Annals of Botany **95**: 901–915, 2005
doi:10.1093/aob/mci100, available online at www.aob.oupjournals.org

INVITED REVIEW

Ethylene Signal Transduction

YI-FENG CHEN, NAOMI ETHERIDGE and G. ERIC SCHALLER*
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

Received: 11 November 2004 Returned for revision: 11 December 2004 Accepted: 17 December 2004 Published electronically: 7 March 2005

• *Background* The phytohormone ethylene is a key regulator of plant growth and development. Components of the pathway for ethylene signal transduction were identified by genetic approaches in *Arabidopsis* and have now been shown to function in agronomically important plants as well.
• *Scope* This review focuses on recent advances in our knowledge on ethylene signal transduction, in particular on recently proposed components of the pathway, on the interaction between the pathway components and on the roles of transcriptional and post-transcriptional regulation in ethylene signalling.
• *Conclusions* Data indicate that the site of ethylene perception is at the endoplasmic reticulum and point to the importance of protein complexes in mediating the initial steps in ethylene signal transduction. The expression level of pathway components is regulated by both transcriptional and post-transcriptional mechanisms, degradation of the transcription factor EIN3 being a primary means by which the sensitivity of plants to ethylene is regulated. EIN3 also represents a control point for cross-talk with other signalling pathways, as exemplified by the effects of glucose upon its expression level. Amplification of the initial ethylene signal is likely to play a significant role in signal transduction and several mechanisms exist by which this may occur based on properties of known pathway components. Signal output from the pathway is mediated in part by carefully orchestrated changes in gene expression, the breadth of these changes now becoming clear through expression analysis using microarrays.

Key words: Ethylene, signal transduction, *Arabidopsis thaliana*, receptor, ETR1, CTR1, protein complex, transcriptional regulation, post-transcriptional regulation.

INTRODUCTION

The phytohormone ethylene plays roles in physiological processes throughout the life cycle of the plant (Mattoo and Suttle, 1991; Abeles *et al*., 1992). Its involvement in such agronomically important processes as senescence, abscission and fruit ripening has made ethylene a target for manipulation by chemical and biotechnological methodologies (Mattoo and Suttle, 1991; Abeles *et al*., 1992; Schaller, 2003). Ethylene has also been implicated in developmental processes such as the formation of the apical hook in dark-grown seedlings (this hook protects the apical meristem as the young seedling forces its way through soil toward the light), the regulation of cell expansion and flower development. Ethylene also regulates plant responses to biotic stresses such as those induced by pathogens, and to abiotic stresses such as those induced by flooding or drought (Mattoo and Suttle, 1991; Abeles *et al*., 1992; Roman *et al*., 1995; O'Donnell *et al*., 1996, 2003; Penninckx *et al*., 1998). There is cross-talk between the ethylene signalling pathway and other hormone signalling pathways, particularly with auxin, whose effects are often mediated by ethylene, but also with ABA, cytokinins, gibberellins and brassinosteroids. The wide-ranging effects of ethylene have made it a topic of intense research for decades, and although many components of the biosynthesis and signalling pathways are now known, much remains to be learned about the pathways and the complex regulation of proteins involved. In this review, we focus on recent advances in our knowledge on ethylene signal transduction, in particular on recently proposed components of the pathway, on the interaction between the pathway components, and on the roles of transcriptional and post-transcriptional regulation in ethylene signalling.

GENETICALLY DEFINED COMPONENTS OF THE SIGNAL TRANSDUCTION PATHWAY

Many key components of the ethylene signal transduction pathway were identified from a simple genetic screen that made use of ethylene's effect on dark-grown seedlings known as the 'triple response'. In the model plant species *Arabidopsis thaliana*, the triple response is characterized by inhibition of hypocotyl and root elongation, a thickened hypocotyl and an exaggerated apical hook. Populations of mutagenized *Arabidopsis* were screened for seedlings that displayed an altered triple-response phenotype, and this approach resulted in the identification of several ethylene-insensitive mutants. These mutants include *etr1* (*et*hylene *r*esponse) (Bleecker *et al*., 1988; Chang *et al*., 1993), *etr2* (Sakai *et al*., 1998), *ein2* (*e*thylene *in*sensitive) (Guzmán and Ecker, 1990; Alonso *et al*., 1999), *ein3* (Roman *et al*., 1995; Chao *et al*., 1997), *ein4*, *ein5*, *ein6* (Roman *et al*., 1995), *hls1* (*h*ook*l*e*s*s) (Guzmán and Ecker, 1990), and *eir1* (*e*thylene *i*nsensitive *r*oot) (Roman *et al*., 1995). Mutants were also identified that exhibited a triple response in the absence of ethylene. These include *ctr1* (*c*onstitutive *t*riple *r*esponse) (Kieber *et al*., 1993) and *ran1* (*r*esponsive to *an*tagonist) (Hirayama *et al*., 1999; Woeste and Kieber, 2000). Genetic and molecular analyses of these mutants have defined a pathway for ethylene signal

* For correspondence. E-mail george.e.schaller@dartmouth.edu

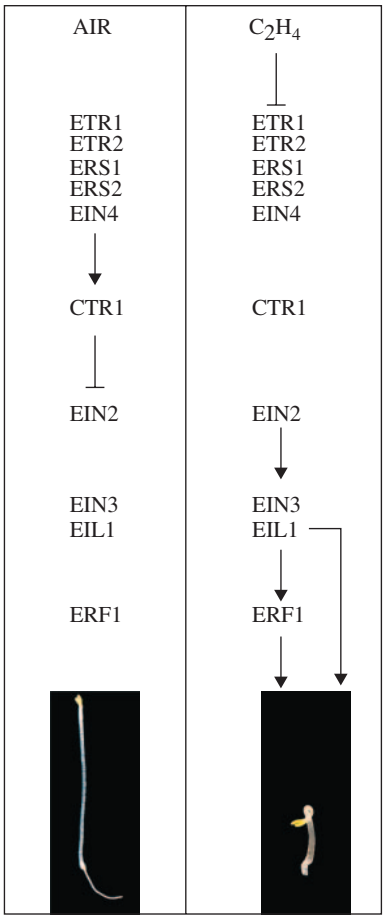

FIG. 1. Pathway for ethylene signal transduction based on genetic analysis. Key pathway components are shown. In air, the negative regulator CTR1 suppresses the pathway so that downstream positive regulators are inactive, thereby resulting in seedlings that show an air-grown phenotype. Binding of ethylene serves to inactivate CTR1, so that the downstream positive regulators are now active. Activation of ethylene responses results in seedlings that show the triple-response phenotype.

transduction leading from initial hormone perception to transcriptional regulation (Fig. 1). These components form the backbone on which our understanding of ethylene signal transduction is based, and much current research is aimed at determining how these components function in the pathway. A brief outline on these signalling components follows (Fig. 2). Readers who desire more detailed information are referred to other recent reviews (Bleecker and Kende, 2000; Alonso and Ecker, 2001; Schaller and Kieber, 2002; Chang and Bleecker, 2004).

Ethylene is perceived by a family of five membrane-bound receptors (ETR1, ETR2, ERS1, ERS2, EIN4) that have similarity to two-component regulators from bacteria (Bleecker, 1999; Schaller and Kieber, 2002). Loss-of-function (LOF) mutations in any single ethylene receptor have little or no effect upon seedling growth, consistent with functional overlap within the receptor family (Hua and Meyerowitz, 1998). Plants with multiple LOF mutations in the receptors show a constitutive ethylene response, indicating that the receptors are negative regulators of

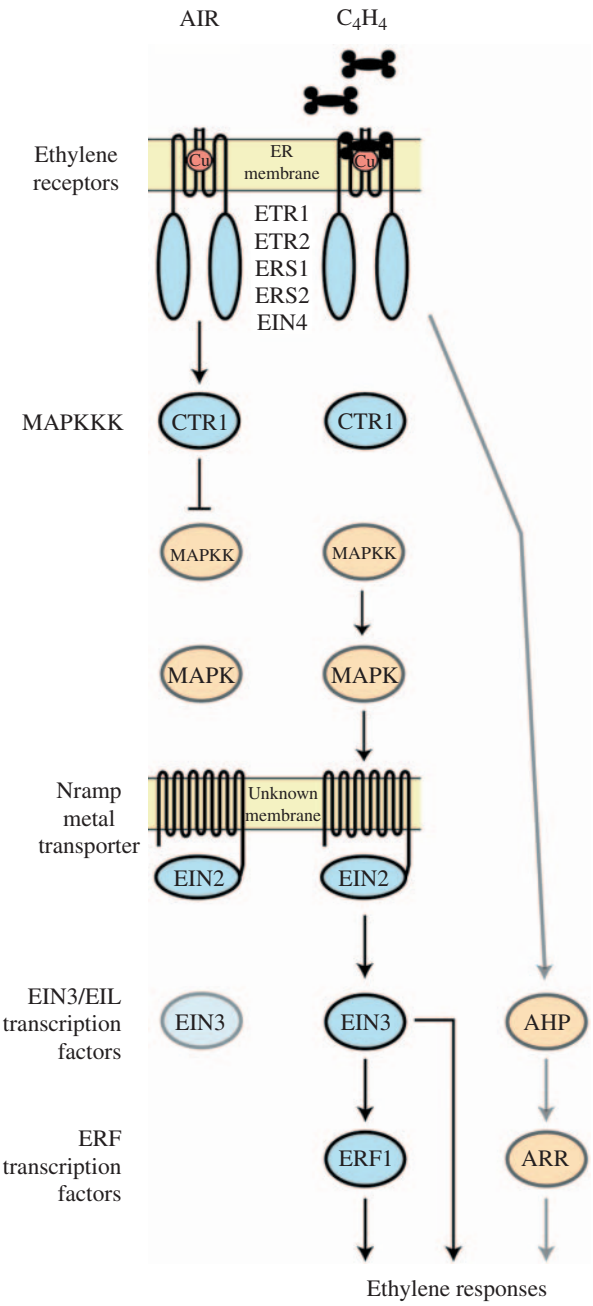

FIG. 2. Model for ethylene signal transduction that incorporates biochemical features of the pathway components. Soluble protein domains are shown as circles and predicting transmembrane structures are shown as lines. Confirmed components of the pathway are shown in blue; more recently proposed components are shown in orange. In air, ethylene receptors maintain CTR1 in an active state that serves to repress ethylene responses. In ethylene, the repression is relieved. Binding of ethylene inactivates the receptors, thereby inactivating CTR1. As a result, EIN2 is activated and a transcriptional cascade involving the EIN3/EIL and ERF transcription factors is initiated. Both families of transcription factors are involved in regulating ethylene responses. The protein level of EIN3 is lower in the absence of ethylene than in the presence of ethylene, due to degradation by the ubiquitin-proteasome pathway. The figure also incorporates components about which conflicting data has been reported, namely a MAPK module operating downstream of CTR1, and a two-component signalling pathway (AHP and ARR) functioning independently of the CTR1-mediated pathway.

ethylene signalling (Hua and Meyerowitz, 1998). As discussed later, this effect of LOF mutations in the receptors is probably due to their interaction with the downstream component CTR1. Interestingly, whereas most receptors are localized to the plasma membrane, analysis of the ethylene receptor ETR1 supports localization to the endoplasmic reticulum (ER) (Chen *et al*., 2002). Such a location is compatible with the ready diffusion of ethylene in both aqueous and lipid environments (Abeles *et al*., 1992).

