Chapter 4
The Role of Protein–Protein Interactions in Signaling by the Ethylene Receptors

Brad M. Binder and G. Eric Schaller

Abstract Protein–protein interactions of the ethylene receptors are involved in propagation and modulation of the ethylene signal. Interactions of the receptors with critical pathway components such as CTR1 and EIN2 are likely to involve multiple members of the receptor family. Additional interactions, such as that involving the receptor ETR1 and regulatory protein RTE1, may allow for isoform-specific signal output. Ethylene receptors also form higher order complexes with each other, suggesting a cooperative mechanism for amplification of the ethylene signal. A model incorporating the role of physical interactions in signal transmission by the receptors is described.

Keywords Ethylene receptors · Histidine kinase · Cooperativity · Endoplasmic reticulum · ETR1 · CTR1

4.1 Introduction

Ethylene is perceived in plants by receptor families. In *Arabidopsis thaliana*, where the receptors have been studied in most detail, there are five members to the receptor family: ETR1 (ETHYLENE RESPONSE1), ETR2, ERS1 (ETHYLENE RESPONSE SENSOR1), ERS2, and EIN4 (ETHYLENE INSENSITIVE4) (Fig. 4.1). The ethylene receptors of dicots and monocots fall into two subfamilies based upon structure and amino acid sequence, all containing an ethylene-binding domain, a GAF (cGMP-specific phosphodiesterases, adenyl cyclases, FhlA)

B.M. Binder (✉)
Department of Biochemistry, Cellular, and Molecular Biology, University of Tennessee, Knoxville, TN 37996-0840, USA
e-mail: bbinder@utk.edu

G.E. Schaller
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755-3563, USA
e-mail: George.E.Schaller@Dartmouth.EDU

C.-K. Wen (ed.), *Ethylene in Plants*, DOI 10.1007/978-94-017-9484-8_4

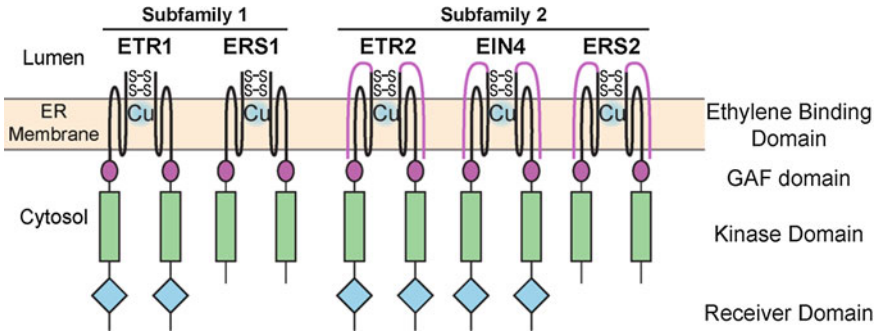

Fig. 4.1 Domain structures of the ethylene receptors from *Arabidopsis thaliana*. The receptors are disulfide-linked homodimers localized to the endoplasmic reticulum. Each receptor contains an ethylene-binding, a GAF, and a histidine kinase-like domain. Three of the five receptors also contain a receiver domain. The ethylene-binding domain includes a copper cofactor (Cu). The receptors are present as two subfamilies based on phylogenetic analysis and structural features. The subfamily-2 receptors contain additional amino acids at the N-terminus that may function as a fourth transmembrane helix or a cleavable signal peptide (*highlighted in purple*)

domain, and a histidine kinase-like domain. Three of the five receptors in *Arabidopsis*, also contain a receiver domain (Fig. 4.1). Biochemical characteristics of the receptors are detailed in Chap. 3. In this chapter, we focus on the roles that protein–protein interactions play in signaling by the receptors. To this end, we discuss (1) the interactions between the receptors and the primary downstream signaling components, CTR1 (CONSTITUTIVE TRIPLE RESPONSE1) and EIN2; (2) the specific interaction between ETR1 and RTE1 (REVERSION-TO-ETHYLENE-SENSITIVITY1); (3) the role that cooperative receptor–receptor interactions may play in amplifying the ethylene signal; and (4) additional interactions, the significance of which is only beginning to be understood.

4.2 Interactions with Downstream Components of the Primary Signal Transduction Pathway

The ethylene receptors are predominantly localized to membranes of the endoplasmic reticulum (ER), their topology being such that the ethylene-binding domain is present within the ER membrane itself and the signal output domain is cytosolic. Not surprisingly, other proteins involved in the primary response to ethylene such as CTR1 and EIN2 are also localized to the ER (Fig. 4.2) (Chen et al. 2002; Gao et al. 2003; Grefen et al. 2008; Bisson et al. 2009; Bisson and Groth 2010).

