Plant Molecular Biology **26**: 1579–1597, 1994.
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Ethylene biosynthesis and action: a case of conservation

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Received and accepted 1 July 1994

Key words: aminotransferase, dioxygenase, ethylene, kinase cascade, raf kinase, two-component system

Introduction

Ethylene is one of the simplest organic molecules with biological activity. At concentrations as low as 0.1 ppm in air, it has been shown to have dramatic effects on plant growth and development [1]. Neljubov [78] was the first to show that ethylene has three major effects in etiolated pea seedlings called the triple response: (1) diageotropic growth, (2) thickening of stem and inhibition of stem elongation, and (3) exaggeration of apical hook curvature. Since then, numerous ethylene effects have been described in light-grown plants such as sex determination in curcurbits, fruit ripening in climacteric fruits, epinastic curvature, flower senescence, and root initiation [1]. Interestingly, ethylene has also been shown to have opposite effects in some plants; for instance, it inhibits stem elongation in most dicots, whereas in some aquatic dicots and rice, it stimulates growth [1, 45, 72]. Such growth is essential for the survival of such plants so as to keep its foliage above water [45, 72].

Until the early 1970s very little was known about how ethylene is biosynthesized, how its production is regulated, and how a plant perceives its presence in nanoliter quantities. Since then, a large amount of biochemical and genetic data has been gathered, indicating that every component of the ethylene production and action pathways in plants so far studied has a homologue found in other prokaryotic or eukaryotic systems (see Table 1). Many excellent reviews on ethylene production and perception have been published [23, 27, 46, 47, 48, 108, 109, 122, 123] and the reader is encouraged to read them for additional information. This review will focus on data obtained over the past five years during which some of the genes responsible for ethylene biosynthesis and perception were cloned.

Ethylene biosynthesis

Introduction

The pathway for ethylene biosynthesis was elucidated by Shang Fa Yang and his collaborators in the late 1970s [123] and has provided the basis for all subsequent biochemical and molecular genetic analysis of the pathway (see Fig. 1). Methionine is the biological precursor of ethylene; it is converted to *S*-adenosylmethionine (AdoMet) by the enzyme methionine adenosyl transferase

Table 1. Ethylene biosynthetic and signal transduction proteins and their homologues.

Gene	Homologue	References
ACS	Aspartate aminotransferase	95, 104
ACO	2-oxoglutarate-dependent dioxygenases	38, 121
ETR1	prokaryotic 2-component modules	16, 82
CTR1	Raf protein kinases	32, 49

ACS, ACC synthase; ACO, ACC oxidase.