CTR1 is the next downstream component identified in the signalling pathway (Kieber *et al*., 1993; Huang *et al*., 2003). CTR1 is a Raf-like ser/thr kinase with similarity to a mitogen-activated protein kinase kinase kinase (MAPKKK), suggesting the involvement of a MAP-kinase-like signalling cascade in the regulation of ethylene signalling. LOF mutations in CTR1 result in a constitutive ethylene-response phenotype, indicating that CTR1 is a negative regulator of ethylene signalling.

EIN2 has similarity to members of the Nramp metal-ion transporter family (Alonso *et al*., 1999). EIN2 plays a major role in the ethylene response as LOF mutations result in complete ethylene insensitivity for all ethylene responses tested, indicating that EIN2 is a positive regulator of the pathway. Also consistent with the action of EIN2 as a positive regulator is the finding that expression of the C-terminal soluble portion of EIN2 confers a constitutive ethylene-response phenotype upon plants grown in the light, although interestingly not upon dark-grown seedlings (Alonso *et al*., 1999). Based on the similarity to Nramp, it is tempting to speculate that EIN2 may regulate ethylene responses in part by altering ion concentrations. One possibility would be calcium, which has been implicated in ethylene responses (Raz and Fluhr, 1992; Kwak and Lee, 1997), but to date no ion transport activity has been demonstrated for EIN2. In addition, although EIN2 is predicted to be membrane-localized, the specific membrane system has not yet been determined. Thus the actual function of EIN2 in the pathway is still a mystery.

Functioning downstream of EIN2 is a small family of transcription factors that includes EIN3 and various EIN3-like (EIL) proteins (Roman *et al*., 1995; Chao *et al*., 1997). LOF mutations in *EIN3* cause partial ethylene insensitivity. This insensitivity can be rescued by expression of *EIN3*, *EIL1* or *EIL2* indicating that, along with EIN3, at least these two EILs can mediate an ethylene response (Chao *et al*., 1997). The EIN3/EIL family are involved in a regulatory cascade and stimulate the transcription of other transcription factors such as ERF1 (*e*thylene *r*esponse *f*actor) (Solano *et al*., 1998; Alonso *et al*., 2003*a*), a member of the ERF family of transcription factors (also sometimes called the EREBP family for *e*thylene *r*esponse *e*lement *b*inding *p*rotein) (Fujimoto *et al*., 2000). These transcription factors have been shown to act as activators or repressors of additional downstream ethylene-responsive genes (Ohme-Takagi and Shinshi, 1995). An *ein3*/*eil1* double mutant eliminates virtually all the transcriptional response to ethylene, indicating the key role this family of transcription factors plays in the immediate response of plants to ethylene.

BASIC MODEL FOR ETHYLENE SIGNAL TRANSDUCTION

The genetically defined elements have been ordered into a proposed pathway (Figs 1, 2) for ethylene signal transduction based on double-mutant analysis (Kieber *et al*., 1993; Hua *et al*., 1995, 1998; Roman *et al*., 1995; Sakai *et al*., 1998). A pathway based on genetic interactions may not always correspond to a biochemical or mechanistically based pathway. What we know about the biochemical nature of these elements does, however, support much of what has been defined genetically. For example, genetics places the receptors early in the pathway and the transcription factors at the end of the pathway. The one signalling element whose position in the pathway is hard to reconcile based on its biochemical characteristics is EIN2, because genetics places this membrane-localized protein in the middle of the pathway.

An important feature of the ethylene signalling pathway is that it contains both positive and negative regulators, some proteins thereby serving to induce the responses while others suppress them. The working model for ethylene signalling is shown in Figs 1 and 2. Figure 1 shows the genetic relationships among key players in the pathway whose role in ethylene signalling is substantiated by multiple lines of research. Figure 2 incorporates genetic relationships, biochemical data and some recently proposed players in the pathway about which contradictory data exists. According to the basic working model, the ethylene receptors activate the kinase activity of CTR1 in the air (absence of ethylene). CTR1 then actively suppresses the downstream responses, such that EIN2 and the EIN3/EIL transcription factors remain inactive. Upon binding ethylene, the receptors no longer activate CTR1, and so CTR1 no longer suppresses the pathway. This relief of suppression allows for activation of EIN2, induction of the transcriptional cascade, and the establishment of ethylene responses. The presence of both positive and negative regulators results in a model for ethylene signalling that sometimes seems counterintuitive, because the binding of ethylene inactivates early components in the pathway.

Evidence indicates that the basic elements and mechanism of the ethylene signal transduction pathway are conserved in agronomically important dicots and monocots, although some differences are observed (Adams-Phillips *et al*., 2004*a*; Klee, 2004). Ethylene receptors have been identified in many other plant species, including six genes in tomato (*Lycopersicon esculentum*) (Klee, 2002), five in rice (*Oryza sativa*) (Yau *et al*., 2004), four in tobacco (*Nicotiana tabacum*) (Terajima *et al*., 2001), three in cucumber (*Cucumis sativus*) (Yamasaki *et al*., 2000), carnation (*Dianthus caryophyllus*) (Shibuya *et al*., 2002) and pears (*Pyrus communis*) (El-Sharkawy *et al*., 2003), two in muskmelon (*Cucumis melo*) (Sato-Nara *et al*., 1999), passion fruit (*Passiflora edulis*) (Mita *et al*., 1998), peach (*Prunus persica*) (Rasori *et al*., 2002) and maize (*Zea mays*) (Gallie and Young, 2004), and one in wheat (*Triticum aestivum*) (Ma and Wang, 2003). Where examined, these receptors display properties in keeping with what has been found with the *Arabidopsis* ethylene

receptors. The receptors have (1) homology to prokaryotic two-component sensors; (2) redundancy within a single plant species; (3) act genetically as negative regulators of downstream responses; (4) show differential expression throughout development with a subset of the genes induced by ethylene; and (5) regulate a broad spectrum of physiological processes.

Less information is available about the conservation of the downstream components of the ethylene signal transduction pathway such as CTR1, EIN2 and EIN3 in other plant species, although these have been found when looked for. Interestingly, in tomato there appears to be a three-member *CTR1*-like gene family (Adams-Phillips *et al*., 2004*b*), compared to the single *Arabidopsis CTR1* gene. Recent reviews provide an up-to-date evaluation of the status of ethylene signalling elements in species of agronomic importance (Adams-Phillips *et al*., 2004*a*; Klee, 2004).

AN ER-LOCALIZED SIGNALLING COMPLEX BETWEEN THE ETHYLENE RECEPTORS AND CTR1

Localization of ethylene receptors to the endoplasmic reticulum (ER)

In most cases signal perception occurs either on the surface of the plasma membrane (e.g. receptors for peptide growth factors) or in the nucleus (e.g. receptors for steroid hormones). Unexpectedly, based on several independent lines of evidence, the ethylene receptor ETR1 was determined to localize to the ER (Chen *et al*., 2002). The ER-localization is supported by biochemical fractionation of *Arabidopsis* membranes as well as by immuno-electron microscopy. Localization of ETR1 to the ER was stable and was not influenced by ethylene binding. Preliminary results indicate that ERS1 and ETR2, two additional members in the ethylene receptor family of *Arabidopsis*, also localize to the ER (Y.-F. Chen and G. E. Schaller, unpubl. data). These results support the conclusion that the site of ethylene perception exists at the ER.

Because the gas ethylene can diffuse in both aqueous and lipid environments (Abeles *et al*., 1992), there is no particular requirement for cell surface localization for the ethylene receptors. The ER localization of the ethylene receptors, however, raises the question as to why this particular location and not another. Is there some particular quality of the ER that aids in ethylene signal transduction? A number of possibilities exist. First, it might be energetically efficient to have an ER-localized receptor, as the receptor is not exported all the way through the secretory system to the plasma membrane, which would expend ATP energy. Second, receptors are rapidly delivered to their site of action, which may be important for members of the *Arabidopsis* ethylene receptor family such as ERS1, ERS2 and ETR2 whose expression is induced by ethylene (Hua *et al*., 1998). Third, the ER is the multi-functional site of, for example, calcium homeostasis, protein biosynthesis and modification, lipid metabolism and stress responses (Staehelin, 1997; Vitale and Denecke, 1999; Hara-Nishimura and Matsushima, 2003). Ethylene may regulate these activities by co-localization of the signalling pathway with these other pathways. Fourth, the ER is a network-like organelle that contacts almost all other organelles and endomembrane systems of the cell (Staehelin, 1997). This feature may confer upon the ER the function as a 'scaffold' or a 'highway' to integrate a variety of signalling pathways as well as communication between various organelles within the cell. Ethylene regulates a broad spectrum of processes in the cell, and a site for ethylene perception at the ER may have evolved to meet such diversity.

Whether the ER is the exclusive site for ethylene perception remains an open question. Based on the analysis of multiple ethylene receptor isoforms in *Arabidopsis*, the ER is the predominant site of localization. It is possible, however, that a subset of receptors is present at one or more other membrane systems, or perhaps moves to the Golgi, plasma membrane or vacuole under specific cellular or environmental conditions that remain to be elucidated. It is also possible that differences may exist among different plant species. For example, transient expression of GFP fusions to the tobacco ethylene receptor NTHK1 in either insect or tobacco protoplasts resulted in fluorescence apparently associated with the plasma membrane (Xie *et al*., 2003). The physiological relevance of this result is unclear because expression of the genes was controlled by the 35S promoter, rather than the native promoter, and overexpression of membrane proteins can result in mislocalization. Additionally, fluorescence extended further inward from the plasma membrane than would be expected for a PM-localized protein and could thus potentially represent localization to the cortical ER that underlies the PM. Nevertheless, this study points to the necessity for further research to determine how common ER-localization of the receptors is in plants.