CTR1 is a Ser/Thr protein kinase that acts just downstream of the receptors to inhibit downstream signaling (Kieber et al. 1993). Several lines of evidence indicate that CTR1 interacts with ethylene receptors. First, although CTR1 contains no transmembrane domains itself, it is found associated with membranes of the ER.

Fig. 4.2 Primary ethylene signal transduction pathway as defined by genetic interactions. Initial signaling elements in the pathway are shown. Those gene products associated with the ER as either integral or peripheral membrane proteins are highlighted in *gray*

The ER association of CTR1 is dependent on the ethylene receptors, mutations of both subfamily-1 and subfamily-2 receptors reducing the levels of membrane-associated CTR1 (Gao et al. 2003). Second, a physical association of CTR1 with the ethylene receptor ETR1 is supported by two-hybrid analysis, in vitro-binding experiments, and co-purification analysis from *Arabidopsis* extracts (Clark et al. 1998; Gao et al. 2003; Huang et al. 2003). Studies on the interaction of CTR1 with other members of the receptor family are more limited than with ETR1, although interactions are observed with ERS1 and ETR2 based on yeast two-hybrid analysis (Clark et al. 1998; Cancel and Larsen 2002). The two-hybrid studies demonstrate that both the kinase and receiver domains of the receptors can interact with CTR1 (Clark et al. 1998). Interaction of CTR1 with the kinase domain could potentially allow CTR1 to interact with all five members of the receptor family; the functional significance of interaction with the receiver domain is unknown.

Studies suggest that differences exist among the receptors and their ability to interact with and regulate CTR1. In yeast two-hybrid studies, the strongest CTR1 interaction is observed with ETR1, the interactions with ERS1 and ETR2 being progressively weaker (Clark et al. 1998; Cancel and Larsen 2002). A greater affinity of CTR1 for subfamily-1 receptors compared to subfamily-2 receptors may explain the predominant role of ETR1 and ERS1 in the regulation of ethylene signal transduction in *Arabidopsis* (Wang et al. 2003; Qu et al. 2007). Studies on the receptor-dependent association of CTR1 with membranes also indicate that the amount of CTR1 associated with the ER does not always correlate with signaling from the receptor/CTR1 complex (Gao et al. 2003). For instance, loss of ETR1 results in an increase in membrane-associated CTR1, which is opposite to predictions based upon the receptor dependence for association of CTR1 with the ER (Gao et al. 2003; Qu et al. 2007). If the level of membrane-associated CTR1 is directly proportional to the signaling output from the receptors, then this increase in CTR1 levels is predicted to result in stronger suppression of the ethylene response. However, *etr1* null plants actually exhibit an increased sensitivity to ethylene (Cancel and Larsen 2002; Qu et al. 2007). A similar conflict with this model is found in the observation that kinase-inactive ETR1 recruits less CTR1 to the ER than wild-type ETR1 (Hall et al. 2012). Here, plants with lower levels of CTR1 suppress ethylene responses to a greater extent than plants with higher levels (Hall et al. 2012). Taken together, these results indicate that CTR1 associates with receptors in a non-stoichiometric fashion and that, furthermore, there exist isoform-specific differences in the ability of the receptors to regulate CTR1.

EIN2 acts downstream of CTR1 in the ethylene signal transduction pathway and, like the ethylene receptors and CTR1, is localized to the ER membrane (Bisson et al. 2009). EIN2 is an integral membrane protein, its N-terminal portion containing the transmembrane segments and being related to the Nramp family of metal transporters. The C-terminal portion of EIN2 contains a large soluble domain that is cleaved in response to ethylene, after which it translocates to the nucleus to control the transcriptional response to ethylene (Ju et al. 2012; Qiao et al. 2012; Wen et al. 2012). The five *Arabidopsis* receptors can interact directly with the soluble domain of EIN2 (Bisson et al. 2009; Bisson and Groth 2010). The interaction with EIN2 occurs through the kinase domains of the receptors, and studies with ETR1 indicate that autophosphorylation modulates this interaction, a non-phosphorylatable form of ETR1 exhibiting higher affinity for EIN2 (Bisson et al. 2009). As noted in Chap. 3, ETR1 kinase activity modulates but is not required for the well-characterized ethylene responses, making the significance of this receptor–EIN2 interaction unclear. However, it is possible that this represents a further mechanism to modulate ethylene responses. Taken together, the interactions of the ethylene receptors with CTR1 and EIN2 point the existence of a signaling complex at the ER membrane. These interactions, involving the first three elements in the ethylene signaling pathway, could facilitate the regulatory mechanism by which ethylene perception controls proteolytic cleavage of EIN2 in a CTR1-dependent manner.