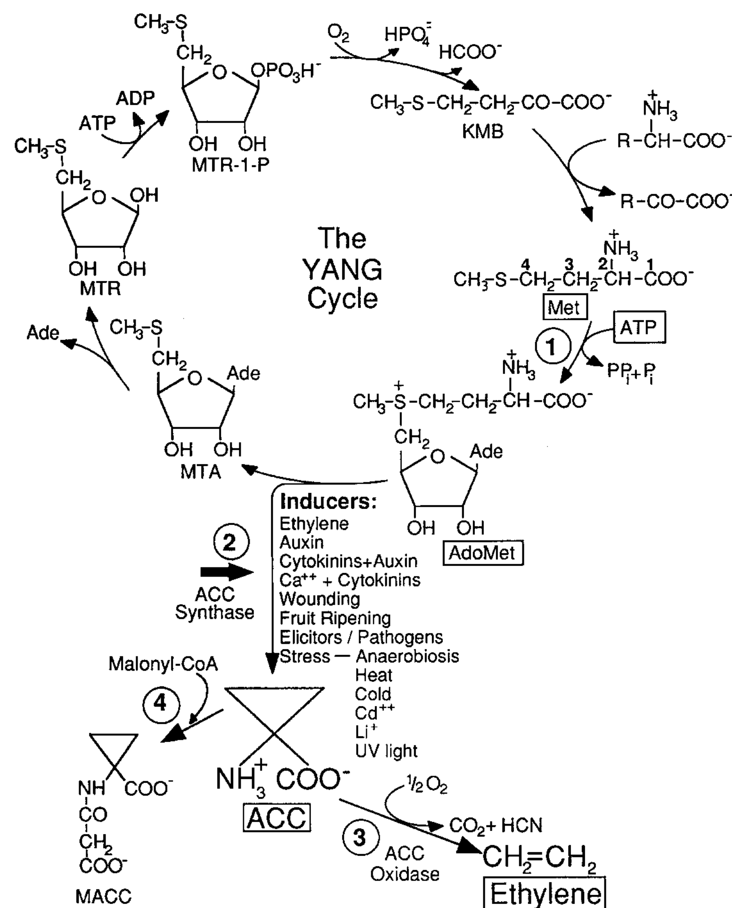

Fig. 1. The ethylene biosynthetic pathway of higher plants. AdoMet, *S*-adenosyl-*L*-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; KMB, 2-keto-4-methylthiobutyrate; MACC, malonyl-ACC; MTA, 5′-methylthioadenosine; MTR, 5′-methylthioribose; MTR-1-P, MTR-1-phosphate (after [123]).

(step 1 in Fig. 1). The rate-limiting step is the conversion of AdoMet to 1-aminocyclopropane-1-carboxylate (ACC) and methylthioadenosine (MTA) which is catalyzed by ACC synthase (step 2, Fig. 1). ACC is then converted to either ethylene, CO_2, and HCN by ACC oxidase (step 3 in Fig. 1, an O_2-dependent process) or N-malonyl-ACC (MACC) by malonyl transferase (step 4, Fig. 1). The latter reaction constitutes a possible regulatory step by inactivating ACC [123]. Interestingly, methionine is recycled through the pathway by converting methylthioadenosine to methionine. The net result is that the ribose moiety of ATP is converted to methionine from which ethylene is derived; the CH_3-S group of MTA is conserved for continued regeneration of methionine. Thus, given a constant pool of CH3-S group and available ATP, a high rate of ethylene production can be achieved without high intracellular concentrations of methionine (a less abundant amino acid).

The enzymes that catalyze steps 3 and 4 in Fig. 1 have been purified to homogeneity [20, 26, 31, 67, 87]. ACC synthase however has only partially been purified because of its low abundance and lability [46]. It was a combination of molecular biological approaches and heterologous expression that allowed the isolation and identification of the genes encoding AdoMet [85, 117], ACC synthase [98, 116], and ACC oxidase [34, 102].

ACC synthase

ACC synthase is a cytosolic enzyme which catalyzes the first committed step in the ethylene biosynthetic pathway. Its half-life is short; the $t1/2$ of tomato ACC synthase is 58 min [51]. There are a multitude of both internal cues and external inducers which elicit *de novo* synthesis of the enzyme [46, 123]. Taken together, ACC synthase represents the key regulatory enzyme in the pathway.