An ethylene receptor-CTR1 signalling complex

Interactions among proteins are limited by diffusion and concentration, which can slow down the rate at which information can be transferred from one element to the next in a signal transduction pathway. One way to circumvent this limitation is to assemble the proteins into complexes, in which two or more of the proteins involved in signal transduction are physically associated. Such complexes can orientate signalling elements as well as establish optimal stoichiometries between the signalling elements. Data indicates that the ethylene receptors form protein complexes with the downstream signalling component CTR1. This physical interaction has been analysed in most detail for ETR1 and CTR1, but evidence suggests that the other ethylene receptors also interact with CTR1.

Like the ethylene receptors, CTR1 has been shown to associate primarily with the ER, as revealed by sucrose density-gradient centrifugation experiments (Gao *et al*., 2003). CTR1 has no predicted transmembrane domains or membrane attachment motifs, suggesting that the membrane association of CTR1 occurs due to interaction with integral membrane proteins, the ethylene receptors being likely candidates. A pull-down assay demonstrated that

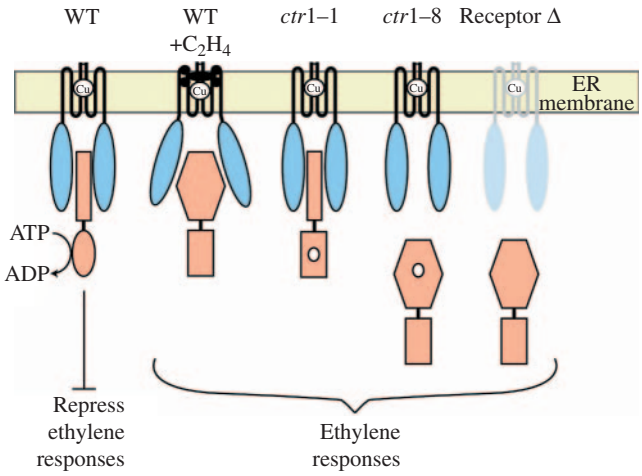

FIG. 3. Model for signalling by the ethylene receptor–CTR1 protein complex. The ethylene receptor (blue) contains one ethylene-binding site per homodimer, with ethylene binding mediated by a single copper ion (Cu) present in the ethylene-binding site. CTR1 (shown in orange) interacts with the soluble domain of the receptor and as a result of this interaction is localized to the ER. In air, the kinase domain of CTR1 actively represses ethylene responses. Binding of ethylene by the receptor leads to a conformational change in CTR1 that reduces its kinase activity, thereby relieving repression of the ethylene response pathway. Mutations in CTR1 (indicated by a white circle) can result in an ethylene-like response in air by two different mechanisms. Mutations such as *ctr1-1* eliminate the kinase activity of CTR1 so that CTR1 is unable to repress the ethylene responses. Mutations such as *ctr1-8* disrupt the interaction of CTR1 with the receptor, resulting in mis-localization of CTR1 to the cytosol. Loss-of-function mutations that eliminate multiple members of the ethylene receptor family (receptor Δ) also result in mis-localization of CTR1 to the cytosol. In the cytosol, CTR1 may adapt a kinase-inactive conformation (as shown here) or may not be proximate to the appropriate phosphorylation substrate (figure adapted from Gao *et al.*, 2003).

CTR1 and ETR1 are in the same signalling complex in *Arabidopsis* (Gao *et al.*, 2003). Several additional lines of evidence support a direct interaction between CTR1 and ETR1. Analysis using the yeast two-hybrid system indicates that CTR1 is able to interact with ETR1 as well as with ERS1 or ETR2 (Clark *et al.*, 1998; Cancel and Larsen, 2002). In addition, CTR1 has been shown to be able to interact with ETR1 and ETR2 using proteins transgenically expressed in *E. coli* and yeast (Clark *et al.*, 1998; Cancel and Larsen, 2002; Gao *et al.*, 2003).

Analysis of various mutations in CTR1 and the ethylene receptors clarify the mechanism of action of CTR1 and demonstrate the importance of the ethylene receptor–CTR1 signalling complex in transmission of the ethylene signal (Fig. 3). CTR1 mutants that lack kinase activity result in the constitutive ethylene-response phenotype, demonstrating the importance of the kinase activity in suppressing ethylene responses (Kieber *et al.*, 1993). Although kinase activity of CTR1 is required, it is not apparently sufficient to suppress ethylene responses. Localization of CTR1 to the ER through its association with the ethylene receptors is also required. A mutation in CTR1 (*ctr1-8*) that disrupts the ability of CTR1 to interact with the ethylene receptors causes a redistribution of CTR1 from the membrane to the cytosol, and results in a constitutive ethylene response (Gao *et al.*, 2003; Huang *et al.*, 2003). Similarly, elimination of ethylene receptors also results in a redistribution of CTR1 from the membrane to the cytosol. Because there is a family of ethylene receptors, elimination of a single family member has little effect upon redistribution of CTR1, but in double- and triple-receptor mutants significant amounts of CTR1 are found in the cytosol instead of at the membrane. Such higher-order receptor mutants show a constitutive ethylene response phenotype (Hua and Meyerowitz, 1998), apparently due to this loss of CTR1 from the membrane. In the cytosol, CTR1 may adapt a kinase-inactive conformation or may not be proximate to the appropriate phosphorylation substrate.

A central question that still remains is the mechanism by which the activity of CTR1 is regulated by the ethylene receptors. Based on the model for signalling by the protein kinase Raf (Heidecker *et al.*, 1990), the N-terminus of CTR1 might be able to autoinhibit its C-terminal Ser/Thr kinase activity. In air (absence of ethylene), the receptors would maintain the N-terminus of CTR1 in a conformation such that CTR1 is active and able to repress downstream ethylene responses. Ethylene binding would induce a conformational change such that the N-terminus of CTR1 could autoinhibit its kinase activity, thereby releasing the repression on downstream ethylene responses. Because of the physical interaction between the ethylene receptors and CTR1, it is possible that regulation of CTR1 could occur due to conformational changes induced in the receptors by binding ethylene then being passed on to CTR1. It is also possible that such regulation could be mediated through changes in the phosphorylation status of the ethylene receptors and/or CTR1. Phosphorylation is a common mechanism used to regulate enzymatic activity, the negative charge of the phosphate group helping contribute to changes in protein conformation. The ethylene receptors ETR1 and ERS1 have been shown to contain the predicted histidine kinase activity (Gamble *et al.*, 1998; Moussatche and Klee, 2004). In addition, members of the ethylene receptor family have been proposed to contain Ser/Thr kinase activity (Moussatche and Klee, 2004; Zhang *et al.*, 2004).

The relative importance of receptor kinase activity in signalling is still unclear. Initial studies failed to uncover a role for kinase activity in ethylene signalling (Gamble *et al.*, 2002; Wang *et al.*, 2003). Recently, mutations that should reduce or eliminate receptor kinase activity were found to have a small effect upon the induction of ethylene responses as well as upon how rapidly seedlings recover their normal growth rate after ethylene is removed (Binder *et al.*, 2004; Qu and Schaller, 2004). These effects, however, are not as large as might be predicted if changes in phosphorylation were the sole regulator of signal transduction between the ethylene receptors and CTR1. Additional research using new mutants should help clarify how activity of CTR1 is regulated.

RECENTLY PROPOSED COMPONENTS OF THE SIGNAL TRANSDUCTION PATHWAY

A number of recent studies have implicated new components in the regulation of ethylene signal transduction. Their role in ethylene signal transduction is, however,

not as clear as those components previously identified by forward genetic analysis. The proposed role of these new components is based on loss-of-function mutant phenotypes that result in slight modifications of ethylene responses or upon phenotypes resulting from over-expression. Thus these components are not necessarily part of the primary ethylene signal transduction pathway. For example, they could represent components of other pathways that impinge upon ethylene signalling. Additional work is needed to determine their contribution to ethylene signalling under physiological conditions, and indeed data conflicting with the original reports has already been found in some instances.

MAPK and MAPKK

A MAPK kinase pathway has been proposed to function in ethylene signalling because CTR1 is similar to Raf, a member of the MAPKKK (mitogen-activated protein kinase kinase kinase) family (Kieber *et al*., 1993; Huang *et al*., 2003). Thus researchers have attempted to determine if a MAPK cascade composed of CTR1, a MAPKK and a MAPK participates in the ethylene signalling pathway. Several studies provide evidence supporting this hypothesis (Novikova *et al*., 2000; Ouaked *et al*., 2003). Ethylene activates MAP kinase-like activity of a 47-kDa protein in *Arabidopsis* (Novikova *et al*., 2000). Similarly, the transient expression of CTR1 activates an endogenous kinase of 44 kDa in maize mesophyll protoplasts (Novikova *et al*., 2000). Ouaked *et al*. (2003) found that the ethylene precursor ACC could activate kinase activity of two *Medicago* MAPKs (SIMK and MMK3), the *Arabidopsis* MPK6 and a *Medicago* MAPKK (SIMKK). In this study, the *Medicago* SIMKK specifically mediated ACC-induced activation of SIMK and MMK3. Transgenic *Arabidopsis* plants over-expressing SIMKK have constitutive MPK6 activation and also show induction of several ethylene-induced target genes. The lines in which SIMKK is ectopically expressed also resemble *ctr1* mutants, showing an apparent constitutive ethylene response phenotype in air (absence of ethylene). Based on the analysis of various *Arabidopsis* mutants, the ACC-induced activation of MPK6 required functional ethylene receptors and CTR1 but occurred upstream or independently of EIN2 and EIN3. Figure 2 shows how this MAPK cassette would fit into the currently understood pathway for ethylene signal transduction.