4.3 Interactions Between ETR1 and RTE1

RTE1 encodes a transmembrane protein that physically associates with ETR1 (Dong et al. 2008, 2010). *RTE1* was first identified as a gene required for the ethylene insensitivity conferred by the dominant *etr1-2* mutation (Resnick et al. 2006). Genes similar to *RTE1* are found in other plants. For instance, the *GREEN-RIPE* (*GR*) gene of tomato appears to have a similar function to *RTE1* (Barry and Giovannoni 2006). Additional *RTE1*/*GR*-like genes are found in tomato and *Arabidopsis* and there may be subfunctionalization within this gene family (Ma et al. 2012).

RTE1 appears to predominantly regulate ETR1 function, having little if any effect on other members of the *Arabidopsis* receptor family. For example, overexpression of *RTE1* results in a reduction in ethylene sensitivity that is largely dependent on ETR1 (Resnick et al. 2006; Zhou et al. 2007). Additionally, *rte1* null mutants phenocopy *etr1* null mutants, resulting in enhanced ethylene sensitivity (Resnick et al. 2006; Zhou et al. 2007). Interestingly, missense mutations like *etr1-2* that confer RTE1-dependent ethylene insensitivity are specific to ETR1. When introduced into the other receptor isoforms, the plants are not ethylene insensitive (Resnick et al. 2006; Rivarola et al. 2009). By contrast, missense mutations in ETR1, such as *etr1-1*, that confer ethylene insensitivity independently of RTE1 are effective at causing ethylene insensitivity when introduced into other receptor isoforms (Rivarola et al. 2009).

Genetic analysis indicated a role of RTE1 in regulating ETR1 activity, the presence of RTE1 facilitating the ability of ETR1 to suppress ethylene responses (i.e., facilitating the role that ETR1 plays in the absence on ethylene). RTE1 physically associates with ETR1 based on in vivo and in vitro assays, truncation analysis indicating that RTE1 interacts with the N-terminal half of ETR1 that contains the transmembrane and GAF domains (Dong et al. 2010). A missense mutation of RTE1 that results in a loss-of-function phenotype decreased the affinity of RTE1 for ETR1, indicating that the RTE1–ETR1 interaction is necessary for function. The interaction of RTE1 with ETR1 may serve to stabilize ETR1 in a state-1 or state-2 conformation (see Chap. 4), whereby the receptor activates CTR1 to suppress the downstream ethylene response. Interestingly, expression of *RTE1* is induced by ethylene, suggesting that RTE1 may be a negative feedback regulator for ETR1, serving to desensitize ETR1 to ethylene. A specific regulator of ETR1 function may have arisen in part due to the predominant role that ETR1 plays in the *Arabidopsis* ethylene response.

Recent research suggests that ETR1 and RTE1 may mediate ethylene signaling in part via a CTR1-independent pathway (Qiu et al. 2012). These researchers found that expression of the N-terminal half of ETR1 containing the ethylene-binding and GAF domains could partially reverse the constitutive triple-response phenotype of *ctr1*. This reversal was RTE1 dependent. This supports the existence of additional ethylene signaling pathways that function independently of the canonical CTR1-dependent pathway, as has been suggested by several independent studies (Kieber et al. 1993; Roman et al. 1995; Larsen and Chang 2001; Hall and Bleecker 2003; Binder et al. 2006). This result also indicates that signaling can occur from the N-terminal half of ETR1 to this pathway. It is unclear whether or not ETR1 is signaling via RTE1 or is simply dependent on RTE1 to maintain the proper conformation for this signaling. It is possible that RTE1 is regulating events occurring within the ER, which raises the possibility that ETR1 has signal outputs to both the ER lumen and cytosol. Support for this hypothesis is a recent study where RTE1 was found to associate with the ER-localized cytochrome b5 to modulate ETR1 function (Chang et al. 2014). This suggests that the ethylene receptors may function in, or be affected by, redox reactions. For instance, ETR1 may mediate H_2O_2 signaling independent of its role as an ethylene receptor (Desikan et al. 2005, 2006). Thus, ETR1 may function in perception and transduction of two signals, ethylene and reactive oxygen species.