The first ACC synthase cDNA was cloned from zucchini using a novel experimental approach [98]. Antibodies to partially purified enzyme from zucchini fruit tissue treated with IAA + LiCl were purified on an affinity matrix containing total proteins from uninduced zucchini fruit. The purified antibodies were highly enriched for those recognizing ACC synthase and were used to screen an expression library. The authenticity of the isolated clones was verified by expression in *Escherichia coli* and yeast [98]. Subsequently, Van Montagu and his colleagues cloned two ACC synthase cDNAs from ripe tomato fruit. ACC synthase was purified from 200 kg of tomatoes and degenerate oligonucleotides from peptide sequences of the purified enzyme were used to screen a tomato fruit cDNA library [115, 116]. Two partial cDNA clones were obtained corresponding to two genes now known as *LE-ACS2* and *LE-ACS4* [116]. Since these initial reports, ACC synthase cDNAs and genomic sequences have been cloned from numerous plant species such as apple [21], zucchini [41], tomato [59, 80, 96, 125], *Arabidopsis* [58, 114], winter squash [76, 77], rice [126], orchid (S.D. O'Neill, GenBank accession number L07882, unpublished), carnation [82], mungbean [8, 9, 10, 50], soybean [62] and tobacco [5]. The emerging picture is that ACC synthase is encoded by a divergent multigene family where each gene is differentially regulated by a different subset of inducers. For example, in tomato the enzyme is encoded by at least nine genes and six of them are induced by auxin (Kawakita and Theologis, unpublished). An interesting aspect of ACC synthase gene expression is its inducibility by protein synthesis inhibitors such as cycloheximide. All the rice [126], *Arabidopsis* [58] and tomato (Kawakita and Theologis, unpublished) genes cloned so far are induced by cycloheximide. Cycloheximide inducibility is the hallmark of primary responsive genes [110]. These results suggest that the expression of the ACC synthase gene may be under the control of a labile repressor(s) molecule or that their transcripts are labile and cycloheximide simply stabilizes them by removing a labile nuclease. Some of the ACC synthase genes have been mapped in tomato [96], *Arabidopsis* [58, 114], and rice [126]; their map positions do not correspond to any known ethylene biosynthesis or action mutants.

Comparison of the primary sequences of ACC synthase genes cloned so far reveals a great deal about the enzyme's evolution. Phylogenetic analysis has shown that there are three major branches in the phylogenetic tree, indicating three major classes of ACC synthase polypeptides [59]. Furthermore, this trifurcation had to occur before the divergence of monocots and dicots, since monocot sequences exist in two separate branches [58, 59, 126].

Biochemical and sequence comparisons show that all ACC synthases have striking similarities to two aminotranferases in particular, *Bacillus* sp. strain YM-2 aspartate [105] and rat tyrosine [37, 69] aminotransferases (see Fig. 2). Like the aminotransferases, ACC synthase is a pyridoxal phosphate-dependent enzyme [123] that functions as a dimer in zucchini [98], winter squash [99], and tomato [57]. Sequence analysis of the ACC synthase genes as well as sequencing of the dodecapeptide containing the lysine residue which forms a Schiff base with pyridoxal phosphate [124] has indicated high sequence similarity to the same region in aminotransferases [77, 107]. There is also extensive conservation between the predicted secondary structure of ACC synthase and that of the aspartate aminotransferase obtained by X-ray crystallography [127, 128]. It has been previously found that eleven out of the twelve residues conserved among all aminotransferases are also present in all ACC synthases [41, 96]. However, on the basis of a recent comprehensive alignment of 51 aminotransferases, it was found

that only four residues are invariant (Gly-197, Asp-222, Lys-258, Arg-386) [70]. All four are also present in all ACC synthases corresponding to residues Gly-212, Asp-237, Lys-278, and Arg-412 (see Fig. 2). Interestingly, the bacillus aspartate aminotransferase is more similar to ACC synthase (19–28% identity) than to the *E. coli*, pig cytosolic and mitochondrial enzymes (13–14% identity). Despite these similarities, ACC synthase and aspartate aminotransferases have distinct substrate specificities determined by complementation of *E. coli* aminotransferase mutants (Zarembinski and Theologis, unpublished).

Closer examination of all ACC synthase amino acid sequences shows that while they share significant sequence similarity, their carboxyl termini are quite divergent (Fig. 2). This hypervariable positively charged carboxyl terminus of ACC synthase is critical for dimerization and for determining some kinetic parameters of the enzyme [57]. There are at least two domains in the carboxyl terminus of ACC synthase that influence its activity. One of them is responsible for the substrate-based (AdoMet) inhibition [57]. Furthermore, ACC synthase appears to be active as a dimer and as a monomer; the wild-type tomato LE-ACS2 isoenzyme is a dimer whereas a carboxyl terminal deletion mutant (last 52 residues deleted) has a nine-fold higher V_{max} and functions as a monomer [57]. Contrastingly, it has been previously reported that wild-type tomato ACC synthase is a dimer when expressed in *E. coli*, but is a monomer when purified from tomato pericarp tissue [99]. A possible explanation of these results is that during purification there is proteolysis of the carboxyl terminus [96, 97], thus producing monomers like the above deletion mutant.

