protein as a downstream target according to the genetic evidence. The sequence of EIN2 has not yet been published, but it has been reported to code for a protein with 12 putative membrane-spanning domains, indicating a possible function as a transporter. A reasonable model for ethylene signalling would be that the kinase cascade initiated by CTR1 negatively regulates the activity of this transporter, and that the substrate for the transporter could act as a second messenger for ethylene signalling. A family of putative transcription factors represented by EIN3 would mediate changes in gene expression in response to the second messenger. This speculative model for ethylene signalling is shown in figure 5.

## 9. THE MECHANISM OF GENETIC DOMINANCE OF RECEPTOR MUTANTS

One of the more intriguing questions about the ethylene perception system that remains unanswered is: by what mechanism do single point mutations in any one of five related genes cause dominant insensitivity to ethylene? To answer this question, two different classes of mechanisms have been suggested (Bleecker & Schaller 1996): dominant-negative mechanisms and gain-of-function mechanisms. Models that favour either class must take into account the observation that dominant mutations tend to disrupt ethylene-binding activity. This implies that these mutants are somehow locked into a signalling mode that is insensitive to ethylene.

As indicated in figure 6*a*, a dominant-negative mechanism for ethylene insensitivity is compatible with a model for signalling in which receptors negatively regulate CTR1 when ethylene binds. This is considered a receptor-activation model because ethylene-binding activates signalling downstream. A mutation in any member of the receptor family must somehow block (or reduce below some threshold) the total signal output received by CTR1 from the receptor family. A dominant-negative mechanism would require interactions between receptor isoforms that would allow one mutant form to poison the entire complex and thus prevent signalling downstream. While the receptors do appear to form dimers, perhaps even heterodimers, there is no evidence for the higher-order complexes of receptors that this model requires.

On the other hand, gain-of-function mechanisms for dominance are more compatible with models in which receptors positively regulate CTR1 in the absence of ethylene, and ethylene binding inhibits this interaction (figure 6*b*). These are termed receptor-inhibition models because ethylene acts to inhibit signal output from receptors. In these cases, mutations that disrupt ethylene binding could lock a receptor in a signalling state, thus keeping CTR1 active even in the presence of saturating ethylene concentrations. This model does not require multi-subunit interactions of receptors. A single mutant isoform, particularly if rendered hyperactive by the mutation, could keep the response pathway in an off state.

Support for the receptor-inhibition model was recently obtained by constructing mutant lines of *Arabidopsis* that are null for three or more members of the receptor family. These triple mutant lines showed a constitutive ethylene-response phenotype similar to ctr1-1 (Hua & Meyerowitz 1998). This is the result predicted by model (*b*) in figure 6.

## REFERENCES


Abeles, F. B., Morgan, P. W. & Saltveit, M. E. Jr 1992 *Ethylene in plant biology*, 2nd edn. San Diego: Academic Press.

Aravind, L. & Ponting, C. P. 1997 The GAF domain—an evolutionary link between diverse phototransducting proteins. *Trends Biochem. Sci.* **22**, 458–459.

Bleecker, A. B. & Schaller, G. E. 1996 The mechanism of ethylene perception. *Pl. Physiol.* **111**, 653–660.

Bleecker, A. B., Estelle, M. A., Somerville, C. & Kende, H. 1988 Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241**, 1086–1089.

Burg, S. P. & Burg, E. A. 1965 Ethylene action and the ripening of fruits. *Science* **148**, 1190–1196.

Burg, S. P. & Burg, E. A. 1967 Molecular requirements for the biological activity of ethylene. *Pl. Physiol.* **42**, 144–152.

Chang, C., Kwok, S. F., Bleecker, A. B. & Meyerowitz, E. M. 1993 *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two-component regulators. *Science* **262**, 539–544.

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

Clark, K. L., Larsen, P. B., Wang, X. & Chang, C. 1998 Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. *Proc. Natn. Acad. Sci. USA* **95**, 5401–5406.

Cochran, A. G. & Kim, P. S. 1996 Imitation of *Escherichia coli* aspartate receptor signaling in engineered dimers of the cytoplasmic domain. *Science* **271**, 1113–1116.

Ecker, J. R. 1995 The ethylene signal transduction pathway in plants. *Science* **268**, 667–674.

Falke, J. J. & Koshland, D. E. Jr 1987 Global flexibility in a sensory receptor: a site-directed cross-linking approach. *Science* **237**, 1596–1600.

Fluhr, R. 1998 Ethylene perception: from two-component signal transducers to gene induction. *Trends Pl. Sci.* **3**, 141–146.

Gamble, R. L., Coonfield, M. L. & Schaller, G. E. 1998 Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. *Proc. Natn. Acad. Sci. USA* **95**, 7825–7829.

Guzman, P. & Ecker, J. R. 1990 Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *Pl. Cell* **2**, 513–523.

Hall, A. E., Chen, Q. G. & Bleecker, A. B. 1998 The relationship between ethylene binding and dominant insensitivity in mutant forms of the ETR1 ethylene receptor. (Submitted.)

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

Hua, J., Chang, C., Sun, Q. & Meyerowitz, E. M. 1995 Ethylene insensitivity conferred by *Arabidopsis ERS* gene. *Science* **269**, 1712–1714.