4.4 Higher Order Receptor Complexes and Cooperative Signaling

Most models for ethylene signal transduction suggest a fairly simple linear pathway. However, these models do not explain the ability of plants to respond to ethylene across a concentration range that spans approximately six orders of magnitude (Chen and Bleecker 1995; Binder et al. 2004a). It is likely that multiple mechanisms facilitate this wide range of ethylene responsiveness. One possibility,

as described above, is the presence of negative feedback regulators such as RTE1. Another possibility, inspired by our understanding of how similar systems function in bacteria, is cooperative signaling mediated by receptor–receptor interactions.

The ethylene receptors form homodimers that are stabilized by two disulfide bonds (Schaller and Bleecker 1995; Hall et al. 2000; Gao et al. 2008; Chen et al. 2010). These dimers represent the simplest functional unit of the receptors, in which one ethylene molecule binds per receptor dimer (Rodriguez et al. 1999). However, the receptor dimers can also form higher order complexes with each other via non-covalent interactions that are possibly mediated by the GAF domain (Gao et al. 2008; Grefen et al. 2008). It has been suggested that CTR1 may also facilitate or participate in this clustering (Mayerhofer et al. 2012). If the ethylene receptors exist as clusters, then it is likely that cooperative signaling occurs. Such models of cooperative signaling have been invoked for the evolutionarily related histidine kinase-linked chemoreceptors of bacteria to explain the high sensitivity and wide-dynamic range of the receptors (Bray et al. 1998).

In this model for cooperative signaling, clustering allows for conformational changes that occur in one receptor that binds ethylene to be transmitted to other receptors in the cluster that lack ethylene, thereby amplifying the ethylene signal (Maddock and Shapiro 1993; Gestwicki and Kiessling 2002; Francis et al. 2004; Wolanin and Stock 2004). Cooperative signaling between the ethylene receptors may explain the observation that *Arabidopsis* plants respond to ethylene at levels approximately 300-fold below the K_d of the receptors for ethylene (Schaller and Bleecker 1995; Binder et al. 2004a; McDaniel and Binder 2012). Cooperative signaling may also help explain the dominant ethylene insensitivity conferred by mutant receptors such as *etr1-1* (Gao and Schaller 2009). Ethylene-insensitive mutations in the binding sites of the receptors such as *etr1-1* display stronger dominance than predicted for a lesion solely within one receptor isoform (Gamble et al. 2002). Additionally, a truncated etr1-1 protein lacking the kinase and receiver output domains still confers dominant ethylene insensitivity (Gamble et al. 2002; Xie et al. 2006; Gao et al. 2008; Qiu et al. 2012). One explanation for this is that the truncated receptor influences the signaling state of the surrounding, full-length receptors. Finally, the ethylene insensitivity of *etr2-1* is partially dependent on ETR1 (Cancel and Larsen 2002).

Physical clustering of the receptors may also allow for trans-phosphorylation between the receptor isoforms. For instance, the histidine kinase of ETR1 could phosphorylate the receiver domains of ETR1 and EIN4. Support for this hypothesis comes from studies on seedling growth recovery following treatment then removal of ethylene, this growth recovery response being dependent upon ETR1 histidine kinase activity. Interestingly, the growth recovery response is substantially slower in the *etr1 etr2 ein4* triple loss-of-function mutant, which lacks the three receptor isoforms with receiver domains, than in the *etr1* single mutant (Binder et al. 2004b). Furthermore, the slow growth recovery of the triple mutant can be rescued by any of the three receptor isoforms containing a receiver domain as well as by a chimeric ETR1 receptor containing the EIN4 receiver domain, but is not rescued by ERS1, ERS2, or a truncated ETR1 lacking the receiver domain (Binder et al. 2004b; Kim et al. 2011).

4.5 Additional Interactions of the Ethylene Receptors

Current data support the existence of ethylene–receptor signaling complexes in which the receptors interact with integral and peripheral proteins of the endoplasmic reticulum (Ju et al. 2012). Gel filtration analysis of ethylene receptors solubilized from *Arabidopsis* supports the existence of such complexes and also suggests that the complexes may contain isoform-specific components (Chen et al. 2010). All five receptors from *Arabidopsis* were identified as components in large protein complexes but the size of these complexes varied depending on which receptor was examined. Interestingly, the complex size associated with ERS1 was affected by ethylene binding, while the complex size associated with ETR1 was not, suggesting that ethylene may regulate composition of the complexes in an isoform-specific manner (Chen et al. 2010).