There are, however, serious reservations about these studies. First, strictly on theoretical grounds, the evidence suggests an unusual form of the MAPK pathway not previously observed in other eukaryotes. CTR1 is a negative regulator of ethylene signal transduction, and is inactivated by ethylene (Kieber *et al*., 1993; Huang *et al*., 2003). In contrast, SIMKK and MPK6 both appear to be activated by ethylene (Ouaked *et al*., 2003). This would suggest a regulatory cascade operating in the form of MAPKKK ⊣ MAPKK → MAPK, rather than the generally accepted MAPKKK → MAPKK → MAPK cascade (i.e. inhibition of a MAPKK by a MAPKKK). Second, it has not been possible to replicate all the data found in the paper by Ouaked *et al*. (2003). Specifically, in a separate study, no significant effect of ACC upon activation of MPK6 was observed in wildtype or mutant *Arabidopsis* plants (Liu and Zhang, 2004). Examination of the data does suggest that a slight stimulation may occur, but this is not nearly as pronounced as that found in a stress response such as occurs with wounding. Third, reduction in the expression of *Arabidopsis MPK6* by RNA interference or through T-DNA insertions does not cause appreciable effects upon ethylene responses (Ecker, 2004; Menke *et al*., 2004).

A likely explanation for the conflicting data arises due to the role of MAPK pathways in stress responses. Plants under either biotic or abiotic stress produce increased levels of ethylene, called stress ethylene, which is able to initiate various ethylene-regulated responses (Mattoo and Suttle, 1991; Abeles *et al*., 1992). Recently Kim *et al*. (2003) provided evidence to indicate that an active NtMEK2 (MAPKK) and SIPK (MAPK) cascade mediated the production of stress ethylene in tobacco. The activation of SIPK coincided with a dramatic increase in ACC synthase (ACS) activity, which was followed by the activation of a subgroup of ACS and ACC oxidase genes, all of which would result in a rapid increase in ethylene production (Kim *et al*., 2003). A similar role for MPK6 in the production of stress ethylene by *Arabidopsis* plants has also now been demonstrated (Liu and Zhang, 2004). The proposed role of MAPK pathways in the production of stress ethylene makes it more difficult to ascribe a specific role for a MAPK pathway functioning in the primary pathway for ethylene signal transduction. For example, increased production of ethylene by the stress pathway would activate ethylene signal transduction, resulting in various ethylene responses, but this would be a secondary consequence of activating the MAPK pathway. Thus, while a MAP kinase pathway may function downstream of the ethylene receptors, it is no longer considered likely to involve SIMK and SIMKK-like proteins.

ARR2

The *Arabidopsis* ethylene receptors are similar to histidine kinases that function in bacterial two-component signalling systems (Bleecker, 1999; Schaller and Kieber, 2002), histidine kinase activity having been confirmed for some of the ethylene receptors (Gamble *et al*., 1998; Moussatche and Klee, 2004). Two-component systems are signal transduction systems that transfer a phosphate between a series of defined proteins acting in sequence: a phospho-relay (Schaller, 2000; Schaller *et al*., 2002). The simplest system involves a histidine kinase and a response regulator (the ARR family in *Arabidopsis*); a more complicated system also involves a third protein known as a His-containing phospho-transfer protein (the AHP family in *Arabidopsis*). The clearest role for two-component systems in *Arabidopsis* is in cytokinin signal transduction, which incorporates all elements of the two-component signalling system (Hwang and Sheen, 2001; Haberer and Kieber, 2002; Heyl and Schmülling, 2003; Kakimoto, 2003).

Because some of the ethylene receptors are histidine kinases, there is interest in determining if a traditional two-component signalling system contributes to ethylene signal transduction as well. Recently, Hass *et al*. (2004) examined the effects of a mutation in the response regulator

ARR2 upon ethylene signal transduction. They reported a slight reduction in the ethylene sensitivity of seedlings containing an *arr2* loss-of-function mutation, and that over-expression of *ARR2* complemented the ethylene hyposensitive phenotype. They also report that an activated form of ARR2 can induce a constitutive ethylene-response-like phenotype in *Arabidopsis* seedlings. The authors propose that an ETR1-initiated phospho-relay regulates activity of ARR2, with ARR2 representing the final step in this phospho-relay (Hass *et al*., 2004). This would represent a new branch of the ethylene signalling pathway, involving both an AHP and an ARR, operating independently of the branch that contains CTR1 (Fig. 2).

There are, however, reservations on how far the interpretations of this study can be carried. First, we have tested three independent *arr2* T-DNA insertion mutants, including the mutant examined by Hass *et al*. (2004), but not observed a significant effect upon the seedling ethylene response (M.G. Mason and G.E. Schaller, unpubl. data). Second, the phenotype of seedlings expressing the activated form of ARR2, while dwarfed, do not show the apical hook or shortened root that one would expect upon activation of the ethylene signalling pathway (Hass *et al*., 2004). Thus, *ARR2* would appear to have only limited involvement in the regulation of accepted ethylene responses. It seems reasonable to suppose, given the histidine kinase activity of the receptors, that they would participate in a phospho-relay, but it is still unclear as to what role this plays, whether it allows for a CTR1-independent change in gene regulation in response to ethylene or potentially allows for cross-talk with the cytokinin signalling pathway.

PP2A

The mutant *enhanced ethylene response 1* (*eer1*) displays both increased sensitivity and increased amplitude of response to ethylene (Larsen and Chang, 2001). Molecular cloning of *eer1* revealed that its mutant phenotype results from a loss-of-function mutation in the previously characterized *RCN1*, which encodes a regulatory subunit for the phosphatase PP2A (Larsen and Cancel, 2003). Loss-of-function mutations affecting PP2A activity increased ethylene responses. Blockage of PP2A activity with inhibitors caused exaggeration of ethylene responses. Intriguingly, a catalytic subunit of PP2A (PP2A-1C), but not the regulatory subunit RCN1, was found capable of interacting with the kinase domain of CTR1 *in vitro* (Larsen and Cancel, 2003). CTR1 did not phosphorylate RCN1 or PP2A-1C, suggesting that they are not substrates of CTR1. It is hypothesized that PP2A might reduce CTR1 activity such that a lower threshold of ethylene is required for manifestation of ethylene response, a situation similar to that of mammalian Raf whose activation requires PP2A activity (Larsen and Cancel, 2003). On the other hand, previous analyses of RCN1 uncovered a role for this gene in auxin signalling (Garbers *et al*., 1996; Rashotte *et al*., 2001), and so the role of this gene in ethylene signalling may not be direct but may arise due to the cross-talk between the auxin and ethylene signalling pathways.

Other candidates

Clues from physiological and biochemical analyses indicate that monomeric GTP-binding proteins and nucleoside diphosphate kinase might regulate the ethylene signalling pathway (Moshkov *et al*., 2003*a*, *b*; Novikova *et al*., 2003). In addition, genetic analysis may still uncover new genes that function either directly in the pathway or that interact with the pathway. Mutant screens are probably saturated for the identification of individual mutants that have substantial effects upon ethylene signal transduction. The search for weakly ethylene-insensitive mutants, however, is still a promising direction for uncovering new genes that affect ethylene signal transduction (Alonso *et al*., 2003*b*).

POTENTIAL MECHANISMS FOR SIGNAL AMPLIFICATION IN THE PATHWAY

In signal transduction pathways, small amounts of an initial signal are recognized by the receptor and this information is then passed along a signalling circuit to regulate downstream responses, such as changes in gene expression and enzyme activity. Transfer of the signal along the circuit depends on the activity of elements that make up the signalling circuit, the affinity between the elements and concentration of the elements. Activity and/or affinity can be altered by post-translational modifications such as phosphorylation. The concentration of each signalling element can be altered by changes in biosynthesis (transcriptional and post-transcriptional regulation) and/or degradation. Alteration of any of these parameters can be used to modify how effectively the signal is transduced. In most cases, the signal is amplified during signal transduction. Thus the concentration of initial elements in the pathway (hormone and receptor) can be quite low, but still result in the activation of many downstream signalling elements. In this section we will consider some of the possibilities by which the ethylene signal can be amplified based on what we know about elements in the signalling circuit.

One basis for postulating that amplification occurs during ethylene signal transduction comes from a consideration of the wide concentration range over which ethylene affects plant development. In dark-grown *Arabidopsis* seedlings, changes in the growth response to ethylene have been observed at ethylene concentrations as low as 0·2 nL L^{-1} (Binder *et al*., 2004*a*, *b*); in contrast fruit ripening involves ethylene production that exceeds 100 μL L^{-1} (Abeles *et al*., 1992). Transcriptional changes have been shown to occur over a range of ethylene concentrations from 0·1 to 1000 μL L^{-1} (Chen and Bleecker, 1995). Not only are plants able to respond to ethylene across a broad concentration range, but they are able to do so at both extremely low concentrations (the 0·2 nL L^{-1} threshold level given above is over 100-fold below the calculated K_d for ethylene binding by the receptor ETR1) and extremely high concentrations (1000 μL L^{-1} being over 10 000-fold above the K_d for ethylene binding). This suggests that the receptors have a mechanism by which to recognize and transduce information in response to very subtle changes in levels of receptor

occupancy by ethylene, indicative of some form of signal amplification.

One possibility by which the receptors might accomplish this task is to have receptors with widely differing affinities for ethylene. Affinities for only two of the five ethylene receptors (ETR1 and ERS1) in *Arabidopsis* have been determined, both of these, however, giving very similar values (Schaller and Bleecker, 1995; Hall *et al*., 2000). These values were determined using receptors transgenically expressed in yeast, and it is thus possible that affinity of the receptors in plants could be modified, potentially as part of a feedback mechanism to regulate the plant's responsiveness to ethylene. Two additional possibilities for signal amplification can be postulated based on what has been found with the histidine-kinase-linked chemotaxis receptors of bacteria. These receptors employ a feedback mechanism, involving methylation of the receptor, by which the signal output of the receptor can be modified so that the receptor adapts to increasing concentrations of the ligand (Parkinson, 1993). No such methylation has been noted for the ethylene receptors, but it is possible that other modifications such as phosphorylation could adjust and reset signal output so that the receptors amplify signals from a wider spectrum of ligand concentrations. The chemotaxis receptors also form higher-order clusters (Bray *et al*., 1998; Ames *et al*., 2002; Gestwicki and Kiessling, 2002). Binding of the ligand by one receptor can affect signal output by other receptors in the cluster even when these other receptors have no bound ligand. This occurs because conformational changes induced in the receptor upon ligand binding are propagated among the surrounding receptors through direct physical interactions. Such clustering could also potentially serve to amplify signal output from the ethylene receptors.