ACC oxidase

ACC oxidase was far more difficult to study than ACC synthase because an *in vitro* enzyme assay was missing [47]. It was only after cloning its gene and discovering sequence similarity to the iron and ascorbate-dependent dioxygenases [35] it was deduced that the enzyme probably requires cofactors iron and ascorbate for its activity [118]. Since then, ACC oxidase has been purified and biochemically characterized from apple [20, 26, 87] and avocado [67]. Biochemical experiments have confirmed that ACC oxidase requires iron, ascorbate, and CO_2 for activity [20, 26, 67, 87]. ACC oxidase activity is not as highly regulated as that of ACC synthase. It is constitutive in most vegetative tissues [123] but it is induced during fruit ripening [68], senescence [120] and wounding [14], and by fungal elicitors [102]. Its subcellular location is still a point of controversy. The primary sequence suggests ACC oxidase to be a cytosolic enzyme since it does not contain putative membrane-spanning domains or a signal peptide [34]. However, there is a large body of data indicating that the enzyme is either associated with the plasma membrane [47] or that it is apoplastic [4, 55, 94]. Like ACC synthase, it is an unstable enzyme; the $t1/2$ of apple ACC oxidase is 2 h [87].

The isolation of the first ACC oxidase gene was somewhat fortuitous; it was isolated in tomato by differential screening [100]. Its authenticity was verified by a combination of antisense experiments [35] and *in vivo* expression in two heterologous systems: *Xenopus* oocytes [102] and yeast [34, 121]. Subsequently, ACC oxidase was cloned from numerous plants (see legend to Fig. 3) and,

Fig. 2. Amino acid sequence alignments of ACC synthases and aminotransferases. ACC synthases: cm1 [77], cm2 [76], cp1a [97], cp1b [41], le1a, le1b, le2, le3, le4 [96], nt1 [5], dc1 [82], ph1 (S. O'Neill, unpubl), ms1 [21], gm1 [62], vr1, vr4, vr5 [8, 9, 10], at1 (X. Liang and A. Theologis, unpublished), at2 [58], at4 (S. Abel and A. Theologis, unpublished), at5 (X. Liang and A. Theologis, unpublished), os1 [126]. Aminotransferases: bacs [105], ty–r [37]. Residues conserved between both aminotransferases and ACC synthases are shaded in blue. Residues conserved between only *Bacillus* sp. strain YM-2 aspartate aminotransferase (bacs) or rat tyrosine aminotranferase (ty–r) and the ACC synthases are shaded in orange and red, respectively. The filled stars designate the residues which represent the active site residues that play functional and structural roles as described on the basis of the X-ray structure of vertebrate aspartate aminotransferases [105]. Unfilled stars represent the four invariant residues present in all aminotransferases and ACC synthases. The numbering is with respect to the pig cytosolic aspartate aminotransferase.

like ACC synthase, is encoded by a multigene family but with limited divergence (90% sequence similarity in the petunia four-member family [106] and 88% similarity between the two functional tomato ACC oxidases pHTOM5 and pTOM13 [102]). Primary sequence comparison (Fig. 3) has shown that ACC oxidase is a member of the family of iron- and ascorbate-dependent dioxygenases ([38, 106], Fig. 3).

Ethylene and fruit ripening

Fruit ripening in the climacteric tomato fruit has been one of the most intensely studied ethylene-mediated developmental processes [27, 111]. The reason is two-fold: first, a large number of dramatic changes occur within a short period of time in the tomato fruit during ripening, many of which are under ethylene control or are initiated by ethylene exposure. Autocatalysis of ethylene production is a characteristic feature of ripening fruits (including tomato) and other senescing tissues in which a massive increase in ethylene production is triggered by exposure to ethylene. Color changes, softening, and conversion of starch to sugar are also associated with the ripening process. Thus, ripening fruit represents an interesting model system to study ethylene biosynthesis and perception. Second, ripening fruit has economic importance; billions of dollars worth of fruits and vegetables rot (or overripen) before they can reach the consumer. Therefore, an understanding of the ripening process is important for learning how to control it during shipping and storage [107].