Analysis of the receptor complexes suggested that additional components besides CTR1, EIN2, RTE1, or additional members of the receptor family participate in these signaling complexes (Chen et al. 2010). Additional proteins have been identified that form physical interactions with the ethylene receptors, however, little is known about the functional implications of these interactions. In *Arabidopsis*, the ethylene receptors interact with phosphotransfer proteins and the affinity of ETR1 for at least one of these is phosphorylation dependent (Urao et al. 2000; Scharein et al. 2008; Scharein and Groth 2011). One possibility is that these phosphotransfer proteins represent downstream targets for the receptor histidine kinase that functions to modulate ethylene responses. This possibility is supported by the observation that the *Arabidopsis* Response Regulatory Protein2 (ARR2) modulates ethylene responses (Hass et al. 2004; Mason and Schaller 2005). A phosphorylation-dependent two-component signaling pathway, along with the potential RTE1-dependent pathway described earlier, implies that ETR1 may signal to several pathways outside of the canonical CTR1-dependent signaling pathway. It also suggests that different domains of ETR1 may signal to different downstream components.

There is also evidence that the receptors of *Arabidopsis* and tomato interact with tetratricopeptide repeat (TPR) proteins (Lin et al. 2008, 2009). These proteins are poorly characterized in plants but are related to proteins in humans that interact with heterotrimeric G-proteins and the small GTPase Ras. It remains to be determined if this interaction could represent another signaling pathway and/or modulate the receptor–CTR1 interaction.

4.6 A Model for Signal Output from the Receptors

In Fig. 4.3, we present a model that emphasizes the significance of physical interactions in mediating signaling from the ethylene receptors. In this model, ethylene is perceived by a family of receptors (ETR1, ETR2, ERS1, ERS2, and

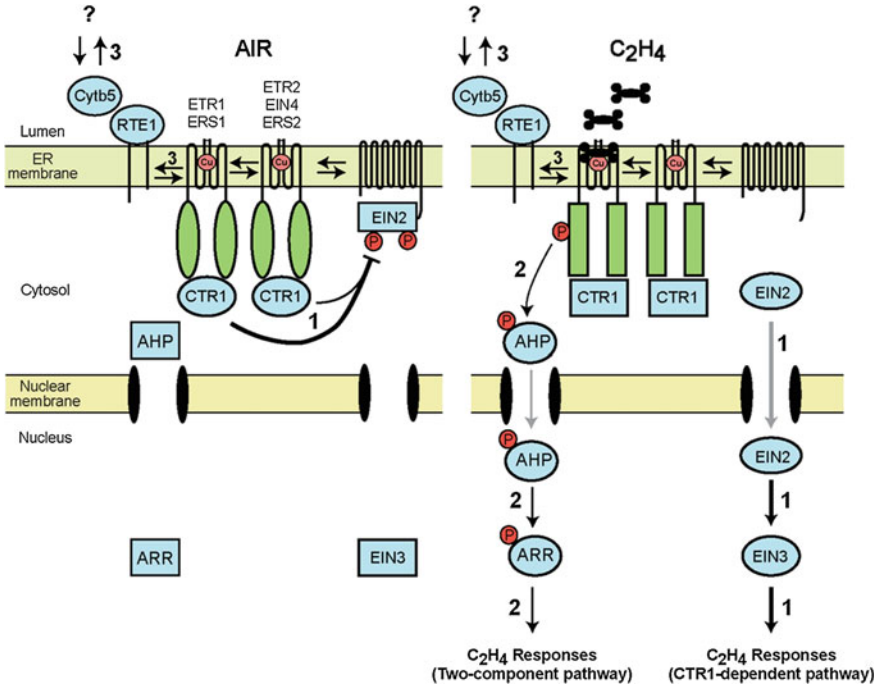

Fig. 4.3 A model for ethylene signal transduction in *Arabidopsis.* Interactions between the receptors and signaling elements of the pathway are indicated. Potential signaling pathways from the receptors are indicated by the numerals 1, 2, and 3. The canonical pathway (*pathway 1*) involves interaction of the receptors with CTR1. In air, the ethylene receptors activate CTR1, which in turn, phosphorylates and inhibits EIN2. In the presence of ethylene, the receptors are inactivated and CTR1 activity is reduced, potentially via conformational changes in the receptors. As a result, EIN2 phosphorylation decreases and is proteolytically cleaved, its C-terminus translocating to the nucleus to initiate the ethylene response. The receptors may also physically associate with EIN2. Several CTR1-independent signaling pathways may also exist. A two-component pathway (*pathway 2*) initiated from the subfamily-1 receptors involves phosphotransfer (AHP) and response regulator (ARR) proteins. RTE1 stabilizes ETR1 through physical interactions and may also mediate signaling to cytochrome b5 in the ER lumen (*pathway 3*). The RTE1-cytochrome b5 pathway could also mediate responses to other signals such as reactive oxygen species. The *thickness of arrows* indicates the relative contributions of these pathways to the ethylene response. Cooperativity in signaling by the receptors may occur due to their physical interaction, such that binding of ethylene to one receptor affects the signaling state of neighboring receptors that do not have ethylene bound. *Ovals* and *squares* indicate different conformations of the proteins, with ovals generally indicating the active form of the protein where known. *Thicker arrows* indicate a greater relative contribution to ethylene signaling. *Gray arrows* indicate translocation to the nucleus of signaling elements