A common means for signal amplification is through kinase-mediated phosphorylation. An enzyme can catalyse multiple reactions, so a single protein kinase may phosphorylate and thereby regulate activity of many copies of its substrate. Phosphorylation is apparently a key mechanism by which the ethylene signal is transduced, this being most clear in the case of the ser/thr protein kinase CTR1. Mutations that eliminate kinase activity of CTR1 result in the constitutive ethylene response phenotype, indicating the CTR1-mediated phosphorylation is required for suppressing ethylene responses in the air (Kieber *et al*., 1993; Huang *et al*., 2003). The possibilities for signal amplification become even greater if one has a cascade of protein kinases acting sequentially, as in the case of the MAP kinase cascade proposed to function downstream of CTR1 (Ouaked *et al*., 2003).

A transcription-factor cascade represents yet another means by which a signal might be amplified. In this case one transcription factor would induce transcription of a second group of transcription factors that would in turn induce transcription of target genes. Evidence supports such a cascade operating in the regulation of ethylene responses in *Arabidopsis*. In *Arabidopsis*, the first elements operating in this cascade are the EIN3/EIL transcription factors (Chao *et al*., 1997). Among their targets are ERF1 and the EREBP family of transcription factors (Solano *et al*., 1998; Alonso *et al*., 2003*a*). It is also postulated that ERF1 may induce transcription of additional transcription factors. Such a transcription-factor cascade serves as a means to amplify the signal as well as allowing for fine-tuning of signal output.

REGULATION OF THE LEVELS OF COMPONENTS IN THE ETHYLENE SIGNALLING PATHWAY

Ethylene signalling, from biosynthesis to response, is highly regulated at both the transcriptional and post-transcriptional levels. Regulation of either hormone levels or that of key proteins in the signal transduction pathway can regulate flux through the pathway, and thereby regulate the final level of ethylene response. The identification of the genes involved in ethylene biosynthesis and signalling has allowed a direct assessment as to what factors regulate their expression. Not surprisingly, ethylene itself is often an important regulator of expression. Thus the signalling pathway is able to feed back on itself to regulate its own sensitivity to ethylene.

Transcriptional regulation

The final two steps in ethylene biosynthesis are catalysed by ACC synthase (ACS) and ACC oxidase (ACO). ACS and ACO are each encoded by gene families, and there is thus the possibility for differences in the regulation of expression amongst the different family members. Transcription of the *ACS* family members in *Arabidopsis* is differentially regulated during development, in different tissues and in response to different stimuli such as ozone and anaerobis (Liang *et al*., 1992; Vahala *et al*., 1998). One *Arabidopsis* ACS isoform (*ACS4*) is transcriptionally induced by auxin and may be a component involved in the cross-talk between this hormone and ethylene (Liang *et al*., 1992). Members of the *ACO* gene family are also differentially regulated, indicating that although ACS is the key regulatory point for ethylene biosynthesis, regulation of *ACO* expression also has functional significance (Prescott and John, 1996). At least two *ACO* genes in *Arabidopsis* are ethylene inducible (Alonso *et al*., 2003*a*; Zhong and Burns, 2003), suggesting that a feedback mechanism is in place to ensure that there will be no limitations to ethylene production once the precursor ACC is produced. One of these genes, *ACO2*, is induced primarily in the apical region of seedlings, which results in differential cell expansion in this area, and thus formation of the apical hook (Silk and Erickson, 1978; Ecker, 1995; Raz and Ecker, 1999). Gibberellins are also essential for hook formation and may be involved in cross-talk with ethylene to control this process (Vriezen *et al*., 2004). This type of multi-hormone regulation appears to be a common theme in ethylene biosynthesis as there is also cross-talk between auxin and ethylene to regulate two *ACO* genes in rice; *OsACO3* is induced by ethylene, but not in the presence of auxin and *OsACO2* is induced by auxin, but to a reduced level in the presence of ethylene (Chae *et al*., 2000).

The ethylene receptors are expressed throughout the plant, but with variations in levels in different tissues based on the analysis of *Arabidopsis* and tomato (Hua *et al*., 1998; Lashbrook *et al*., 1998; Tieman and Klee, 1999).

Three receptor genes in *Arabidopsis* (*ERS1*, *ETR2* and *ERS2*) are induced by ethylene (Hua *et al*., 1998). Because the receptors are negative regulators of the pathway (Hua and Meyerowitz, 1998), an increase in the number of receptors could result in de-sensitization of the pathway. This mechanism of reducing ethylene sensitivity has been proposed to limit the spread of necrosis following pathogen infection in tomato (Ciardi *et al*., 2000). Ethylene-induced biosynthesis of new receptors could also play another role in ethylene signal transduction. Evidence indicates that the ethylene receptors are capable of binding ethylene very tightly, the apparent half-life for dissociation of ethylene from ETR1 is at least 12·5 h (Schaller and Bleecker, 1995). However, plants respond within minutes to a decrease in ethylene levels (Abeles *et al*., 1992; Binder *et al*., 2004*b*), an observation difficult to account for if all receptors are still occupied by ethylene. The production of new 'empty' receptors may account for the re-sensitization of the plant to ethylene. If ethylene levels have decreased, the newly synthesized receptors will not bind ethylene and consequently, being negative regulators, will suppress ethylene responses in the plant.

There is also evidence that receptor levels can be reduced at the transcriptional level in response to stimuli. The receptor *ETR1*, on which ethylene has little stimulatory effect at the transcriptional level, is apparently repressed by ethylene in the apical hook of etiolated seedlings (Raz and Ecker, 1999). Recently transcription of *ETR1* has also been shown to be repressed by salt and osmotic stress, suggesting that abiotic stresses may not only alter the production of ethylene as a stress signal, but may also alter expression of components in the signalling pathway (Zhao and Schaller, 2004).

There is little experimental data describing transcriptional regulation of downstream components of the ethylene response pathway. The *Arabidopsis CTR1* gene is not ethylene inducible (Kieber *et al*., 1993; Gao *et al*., 2003), which is in contrast to a member of the tomato family, *LeCTR1*, that is induced by ethylene in immature fruits and in leaves (Adams-Phillips *et al*., 2004*b*). In addition, there is no evidence for transcriptional regulation of *EIN2* in *Arabidopsis* and tomato (Klee, 2004), although in maize expression of *EIN2* is differentially regulated in embryos (Gallie and Young, 2004). The *EIN3/EIL* transcription factors do not appear to be primarily regulated by ethylene at the transcriptional level (Chao *et al*., 1997; Tieman *et al*., 2001; Lee and Kim, 2003; Rieu *et al*., 2003*a*), although ethylene does have an effect on expression of *EIL1* from *Arabidopsis* (De Paepe *et al*., 2004). Instead, as discussed below, most of the regulation within the *EIN3/EIL* gene family appears to be through manipulation of protein concentrations at the post-transcriptional level.

Post-transcriptional regulation

Ethylene signalling is highly regulated at the post-transcriptional level via ubiquitin/26S proteasome-mediated degradation. This mechanism is widely used in eukaryotes to control the levels and thus the activity of key proteins. Components of the ubiquitin/26S proteasome system are encoded by approximately 1500 genes in the *Arabidopsis* genome, and control numerous aspects of the plant's life cycle, from growth and development to defence responses (Sullivan *et al*., 2003; Vierstra, 2003). Degradation is initiated by the covalent attachment of ubiquitin (Ub) moieties to the targeted protein, as regulated by the actions of Ub-conjugating and Ub-ligase enzymes. The 26S proteasome then recognizes the polyubiquitinated protein and degrades it.

Post-transcriptional regulation is involved in ethylene biosynthesis where, after production, ACC synthase (ACS) is rapidly degraded, thereby causing a decline in ACS activity (Kim and Yang, 1992). Biochemical studies revealed that this turnover is controlled by phosphorylation of a component other than ACS (Felix *et al*., 1991; Spanu *et al*., 1994). Recent genetic studies in *Arabidopsis* have begun to shed light on how this process may occur. Several ethylene-overproducing mutants (*eto1* to *3*) were identified in *Arabidopsis* and shown to arise from a change in the activity of ACS. For example, it was found that ACS5 protein levels are negatively regulated by ETO1 (Woeste *et al*., 1999; Chae *et al*., 2003), and this regulation may occur via ubiquitin/26S proteasome degradation (Wang *et al*., 2004). One model suggests that ETO1 promotes ubiquitination, and thus turnover, of ACS5 through interaction with both ACS5 and a scaffold protein of the ubiquitin-conjugating complex (Wang *et al*., 2004). Phosphorylation has been implicated in targeting proteins for ubiquitination in animal systems (Fuchs *et al*., 1998) and it may thus be that phosphorylation plays a role in the proteasome-mediated degradation of ACS5.

ACC oxidase (ACO) levels may also be regulated by proteasome-mediated degradation through the activity of RUB (related to ubiquitin) and RCE (RUB-congugating enzyme), which are involved in activating ubiquitin ligase to promote ubiquitination (Larsen and Cancel, 2004). Reduction in the mRNA levels of *rce1* (Larsen and Cancel, 2004) or *rub1* and *rub2* (Bostick *et al*., 2004) results in greatly increased levels of ethylene, and this increase appears, at least in the case of *rce1*, to be a result of increased ACO activity (Bostick *et al*., 2004). These results suggest that some of the ACS and ACO family members are post-transcriptionally regulated through proteasome-mediated degradation.

The ethylene receptor ETR1 is post-transcriptionally regulated, although it is not known by what mechanism. Certain mutations in the ethylene-binding region of the *ETR1* gene cause increases in receptor protein levels that are not reflected by changes in mRNA levels (Zhao *et al*., 2002). Similar results are obtained when the ethylene-binding site is perturbed by silver ions suggesting that the post-transcriptional regulation of ETR1 is controlled by ligand binding (Zhao *et al*., 2002). Ligand-mediated turnover is a common mechanism for regulating receptor levels in animals (Wiley, 1992), but further studies are needed to fully clarify the details of post-transcriptional ethylene receptor regulation in plants.

The clearest role for post-transcriptional regulation in the pathway for ethylene signal transduction is in regulation of the key transcription factor EIN3. In the absence of

ethylene, EIN3 is continuously degraded through the proteasome-mediated pathway, thereby preventing activation of its transcriptional targets. In the presence of ethylene, degradation of EIN3 is suppressed, thereby allowing EIN3 protein levels to increase and thus promote the ethylene response (Fig. 2) (Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). Degradation of EIN3 is regulated by two ubiquitin-ligases, EBF1 and EBF2 (*E*IN3 *b*inding *F* box), which promote the ubiquitination and turnover of EIN3. Mutants in either *EBF1* or *EBF2* result in increased EIN3 accumulation. Accumulation is even greater in the double *ebf1 ebf2* mutant; this mutant exhibits a constitutive ethylene response phenotype in air due to stabilization of EIN3 (Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). These proteins appear to work together to control EIN3 levels; however they are differentially regulated by ethylene so they may have subtly different roles during the course of the ethylene response. EBF1 appears to be involved in controlling EIN3 at low ethylene concentrations, possibly to keep EIN3 levels reduced until a certain ethylene threshold has been reached (Gagne *et al*., 2004). *EBF2* is induced at higher ethylene levels (Alonso *et al*., 2003*a*; Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004), which may facilitate EIN3 degradation once the signal is gone and so allow a rapid response to changing ethylene concentrations.