Hua, J., Sakai, H. & Meyerowitz, E. M. 1997 The ethylene receptor gene family in *Arabidopsis*. In *Biology and biotechnology of the plant hormone ethylene*, vol. 34 (ed. A. K. Kanellis, C. Chang, H. Kende & D. Grierson), pp. 71–76. NATO ASI series. Dordrecht: Kluwer.

Hua, J., Sakai, H., Nourizadeh, S., Chen, Q. G., Bleecker, A. B., Ecker, J. R. & Meyerowitz, E. M. 1998 *EIN4* and *ERS2* are members of the putative ethylene receptor gene family. *Pl. Cell*. (In the press.)

Kehoe, D. M. & Grossman, A. R. 1996 Similarity of a chromatic adaption sensor to phytochrome and ethylene receptors. *Science* **273**, 1409–1412.

Kieber, J. J. 1997 The ethylene response pathway in *Arabidopsis*. *A. Rev. Pl. Physiol. Molec. Biol.* **48**, 277–296.

Kieber, J. J., Rothenberg, M., Roman, G., Feldmann, K. A. & Ecker, J. R. 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.

Maeda, T., Wurgler-Murphy, S. M. & Saito, H. 1994 A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* **369**, 242–245.

Milligan, D. L. & Koshland, D. E. Jr 1988 Site-directed cross-linking: establishing the dimeric structure of the aspartate receptor of bacterial chemotaxis. *J. Biol. Chem.* **263**, 6268–6275.

Muhs, M. A. & Weiss, F. T. 1962 Determination of equilibrium constant of silver-olefin complexes using gas chromatography. *J. Am. Chem. Soc.* **84**, 4698–4705.

Ota, I. M. & Varshavsky, A. 1993 A yeast protein similar to bacterial two-component regulators. *Science* **262**, 566–569.

Pan, S. Q., Charles, T., Jun, S., Wu, Z.-L. & Nester, E. W. 1993 Preformed dimeric state of the sensor VirA is involved in plant-*Agrobacterium* signal transduction. *PNAS* **90**, 9939–9943.

Parkinson, J. S. 1993 Signal transduction schemes of bacteria. *Cell* **73**, 857–871.

Posas, F., Wurgler-Murphy, S. M., Maeda, T., Witten, E. A., Thai, T. C. & Saito, H. 1996 Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1-YPD1-SSK1 'two-component' osmosensor. *Cell* **86**, 865–875.

Roman, G., Lubarsky, B., Kieber, J., Rothenbeg, M. & Ecker, J. R. 1995 Genetic analyisis of ethylene signal transduction in *Arabidopsis thaliana*: five novel loci integrated into a stress response pathway. *Genetics* **130**, 1393–1409.

Sakai, H., Hua, J., Chen, Q. G., Chang, C., Bleecker, A. B., Medrano, L. J. & Meyerowitz, E. M. 1998 *ETR2* is an ETR1-like gene controlling ethylene signal transduction. *Proc. Natn. Acad. Sci. USA* **95**, 5812–5817.

Sanders, I. O., Smith, A. R. & Hall, M. A. 1989 The measurement of ethylene binding and metabolism in plant tissue. *Planta* **179**, 97–103.

Sanders, I. O., Harpham, N. V. J., Raskin, I., Smith, A. R. & Hall, M. A. 1991 Ethylene binding in wild type and mutant *Arabidopsis thaliana* (L.) heynh. *Ann. Bot.* **68**, 97–103.

Schaller, G. E. & Bleecker, A. B. 1995 Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. *Science* **270**, 1809–1811.

Schaller, G. E., Ladd, A. N., Lanahan, M. B., Spanbauer, J. M. & Bleecker, A. B. 1995 The ethylene response mediator ETR1 from *Arabidopsis* forms a disulfide-linked dimer. *J. Biol. Chem.* **270**, 12 526–12 530.

Schaller, G. E., Hall, A. E., Findell, J. L., Coonfield, M. L., Sisler, E. C. & Bleecker, A. B. 1998 Ethylene perception by the ETR1 and ERS1 proteins of *Arabidopsis*. (Submitted.)

Sisler, E. C. 1979 Measurement of ethylene binding in plant tissue. *Pl. Physiol.* **64**, 538–542.

Swanson, R. V., Alex, L. A. & Simon, M. I. 1994 Histidine and aspartate phosphorylation: two component systems and the limits of homology. *Trends Biochem. Sci.* **19**, 485–490.

Thompson, J. S., Harlow, R. L. & Whitney, J. F. 1983 Copper(I)-olefin complexes. Support for the proposed role of copper in the ethylene effect in plants. *J. Am. Chem. Soc.* **105**, 3522–3527.

Wilkinson, J. Q., Lanahan, M. B., Yen, H. C., Giovannoni, J. J. & Klee, J. H. 1995 An ethylene-inducible component of signal transduction encoded by *Never-ripe*. *Science* **270**, 1807–1809.

Yen, H. C., Lee, S., Tanksley, S. D., Lanahan, M. B., Klee, H. J. & Giovannoni, J. J. 1995 The tomato *Never-ripe* locus regulates ethylene-inducible gene expression and is linked to a homolog of the *Arabidopsis ETR1* gene. *Pl. Physiol.* **107**, 1343–1353.