EIN4 in *Arabidopsis*) predominantly localized to the ER membrane. The primary output from the receptors is to CTR1. Here, the receptors function in a largely overlapping manner to physically associate with CTR1 and to regulate CTR1 activity. When ethylene binds, a conformational change in the receptors is

transmitted to CTR1 and CTR1 activity is reduced. Even though all five receptor isoforms are likely involved in regulating CTR1, evidence suggests that the subfamily-1 receptors of *Arabidopsis* play a larger role than the subfamily-2 receptors in this regulation. The reduction in CTR1 activity results in activation of EIN2 via proteolytic cleavage, and stimulation of the ethylene response. The model depicted in Fig. 4.3 also includes several other potential outputs from the receptors. One potential output is based on participation in a two-component signaling pathway dependent on His-Asp phosphorylation, and physical interaction with phosphotransfer proteins. A second potential output is an RTE1-dependent signaling pathway that involves the N-terminal portion of ETR1. The downstream target for this is unknown but could involve a signaling element within the ER lumen such as cytochrome b5. Alternatively, RTE1 may mediating responses from other ligands. Because these additional pathways likely represent outputs that are secondary to the CTR1-mediated pathway, as well as being receptor isoform-specific, thinner arrows are used to indicate their signaling role. This model also depicts cooperative signaling where the binding of ethylene to one receptor may affect the signaling state of neighboring receptors to amplify the signal from a single binding event. Cooperative signaling could regulate signaling to the CTR1-dependent pathway as well as the additional CTR1-independent pathways.

4.7 Concluding Remarks

Ethylene binds to its cognate receptors in plants to mediate the variety of responses associated with this phytohormone. Physical interactions between the receptors and downstream signaling components are vital to signal transduction. The majority of the responses we associate with ethylene are dependent on signaling from the receptors to CTR1 and are likely transmitted via conformational changes in the receptors, transduction to CTR1 facilitated by physical interaction of the receptors and CTR1. The receptors also interact with other proteins. These interactions may serve to modulate signaling through the CTR1-dependent pathway and/or allow for signaling through alternative CTR1-independent pathways. There is increasing evidence for isoform-specific interactions of the receptors with downstream signaling elements. The existence of such interactions supports subfunctionalization of the receptors, explaining in part why ethylene receptors exist as multi-member families in plants. Future studies will undoubtedly reveal additional components of the ethylene–receptor signaling complexes.

Acknowledgments This work was supported by US National Science Foundation grants to BMB (MCB-0918430 and IOS-1254423) and GES (IOS-1022053).

References

Barry C, Giovannoni J. Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci USA. 2006;103:7923–8.

Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB. Short-term growth responses to ethylene in *Arabidopsis* seedlings are EIN3/EIL1 independent. Plant Physiol. 2004a;136:2921–7.

Binder BM, O'Malley RC, Wang W, Moore JM, Parks BM, Spalding EP, et al. *Arabidopsis* seedling growth response and recovery to ethylene. A kinetic analysis. Plant Physiol. 2004b;136:2913–20.

Binder BM, O'Malley RC, Wang W, Zutz TC, Bleecker AB. Ethylene stimulates nutations that are dependent on the ETR1 receptor. Plant Physiol. 2006;142:1690–700.

Bisson MM, Groth G. New insight in ethylene signaling: autokinase activity of ETR1 modulates the interaction of receptors and EIN2. Mol Plant. 2010;3:882–9.

Bisson MMA, Bleckmann A, Allekotte S, Groth G. EIN2, the central regulator of ethylene signalling, is localized at the ER membrane where it interacts with the ethylene receptor ETR1. Biochem J. 2009;424:1–6.

Bray D, Levin MD, Morton-Firth CJ. Receptor clustering as a cellular mechanism to control sensitivity. Nature. 1998;393:85–8.