Several models have been proposed for the mechanism for ethylene-mediated stabilization of EIN3 (Guo and Ecker, 2003; Kepinski and Leyser, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). Ethylene may affect EBF1 and EBF2 stability, activity or location, which would in turn affect EIN3 accumulation. Alternatively, ethylene may promote post-translational modification of EIN3, such as phosphorylation, which could then affect the ability of EBF1/EBF2 to interact with EIN3 and target it for degradation. Regardless of the mechanism involved, these studies make it clear that varying the protein levels of EIN3 serves as an important means to control flux through the signalling pathway. EIN3 is in essence a bottleneck in the pathway such that slight changes in its protein level can have significant effects upon signal output.

CROSS-TALK WITH OTHER SIGNALLING PATHWAYS

Interactions among hormones and other physical-chemical factors have been a topic in plant hormone biology for decades. The identification of mutants that affect individual signalling pathways now allows for a careful analysis of the relative contribution of different signalling pathways to a specific response. These genetic studies on interactions between ethylene and other signalling molecules are providing a picture of the level of interplay among signalling factors in determining plant growth and development. An example of this type of study is the evaluation of interactions between ethylene, ABA and sugar sensing (Gazzarrini and McCourt, 2001). The global analyses of gene expression provides additional clues and implications on cross-talk between signalling pathways (Schenk *et al*., 2000; Alonso *et al*., 2003*a*; Zhong and Burns, 2003; De Paepe *et al*., 2004).

An example of how multiple signals can be co-ordinated to generate effects on plant growth is apparent in the formation of the apical hook in *Arabidopsis* seedlings, which is proposed to occur through integration of ethylene, auxin and light signalling (Li *et al*., 2004). The expression level of *HLS1*, which encodes a putative *N*-acetyltransferase, is a key component in the regulation of hook formation. Ethylene activates *HLS1* transcription and thus stimulates formation of the apical hook. Light causes a decrease in *HLS1* transcription and thus stimulates opening of the apical hook. The actual formation of the apical hook is apparently a result of the asymmetric distribution of auxin within the hook region. Differing auxin levels result in differing amounts of cell elongation, which in turn results in bending and hook formation. HLS1 controls the response to auxin within the hook region by inhibiting the function of the auxin response factor ARF2, a negative regulator of the auxin response. Other proteins may function to integrate signalling from different sets of regulators. For example, DELLA proteins, which act as repressors of growth, are thought to integrate the actions of ethylene, auxin and gibberellin in control of plant growth (Achard *et al*., 2003).

Some known components of the ethylene-signalling pathway could be the sites by which other signalling pathways interplay with ethylene. Gibson *et al*. (2001) isolated a *sugar-insensitive 1* (*sis1*) mutant of *Arabidopsis*, which is insensitive to the inhibitory effects of high concentrations of sucrose on seedling development. The *SIS1* gene turns out to be allelic to *CTR1*, a known component of the ethylene signal transduction pathway (Gibson *et al*., 2001). Another sugar response mutant *glucose-insensitive 4* (*gin4*) is also allelic to *CTR1* (Zhou *et al*., 1998). These genetic studies demonstrate a clear role for the ethylene-signalling pathway in the regulation of plant responses to sugars. From these data alone, however, one cannot conclude that CTR1 is the site at which sugars regulate the ethylene-signalling pathway. Rather, the interpretation is that constitutive output from the pathway results in insensitivity to both sucrose and glucose.

The likely point at which sugars regulate output from the ethylene-signalling pathway is at the transcription factor EIN3, as revealed by a series of recent elegant studies. Yanagisawa *et al*. (2003) discovered an antagonistic interaction between glucose and ethylene in regulation of stability of EIN3 protein levels. EIN3 protein is ubiquitinated and targeted to proteasomes for degradation. Glucose enhances the degradation of EIN3, while ethylene stabilizes EIN3, a process involving regulation of two F-box proteins, essential components of the ubiquitin proteasome system (Guo and Ecker, 2003; Potuschak *et al*., 2003; Gagne *et al*., 2004). The plant glucose sensor hexokinase mediates the glucose effect. The glucose-induced degradation of EIN3 may potentially serve to promote growth by preventing certain ethylene responses such as inhibition of cell elongation (Yanagisawa *et al*., 2003). Cross-talk between ethylene and sugar takes place not via post-transcriptional regulation of EIN3, but in all likelihood at transcriptional levels as well. DNA microarray analysis demonstrated that several ethylene biosynthetic and signal transduction genes are repressed by glucose (Price *et al*., 2004).

GLOBAL ANALYSIS OF PROCESSES REGULATED BY ETHYLENE

This review has focused on the initial steps in ethylene signal transduction, from perception of ethylene by the receptors to regulation of key transcription factors such as EIN3. What we think of in terms of ethylene responses, however, are the result of co-ordinated increases and decreases in expression of many genes and proteins, with the relative levels of these genes and proteins further regulated by inputs from other signalling pathways. The use of microarrays now allows for global analysis of gene expression changes in response to ethylene and is critical to unravelling the relationship between transcriptional regulation and ethylene responses. By using this experimental approach not only can we gain a broad understanding of what genes and processes are regulated by ethylene but, by use of ethylene pathway mutants, determine the relative contributions of known components in the signal transduction pathway to transcriptional regulation. This thus seems a fitting way to conclude this review, for these global approaches will refine what we already know about ethylene signal transduction as well as throw light upon darkened corners of regulation about which we had little if no prior knowledge.

In one of the earliest microarray studies, the effects of ethylene, salicylic acid (SA) and methyl jasmonate (MJ) were examined using a cDNA array of 2375 selected genes (Schenk *et al*., 2000). A fairly high level of coordination was observed among these signals known to be involved in plant defences, with 50 % of the genes induced by ethylene also induced by MJ. Recently, several groups have performed analyses using arrays representing a greater number of genes (Alonso *et al*., 2003*a*; Zhong and Burns, 2003; De Paepe *et al*., 2004). Alonso *et al*. (2003) used Affymetrix gene expression arrays to examine the RNA levels of more than 22 000 genes in response to exogenous ethylene treatment in *Arabidopsis*. They identified 628 genes whose levels of expression were significantly altered by ethylene treatment; 244 genes were induced and 384 genes were repressed by hormone treatment. Zhong and Burns (2003) examined ethylene-regulated gene expression in *Arabidopsis* with an expressed sequence tag-based microarray containing about 6000 unique genes and identified about 7 % of the investigated genes as ethylene-regulated. De Paepe *et al*. (2004) used cDNA-microarray technology as well as cDNA-amplified fragment length polymorphism (AFLP) to show significant differences in gene expression among wildtype plants, the constitutive ethylene-response mutant *ctr1-1* and the ethylene-insensitive mutant *ein2-1*. Cluster analysis indicates that ethylene affects transcription of genes involved in many biological processes, from metabolism to signal transduction, and including, for example, protein degradation mediated by the ubiquitin-proteasome system as well as by proteases, the transport of water, peptides and ions, cell wall metabolism and lipid metabolism.

Expression studies are expected to clarify which genes are involved in well-known processes mediated by ethylene, including the triple response in seedlings, fruit ripening, senescence, abscission and responses to pathogens. It is also likely to enrich our understanding of other physiological processes regulated by ethylene that we are only beginning to be understood. One such example is the role of ethylene in plant reproductive development. Ethylene regulates the transition from vegetative growth to flowering (Ogawara *et al*., 2003), sex determination (Yamasaki *et al*., 2000), ovary and ovule development (De Martinis and Mariani, 1999), pollination (Zhang and O'Neill, 1993), anther development and dehiscence (Rieu *et al*., 2003*b*), as well as senescence and abscission of floral organs (O'Neill, 1997). Interestingly, although the RNA levels of the five receptor genes in *Arabidopsis* are generally low and ubiquitous, higher expression occurs at the reproductive organs and tissues such as young floral primordia, floral organ primordia, the locules of the anthers, the developing carpels, the funiculi and the ovules (Hua *et al*., 1998). If there are increased levels of the receptors in reproductive organs compared to vegetative tissues, there may as a consequence be differences in ethylene sensitivity and signal transduction. It may also be that high expression of the receptors in these tissues assists in functions independent of their role in ethylene perception, one possibility being as a scaffold for other proteins required during development.

From the initial perception of ethylene by a five-member family of receptors (the transduction of this signal being along a pathway conserved in all plant species examined), a truly diverse set of genes are induced, these being finely tuned to mediate responses particular to the cell, the tissue, the developmental state and the environmental condition in which the plant is growing. To date, much of the global analysis of gene expression has analysed changes in gene expression at the whole-plant level, an approach that will essentially average out the changes across multiple tissues and cell types, thereby yielding a picture of what changes in gene expression are common throughout the plant. Such a whole-plant approach has yielded essential information about ethylene signal transduction and the control of gene expression and is certainly not completed even at this point. For example, the timing and duration of ethylene treatment upon gene expression need to be examined in more detail at the whole-plant and tissue level. It is clear, however, given the complexity of ethylene's involvement in such processes as reproductive development, that a complete understanding of ethylene's role in plant growth and development will also require more focused approaches capable of resolving changes in gene expression occurring within individual cell types. The recent profiling of patterns of gene expression in different root cell types points to the direction such studies are likely to take in the future (Birnbaum *et al*., 2003).

ACKNOWLEDGEMENTS

We thank Mike Jackson for discussions that inspired portions of this review, Mike Mason for critical reading of the manuscript, and the National Science Foundation and the US Department of Agriculture (NRICGP) for research support.

LITERATURE CITED

Abeles FB, Morgan PW, Saltveit Jr ME. 1992. *Ethylene in plant biology*, 2nd edn. San Diego: Academic Press.

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

Adams-Phillips L, Barry C, Giovannoni J. 2004*a*. Signal transduction systems regulating fruit ripening. *Trends in Plant Science* **9**: 331–338.