Until recently, there existed a great deal of discussion as to whether ethylene is the trigger for ripening in climacteric fruits, or is simply a by-product of the ripening process [35, 52, 79]. Recent data using antisense technology in tomato show that ethylene is the controlling factor for fruit ripening. The best example of such an experiment was done by driving the expression of the tomato ACC synthase *LE-ACS2* cDNA in its antisense orientation with the CaMV 35S promoter in transgenic tomato [79]. The LE-ACS2 antisense fruits produce large amounts of *LE-ACS2* antisense mRNA which completely inhibit the expression of the ACC synthase genes, *LE-ACS2* and *LE-ACS4*, expressed during fruit ripening [79]. More importantly, the antisense fruits produce less than 0.1 nl $g^{-1} h^{-1}$ ethylene, do not show a climacteric rise in respiration, and never ripen. The fruits ripen only when treated for at least six days with exogenous ethylene or propylene (an ethylene analogue) [79]. This result indicates first that the lesion is specific to ethylene production. Second, ethylene is not a trigger, but a rheostat for fruit ripening such that ethylene must be present continuously to induce a rapidly turning-over set of mRNAs and proteins which initiate the ripening process [79, 107]. Other successful attempts to reduce ethylene production in tomato include ACC oxidase antisense experiments [35] and overexpression of the *Pseudomonas syringae* gene encoding ACC deaminase. This enzyme metabolizes ACC before it can be converted to ethylene [52].

The LE-ACS2 antisense plants have also provided a valuable tool to study ethylene-dependent and -independent gene expression during fruit ripening. Northern analysis of antisense and wild-type fruits show that there are at least two signal transduction pathways important for fruit ripening: an ethylene-independent (developmentally controlled) and an ethylene-dependent path-

Fig. 3. Amino acid sequence alignments of ACC oxidases and dioxygenases. ACC oxidases: pTOM13 [53], pHTOM5 [102], GTOMA [39], e8 [18], carnationefe [119], orchidefe (S.D. O'Neill, GenBank accession number L07912, unpublished), appleefe [22], petacola, petaco3a, petaco4a [106], acataccoxi [63], peachefe [14], peaefe (S.C. Peck, D.C. Olson and H. Kende, unpublished), atefe (M. A. Gomez-Lim, unpublished), avocadoefe [68]. Dioxygenases: f13h–horvu (M. Meldgaard, unpublished), hy6h–hyoni [66], ipns–nocla [17]. Residues conserved between ACC oxidases and at least two dioxygenases are shaded in blue. Residues conserved between only flavanone-3-hydroxylase (f13h–horvu), hyoscyamine-6-dioxygenase (hy6h–hyoni), or isopenicillin N synthase (ipns–nocla) and the ACC oxidases are shaded in orange, red, and green respectively. The stars designate amino acids that are conserved across all members of the Fe(II) and ascorbate requiring superfamily of enzymes [106]. The numbering is with respect to the E8 amino acid sequence.

way [111]. Furthermore, ethylene not only affects transcription but translation as well. The gene encoding polygalacturonase (PG) is known to be developmentally regulated during tomato fruit ripening [111]. While antisense fruits express PG mRNA, they do not accumulate PG polypeptide unless continuous ethylene or propylene is added, indicating that either the translatability of the PG mRNA or the turnover rate of the PG polypeptide is under ethylene control [111]. Similar conclusions have also been reached using transgenic *rin* plants that express PG from the E8 promoter [30].