Cancel JD, Larsen PB. Loss-of-function mutations in the ethylene receptor ETR1 cause enhanced sensitivity and exaggerated response to ethylene in *Arabidopsis*. Plant Physiol. 2002;129:1557–67.

Chang J, Clay JM, Chang C. Association of cytochrome b5 with ETR1 ethylene receptor signaling through RTE1 in *Arabidopsis*. Plant J. 2014;77:558–67.

Chen QHG, Bleecker AB. Analysis of ethylene signal-transduction kinetics associated with seedling-growth response and chitinase induction in wild-type and mutant *Arabidopsis*. Plant Physiol. 1995;108:597–607.

Chen Y-F, Randlett MD, Findell JL, Schaller GE. Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of *Arabidopsis*. J Biol Chem. 2002;277:19861–6.

Chen YF, Gao Z, Kerris RJ 3rd, Wang W, Binder BM, Schaller GE. Ethylene receptors function as components of high-molecular-mass protein complexes in *Arabidopsis*. PLoS One. 2010;5:e8640.

Clark KL, Larsen PB, Wang X, Chang C. Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. Proc Natl Acad Sci USA. 1998;95:5401–6.

Desikan R, Hancock JT, Bright J, Harrison J, Weir I, Hooley R, et al. A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. Plant Physiol. 2005;137:831–4.

Desikan R, Last K, Harrett-Williams R, Tagliavia C, Harter K, Hooley R, et al. Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtrbohF-mediated hydrogen peroxide synthesis. Plant J. 2006;47:907–16.

Dong CH, Rivarola M, Resnick JS, Maggin BD, Chang C. Subcellular co-localization of *Arabidopsis* RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 ethylene signaling. Plant J. 2008;53:275–86.

Dong CH, Jang M, Scharein B, Malach A, Rivarola M, Liesch J, et al. Molecular association of the *Arabidopsis* ETR1 ethylene receptor and a regulator of ethylene signaling, RTE1. J Biol Chem. 2010;285:40706–13.

Francis NR, Wolanin PM, Stock JB, DeRosier DJ, Thomas DR. Three-dimensional structure and organization of a receptor/signaling complex. Proc Natl Acad Sci USA. 2004;101:17480–5.

Gamble RL, Qu X, Schaller GE. Mutational analysis of the ethylene receptor ETR1. Role of the histidine kinase domain in dominant ethylene insensitivity. Plant Physiol. 2002;128:1428–38.

Gao Z, Schaller GE. The role of receptor interactions in regulating ethylene signal transduction. Plant Signal Behav. 2009;4:1152–3.

Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, et al. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. J Biol Chem. 2003;278:34725–32.

Gao Z, Wen C-K, Binder BM, Chen Y-F, Chang J, Chiang Y-H, et al. Heteromeric interactions among ethylene receptors mediate signaling in *Arabidopsis*. J Biol Chem. 2008;283:23801–10.

Gestwicki JE, Kiessling LL. Inter-receptor communication through arrays of bacterial chemoreceptors. Nature. 2002;415:81–4.

Grefen C, Städele K, Růžička K, Obrdlik P, Harter K, Horák J. Subcellular localization and in vivo interaction of the *Arabidopsis thaliana* ethylene receptor family members. Mol Plant. 2008;1:308–20.

Hall AE, Bleecker AB. Analysis of combinatorial loss-of-function mutants in the *Arabidopsis* ethylene receptors reveals that the *ers1 etr1 d*ouble mutant has severe developmental defects that are EIN2 dependent. Plant Cell. 2003;15:2032–41.

Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB. Ethylene perception by the ERS1 protein in *Arabidopsis*. Plant Physiol. 2000;123:1449–58.

Hall BP, Shakeel SN, Amir M, Haq NU, Qu X, Schaller GE. Histidine kinase activity of the ethylene receptor ETR1 facilitates the ethylene response in *Arabidopsis*. Plant Physiol. 2012;159:682–95.

Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, et al. The response regulator 2 mediates ethylene signalling and hormone signal integration in *Arabidopsis*. EMBO J. 2004;23:3290–302.

Huang YF, Li H, Hutchison CE, Laskey J, Kieber JJ. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. Plant J. 2003;33:221–33.

Ju C, Yoon GM, Shemansky JM, Lin D, Yin I, Chang J, et al. CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in *Arabidopsis*. Proc Natl Acad Sci. 2012;109:19486–91.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. Cell. 1993;72:427–41.