Adams-Phillips L, Barry C, Kannan P, Leclercq J, Bouzayen M, Giovannoni J. 2004*b*. Evidence that CTR1-mediated ethylene signal transduction in tomato is encoded by a multigene family whose members display distinct regulatory features. *Plant Molecular Biology* **54**: 387–404.

Alonso JM, Ecker JR. 2001. The ethylene pathway: a paradigm for plant hormone signalling and interaction. *Science STKE* **2001**: RE1.

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

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R *et al.* 2003*a*. Genome-wide insertional mutagenesis of Arabidopsis thaliana. *Science* **301**: 653–657.

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM, Ecker JR. 2003*b*. Five components of the ethylene-response pathway identified in a screen for weak ethylene-insensitive mutants in Arabidopsis. *Proceedings National Academy Sciences USA* **100**: 2992–2997.

Ames P, Studdert CA, Reiser RH, Parkinson JS. 2002. Collaborative signalling by mixed chemoreceptor teams in *Escherichia coli*. *Proceedings National Academy Sciences USA* **99**: 7060–7065.

Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB. 2004*a*. Short-term growth responses to ethylene in Arabidopsis seedlings are EIN3/EIL1 independent. *Plant Physiology* **136**: 2921–2927.

Binder BM, O'Malley RC, Wang W, Moore JM, Parks BM, Spalding EP, Bleecker AB. 2004*b*. Arabidopsis seedling growth response and recovery to ethylene. A kinetic analysis. *Plant Physiology* **136**: 2913–2920.

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN. 2003. A gene expression map of the Arabidopsis root. *Science* **302**: 1956–1960.

Bleecker AB. 1999. Ethylene perception and signalling: an evolutionary perspective. *Trends in Plant Science* **4**: 269–274.

Bleecker AB, Estelle MA, Somerville C, Kende H. 1988. Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241**: 1086–1089.

Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. *Annual Review of Cellular and Developmental Biology* **16**: 1–18.

Bostick M, Lochhead SR, Honda A, Palmer S, Callis J. 2004. Related to ubiquitin 1 and 2 are redundant and essential and regulate vegetative growth, auxin signalling, and ethylene production in Arabidopsis. *Plant Cell* **16**: 2418–2432.

Bray D, Levin MD, Morton-Firth CJ. 1998. Receptor clustering as a cellular mechanism to control sensitivity. *Nature* **393**: 85–88.

Cancel JD, Larsen PB. 2002. Loss-of-function mutations in the ethylene receptor ETR1 cause enhanced sensitivity and exaggerated response to ethylene in Arabidopsis. *Plant Physiology* **129**: 1557–1567.

Chae HS, Cho YG, Park MY, Lee MC, Eun MY, Kang BG, Kim WT. 2000. Hormonal cross-talk between auxin and ethylene differentially regulates the expression of two members of the 1-aminocyclopropane-1-carboxylate oxidase gene family in rice (*Oryza sativa* L.). *Plant and Cell Physiology* **41**: 354–362.

Chae HS, Faure F, Kieber JJ. 2003. The *eto1*, *eto2*, and *eto3* mutations and cytokinin treatment increase ethylene biosynthesis in *Arabidopsis* by increasing the stability of ACS protein. *Plant Cell* **15**: 545–559.

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

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 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 JR. 1997. Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* **89**: 1133–1144.

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

Chen YF, Randlett MD, Findell JL, Schaller GE. 2002. Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of Arabidopsis. *Journal of Biological Chemistry* **277**: 19861–19866.

Ciardi JA, Tieman DM, Lund ST, Jones JB, Stall RE, Klee HJ. 2000. Response to *Xanthomonas campestris* pv. *vesicatoria* in tomato involves regulation of ethylene receptor gene expression. *Plant Physiology* **123**: 81–92.

Clark KL, Larsen PB, Wang X, Chang C. 1998. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS1 ethylene receptors. *Proceedings National Academy Sciences USA* **95**: 5401–5406.

De Martinis D, Mariani C. 1999. Silencing gene expression of the ethylene-forming enzyme results in a reversible inhibition of ovule development in transgenic tobacco plants. *Plant Cell* **11**: 1061–1072.

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

Ecker JR. 1995. The ethylene signal transduction pathway in plants. *Science* **268**: 667–674.

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

El-Sharkawy I, Jones B, Li ZG, Lelievre JM, Pech JC, Latche A. 2003. Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (Pyrus communis L) with/without cold requirement. *Journal of Experimental Botany* **54**: 1615–1625.

Felix G, Grosskopf DG, Regenass M, Boller T. 1991. Rapid changes of protein phosphorylation are involved in transduction of the elicitor signal in plant cells. *Proceedings National Academy Sciences USA* **88**: 8831–8834.

Fuchs SY, Fried VA, Ronai Z. 1998. Stress-activated kinases regulate protein stability. *Oncogene* **17**: 1483–1490.

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M. 2000. *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell* **12**: 393–404.

Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, Yanagisawa S, Vierstra RD. 2004. Arabidopsis EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. *Proceedings National Academy Sciences USA* **101**: 6803–6808.

Gallie DR, Young TE. 2004. The ethylene biosynthetic and perception machinery is differentially expressed during endosperm and embryo development in maize. *Molecular Genetics and Genomics* **271**: 267–281.

Gamble RL, Coonfield ML, Schaller GE. 1998. Histidine kinase activity of the ETR1 ethylene receptor from Arabidopsis. *Proceedings National Academy Sciences USA* **95**: 7825–7829.

Gamble RL, Qu X, Schaller GE. 2002. Mutational analysis of the ethylene receptor ETR1: role of the histidine kinase domain in dominant ethylene insensitivity. *Plant Physiology* **128**: 1428–1438.

Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, Schaller GE. 2003. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of Arabidopsis through participation in ethylene receptor signalling complexes. *Journal of Biological Chemistry* **278**: 34725–34732.

Garbers C, DeLong A, Deruere J, Bernasconi P, Soll D. 1996. A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in Arabidopsis. *EMBO Journal* **15**: 2115–2124.

Gazzarrini S, McCourt P. 2001. Genetic interactions between ABA, ethylene and sugar signalling pathways. *Current Opinion in Plant Biology* **4**: 387–391.

Gestwicki JE, Kiessling LL. 2002. Inter-receptor communication through arrays of bacterial chemoreceptors. *Nature* **415**: 81–84.

Gibson SI, Laby RJ, Kim D. 2001. The sugar-insensitive1 (*sis1*) mutant of Arabidopsis is allelic to ctr1. *Biochemical and Biophysical Research Communications* **280**: 196–203.

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

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

Haberer G, Kieber JJ. 2002. Cytokinins. New insights into a classic phytohormone. *Plant Physiology* **128**: 354–362.

Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB. 2000. Ethylene perception by the ERS1 protein in Arabidopsis. *Plant Physiology* **123**: 1449–1458.

Hara-Nishimura I, Matsushima R. 2003. A wound-inducible organelle derived from endoplasmic reticulum: a plant strategy against environmental stresses? *Current Opinion in Plant Biology* **6**: 583–588.

Hass C, Lohrmann J, Albrecht V, Sweere U, Hummel F, Yoo SD, Hwang I, Zhu T, Schafer E, Kudla J, Harter K. 2004. The response regulator 2 mediates ethylene signalling and hormone signal integration in Arabidopsis. *EMBO Journal* **23**: 3290–3302.

Heidecker G, Huleihel M, Cleveland JL, Kolch W, Beck TW, Lloyd P, Pawson T, Rapp UR. 1990. Mutational activation of c-raf-1 and definition of the minimal transforming sequence. *Molecular and Cellular Biology* **10**: 2503–2512.

Heyl A, Schmülling T. 2003. Cytokinin signal perception and transduction. *Current Opinion in Plant Biology* **6**: 480–488.

Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, Nourizadeh S, Alonso JM, Dailey WP, Dancis A, Ecker JR. 1999. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signalling in Arabidopsis. *Cell* **97**: 383–393.

Hua J, Chang C, Sun Q, Meyerowitz EM. 1995. Ethylene sensitivity conferred by Arabidopsis *ERS* gene. *Science* **269**: 1712–1714.

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

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM. 1998. Ein4 and ERS2 are members of the putative ethylene receptor family in Arabidopsis. *Plant Cell* **10**: 1321–1332.

Huang Y, Li H, Hutchison CE, Laskey J, Kieber JJ. 2003. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signalling in Arabidopsis. *Plant Journal* **33**: 221–233.

Hwang I, Sheen J. 2001. Two-component circuitry in Arabidopsis cytokinin signal transduction. *Nature* **413**: 383–389.

Kakimoto T. 2003. Perception and signal transduction of cytokinins. *Annual Review of Plant Biology* **54**: 605–627.

Kepinski S, Leyser O. 2003. SCF-mediated proteolysis and negative regulation in ethylene signalling. *Cell* **115**: 647–648.

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

Kim CY, Liu Y, Thorne ET, Yang H, Fukushige H, Gassmann W, Hildebrand D, Sharp RE, Zhang S. 2003. Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. *Plant Cell* **15**: 2707–2718.

Kim WT, Yang SF. 1992. Turnover of 1-aminocyclopropane-1-carboxylic acid synthase protein in wounded tomato fruit tissue. *Plant Physiology* **100**: 1126–1131.

Klee HJ. 2002. Control of ethylene-mediated processes in tomato at the level of receptors. *Journal of Experimental Botany* **53**: 2057–2063.

Klee HJ. 2004. Ethylene signal transduction. Moving beyond Arabidopsis. *Plant Physiology* **135**: 660–667.

Kwak SH, Lee SH. 1997. The requirements for Ca2+, protein phosphorylation, and dephosphorylation for ethylene signal transduction in *Pisum sativum* L. *Plant Cell Physiology* **38**: 1142–1149.

Larsen PB, Cancel JD. 2003. Enhanced ethylene responsiveness in the Arabidopsis *eer1* mutant results from a loss-of-function mutation in the protein phosphatase 2A a regulatory subunit, RCN1. *Plant Journal* **34**: 709–718.

Larsen PB, Cancel JD. 2004. A recessive mutation in the RUB1-conjugating enzyme, RCE1, reveals a requirement for RUB modification for control of ethylene biosynthesis and proper induction of basic chitinase and PDF1.2 in Arabidopsis. *Plant Journal* **38**: 626–638.

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

Lashbrook CC, Tieman DM, Klee HJ. 1998. Differential regulation of the tomato *ETR* gene family throughout plant development. *Plant Journal* **15**: 243–252.

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

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

Liang X, Abel S, Keller JA, Shen NF, Theologis A. 1992. The 1-aminocyclopropane-1-carboxylate synthase gene family of Arabidopsis thaliana. *Proceedings National Academy Sciences USA* **89**: 11046–11050.

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

Ma QH, Wang XM. 2003. Characterization of an ethylene receptor homologue from wheat and its expression during leaf senescence. *Journal of Experimental Botany* **54**: 1489–1490.

Mattoo AK, Suttle JC, eds. 1991. *The plant hormone ethylene*. Boca Raton, FA: CRC Press, Inc.

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

Mita S, Kawamura S, Yamawaki K, Nakamura K, Hyodo H. 1998. Differential expression of genes involved in the biosynthesis and perception of ethylene during ripening of passion fruit (Passiflora edulis Sims). *Plant and Cell Physiology* **39**: 1209–1217.

Moshkov IE, Mur LA, Novikova GV, Smith AR, Hall MA. 2003*a*. Ethylene regulates monomeric GTP-binding protein gene expression and activity in Arabidopsis. *Plant Physiology* **131**: 1705–1717.

Moshkov IE, Novikova GV, Mur LA, Smith AR, Hall MA. 2003*b*. Ethylene rapidly up-regulates the activities of both monomeric GTP-binding proteins and protein kinase(s) in epicotyls of pea. *Plant Physiology* **131**: 1718–1726.

Moussatche P, Klee HJ. 2004. Autophosphorylation activity of the arabidopsis ethylene receptor multigene family. *Journal of Biological Chemistry* **279**: 48734–48741.

Novikova GV, Moshkov IE, Smith AR, Hall MA. 2000. The effect of ethylene on MAPKinase-like activity in *Arabidopsis thaliana*. *FEBS Letters* **474**: 29–32.

Novikova GV, Moshkov IE, Smith AR, Hall MA. 2003. Nucleoside diphosphate kinase is a possible component of the ethylene signal transduction pathway. *Biochemistry (Mosc)* **68**: 1342–1348.

O'Donnell PJ, Calvert C, Atzorn R, Wasternack C, Leyser HMO, Bowles DJ. 1996. Ethylene as a signal mediating the wound Response of tomato plants. *Science* **274**: 1914–1917.

O'Donnell PJ, Schmelz E, Block A, Miersch O, Wasternack C, Jones JB, Klee HJ. 2003. Multiple hormones act sequentially to mediate a susceptible tomato pathogen defense response. *Plant Physiology* **133**: 1181–1189.

O'Neill SD. 1997. Pollination regulation of flower development. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 547–574.

Ogawara T, Higashi K, Kamada H, Ezura H. 2003. Ethylene advances the transition from vegetative growth to flowering in *Arabidopsis thaliana*. *Journal of Plant Physiology* **160**: 1335–1340.

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

Ouaked F, Rozhon W, Lecourieux D, Hirt H. 2003. A MAPK pathway mediates ethylene signalling in plants. *EMBO Journal* **22**: 1282–1288.

Parkinson JS. 1993. Signal transduction schemes of bacteria. *Cell* **73**: 857–871.

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

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P. 2003. EIN3-dependent regulation of plant ethylene hormone signalling by two arabidopsis F box proteins: EBF1 and EBF2. *Cell* **115**: 679–689.

Prescott AG, John P. 1996. DIOXYGENASES: Molecular Structure and Role in Plant Metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**: 245–271.

Price J, Laxmi A, St Martin SK, Jang JC. 2004. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. *Plant Cell* **16**: 2128–2150.

Qu X, Schaller GE. 2004. Requirement of the histidine kinase domain for signal transduction by the ethylene receptor ETR1. *Plant Physiology* **136**: 2961–2970.

Rashotte AM, DeLong A, Muday GK. 2001. Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. *Plant Cell* **13**: 1683–1697.

Rasori A, Ruperti B, Bonghi C, Tonutti P, Ramina A. 2002. Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. *Journal of Experimental Botany* **53**: 2333–2339.

Raz V, Ecker JR. 1999. Regulation of differential growth in the apical hook of Arabidopsis. *Development* **126**: 3661–3668.

Raz V, Fluhr R. 1992. Calcium requirement for ethylene-dependent responses. *Plant Cell* **4**: 1123–1130.

Rieu I, Mariani C, Weterings K. 2003*a*. Expression analysis of five tobacco EIN3 family members in relation to tissue-specific ethylene responses. *Journal of Experimental Botany* **54**: 2239–2244.

Rieu I, Wolters-Arts M, Derksen J, Mariani C, Weterings K. 2003*b*. Ethylene regulates the timing of anther dehiscence in tobacco. *Planta* **217**: 131–137.

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

Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM. 1998. *ETR2* is an *ETR1*-like gene involved in ethylene signalling in *Arabidopsis*. *Proceedings National Academy Sciences USA* **95**: 5812–5817.

Sato-Nara K, Yuhashi KI, Higashi K, Hosoya K, Kubota M, Ezura H. 1999. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiology* **120**: 321–330.

Schaller GE. 2000. Histidine kinases and the role of two-component systems in plants. *Advances in Botanical Research* **32**: 109–148.

Schaller GE. 2003. Regulators of growth: ethylene. In: Thomas B, Murphy D, Murray B, eds. *Encyclopedia of applied plant science*. London: Academic Press, 1019–1026.

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

Schaller GE, Kieber JJ. 2002. Ethylene. In: Somerville C, Meyerowitz E, eds. *The Arabidopsis book*, Vol DOI/10.1199/tab.0071. Rockville, MD: American Society of Plant Biologists.

Schaller GE, Mathews DE, Gribskov M, Walker JC. 2002. Two-component signalling elements and histidyl-aspartyl phosphorelays. In: Somerville C, Meyerowitz E, eds. *The Arabidopsis book*, Vol DOI/10.1199/tab.0086. Rockville, MD: American Society of Plant Biologists.

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM. 2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. *Proceedings National Academy Sciences USA* **97**: 11655–11660.

Shibuya K, Nagata M, Tanikawa N, Yoshioka T, Hashiba T, Satoh S. 2002. Comparison of mRNA levels of three ethylene receptors in senescing flowers of carnation (*Dianthus caryophyllus* L.). *Journal of Experimental Botany* **53**: 399–406.

Silk WK, Erickson RO. 1978. Kinematics of hypocotyl curvature. *American Journal of Botany* **65**: 310–319.

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

Spanu P, Grosskopf DG, Felix G, Boller T. 1994. The apparent turnover of 1-aminocyclopropane-1-carboxylate synthase in tomato cells is regulated by protein phosphorylation and dephosphorylation. *Plant Physiology* **106**: 529–535.

Staehelin LA. 1997. The plant ER: a dynamic organelle composed of a large number of discrete functional domains. *Plant Journal* **11**: 1151–1165.

Sullivan JA, Shirasu K, Deng XW. 2003. The diverse roles of ubiquitin and the 26S proteasome in the life of plants. *Nature Reviews Genetics* **4**: 948–958.

Terajima Y, Nukui H, Kobayashi A, Fujimoto S, Hase S, Yoshioka T, Hashiba T, Satoh S. 2001. Molecular cloning and characterization of a cDNA for a novel ethylene receptor, NT-ERS1, of tobacco (*Nicotiana tabacum* L.). *Plant and Cell Physiology* **42**: 308–313.

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

Tieman DM, Klee HJ. 1999. Differential expression of two novel members of the tomato ethylene-receptor family. *Plant Physiology* **120**: 165–172.

Vahala J, Schalgnhaufer CD, Pell EJ. 1998. Induction of ACC synthase cDNA by ozone in light-grown *Arabidopsis thaliana* leaves. *Physiologia Plantarum* **103**: 45–50.

Vierstra RD. 2003. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends in Plant Science* **8**: 135–142.

Vitale A, Denecke J. 1999. The endoplasmic reticulum-gateway of the secretory pathway. *Plant Cell* **11**: 615–628.

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

Wang KL, Yoshida H, Lurin C, Ecker JR. 2004. Regulation of ethylene gas biosynthesis by the Arabidopsis ETO1 protein. *Nature* **428**: 945–950.

Wang W, Hall AE, O'Malley R, Bleecker AB. 2003. Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from Arabidopsis is not required for signal transmission. *Proceedings National Academy Sciences USA* **100**: 352–357.

Wiley HS. 1992. Receptors: topology, dynamics, and regulation. In: Bittar EE, ed. *Fundamentals of medical cell biology*, Vol 5A. Greenwich, CT: JAI Press Inc., 113–142.

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

Woeste KE, Ye C, Kieber JJ. 1999. Two Arabidopsis mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. *Plant Physiology* **119**: 521–530.

Xie C, Zhang JS, Zhou HL, Li J, Zhang ZG, Wang DW, Chen SY. 2003. Serine/threonine kinase activity in the putative histidine kinase-like ethylene receptor NTHK1 from tobacco. *Plant Journal* **33**: 385–393.

Yamasaki S, Fujii N, Takahashi H. 2000. The ethylene-regulated expression of CS-ETR2 and CS-ERS genes in cucumber plants and their possible involvement with sex expression in flowers. *Plant and Cell Physiology* **41**: 608–616.

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

Yau CP, Wang L, Yu M, Zee SY, Yip WK. 2004. Differential expression of three genes encoding an ethylene receptor in rice during development, and in response to indole-3-acetic acid and silver ions. *Journal of Experimental Botany* **55**: 547–556.

Zhang XS, O'Neill SD. 1993. Ovary and gametophyte development are coordinately regulated by auxin and ethylene following pollination. *Plant Cell* **5**: 403–418.

Zhang ZG, Zhou HL, Chen T, Gong Y, Cao WH, Wang YJ, Zhang JS, Chen SY. 2004. Evidence for serine/threonine and histidine kinase activity in the tobacco ethylene receptor protein NTHK2. *Plant Physiology* **136**: 2971–2981.

Zhao XC, Qu X, Mathews DE, Schaller GE. 2002. Effect of ethylene pathway mutations upon expression of the ethylene receptor ETR1 from Arabidopsis. *Plant Physiology* **130**: 1983–1991.

Zhao XC, Schaller GE. 2004. Effect of salt and osmotic stress upon expression of the ethylene receptor ETR1 in Arabidopsis thaliana. *FEBS Letters* **562**: 189–192.

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

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