Kim H, Helmbrecht EE, Stalans MB, Schmitt C, Patel N, Wen CK, et al. Ethylene receptor ETHYLENE RECEPTOR1 domain requirements for ethylene responses in *Arabidopsis* seedlings. Plant Physiol. 2011;156:417–29.

Larsen PB, Chang C. The *Arabidopsis eer1* mutant has enhanced ethylene responses in the hypocotyl and stem. Plant Physiol. 2001;125:1061–73.

Lin Z, Arciga-Reyes L, Zhong S, Alexander L, Hackett R, Wilson I, et al. SlTPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development. J Exp Bot. 2008;59:4271–87.

Lin Z, Ho CW, Grierson D. AtTRP1 encodes a novel TPR protein that interacts with the ethylene receptor ERS1 and modulates development in *Arabidopsis*. J Exp Bot. 2009;60:3697–714.

Ma Q, Du W, Brandizzi F, Giovannoni JJ, Barry CS. Differential control of ethylene responses by GREEN-RIPE and GREEN-RIPE LIKE1 provides evidence for distinct ethylene signaling modules in tomato. Plant Physiol. 2012;160:1968–84.

Maddock JR, Shapiro L. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science. 1993;259:1717–23.

Mason MG, Schaller GE. Histidine kinase activity and the regulation of ethylene signal transduction. Can J Bot. 2005;83:563–70.

Mayerhofer H, Panneerselvam S, Mueller-Dieckmann J. Protein kinase domain of CTR1 from *Arabidopsis thaliana* promotes ethylene receptor cross talk. J Mol Biol. 2012;415:768–79.

McDaniel B, Binder BM. ETHYLENE RECEPTOR1 (ETR1) is sufficient and has the predominant role in mediating inhibition of ethylene responses by silver in *Arabidopsis thaliana*. J Biol Chem. 2012;287:26094–103.

Qiao H, Shen Z, Huang S-sC, Schmitz RJ, Urich MA, Briggs SP, et al. Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. Science. 2012;338:390–3.

Qiu L, Xie F, Yu J, Wen C-K. *Arabidopsis* RTE1 is essential to ethylene receptor ETR1 amino-terminal signaling independent of CTR1. Plant Physiol. 2012;159:1263–76.

Qu X, Hall BP, Gao Z, Schaller GE. A strong constitutive ethylene-response phenotype conferred on *Arabidopsis* plants containing null mutations in the ethylene receptors ETR1 and ERS1. BMC Plant Biol. 2007;7:3.

Resnick JS, Wen CK, Shockey JA, Chang C. REVERSION-TO-ETHYLENE SENSITIVITY1, a conserved gene that regulates ethylene receptor function in *Arabidopsis*. Proc Natl Acad Sci USA. 2006;103:7917–22.

Rivarola M, McClellan CA, Resnick JS, Chang C. ETR1-specific mutations distinguish ETR1 from other *Arabidopsis* ethylene receptors as revealed by genetic interaction with RTE1. Plant Physiol. 2009;150:547–51.

Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science. 1999;283:996–8.

Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*—five novel mutant loci integrated into a stress response pathway. Genetics. 1995;139:1393–409.

Schaller GE, Bleecker AB. Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. Science. 1995;270:1809–11.

Scharein B, Groth G. Phosphorylation alters the interaction of the *Arabidopsis* phosphotransfer protein AHP1 with its sensor kinase ETR1. PLoS One. 2011;6:e24173.

Scharein B, Voet-van-Vormizeele J, Harter K, Groth G. Ethylene signaling: identification of a putative ETR1-AHP1 phosphorelay complex by fluorescence spectroscopy. Anal Biochem. 2008;377:72–6.

Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K. Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system. FEBS Lett. 2000;478:227–32.

Wang W, Hall AE, O'Malley R, Bleecker AB. Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from *Arabidopsis* is not required for signal transmission. Proc Natl Acad Sci USA. 2003;100:352–7.

Wen X, Zhang C, Ji Y, Zhao Q, He W, An F, et al. Activation of ethylene signaling is mediated by nuclear translocation of the cleaved EIN2 carboxyl terminus. Cell Res. 2012;22:1613–6.

Wolanin PM, Stock JB. Bacterial chemosensing: cooperative molecular logic. Curr Biol. 2004;14: R486–7.

Xie F, Liu Q, Wen C-K. Receptor signal output mediated by the ETR1 N-terminus is primarily subfamily I receptor dependent. Plant Physiol. 2006;142:492–508.

Zhou X, Liu Q, Xie F, Wen CK. RTE1 is a Golgi-associated and ETR1-dependent negative regulator of ethylene responses. Plant Physiol. 2007;145:75–86.