Genetic analysis of ethylene biosynthesis

Several mutants have been isolated or constructed using antisense technology which overproduce ethylene in both tomato and *Arabidopsis*. They can be divided into two major classes: the first class are ethylene overproducers with lesions that affect the activity of the ethylene biosynthetic pathway, whereas the second class are ethylene perception mutants that overproduce ethylene and will be discussed in the next section. The major representatives of the first class are the *eto1*, *eto2*, and *eto3* (*Arabidopsis*) mutations. The *eto1* mutation is recessive and is responsible for a ten-fold ethylene overproduction in dark-grown *Arabidopsis* seedlings [33]. The *eto2* and *eto3* mutations are dominant which cause 20- and 100-fold higher ethylene production in etiolated *Arabidopsis* seedlings, respectively [48]. Interestingly, the Eto$^-$ phenotype is specific to etiolated seedlings; light-grown plants do not overproduce ethylene [48]. Therefore, it is of great interest that *ACS2* promoter-GUS fusions in wild-type *Arabidopsis* show higher *ACS2* gene expression in light than in dark wild-type *Arabidopsis* seedlings [93]. Also, the Eto$^-$ phenotype does not affect ACC oxidase activity [33]. ETO1 is probably a regulatory protein since it does not map to any of the five known *Arabidopsis* ACC synthase genes [58]. The possibility exists that ETO1 is a negative regulator of ethylene biosynthesis, probably at the level of ACC synthase gene expression. Interestingly, cycloheximide enhances the level of mRNA from all five *Arabidopsis* ACC synthase genes which suggests that ETO1 may be a labile repressor of ACC synthase whose synthesis is blocked by protein synthesis inhibition [58].

Recently, it was shown that transgenic tomato fruit expressing antisense E8 mRNA produces ten-fold higher levels of ethylene [86]. These results indicate that E8 is a negative regulator of ethylene biosynthesis. Since E8 has homology to ACC oxidase (Fig. 3) the results also indicate that one of the components of the regulatory machinery responsible for monitoring ethylene production requires a redox reaction for its activity. It has been postulated that one or more of the *eto* mutations may be E8 mutations [107].

Ethylene perception

Introduction

Until very recently, the understanding of the ethylene sensing apparatus has lagged behind that of the ethylene biosynthetic pathway. This was due to the inability of classical biochemical techniques to shine light on the ethylene signal transduction pathway and to the absence of good plant genetic models to study ethylene action. Classic physiological studies have pointed out that the ethylene receptor is very particular in the type of ligand it will accept. The preferred ligand is a small aliphatic (two carbons with no large side groups is the best), unsaturated (double bond is preferred) molecule free of resonance forms and with its terminal carbon free of positive charge [13]. Furthermore, it has also been postulated that the ethylene receptor probably contains a metal ion since unsaturated aliphatic molecules bind metals readily [13]. The metal is probably Zn^{2+} since zinc-deficient tomato plants are ethylene-insensitive. Copper and iron-deficient plants still show strong epinasty in the presence of exogenous ethylene [13]. Carbon dioxide appears to be a competitive inhibitor of the ethylene reception site [13]. Finally, oxygen is required for ethylene action, indicating that the receptor's metal ion must

be oxidized by molecular oxygen either directly or indirectly before reception of and activation by ethylene can occur [13]. Ethylene has been shown to act via calcium and protein phosphorylation; both have been shown to be essential for ethylene-dependent expression of pathogenesis-related (PR) proteins [89, 90]. As it will be discussed later, molecular genetic evidence strongly supports the phosphorylation aspect of the ethylene perception pathway.

It was however genetic analysis of the ethylene perception using the small crucifer *Arabidopsis thaliana* as a model system that established the nature of the ethylene-sensing machinery. Etiolated *Arabidopsis* seedlings show each aspect of the triple response clearly and reproducibly. The small size and short generation time of *Arabidopsis* allows the screening of thousands of seedlings very rapidly. Mutants that either (a) fail to respond to exogenous ethylene (ethylene-insensitive (*ein*), ethylene-resistant (*etr*), and ACC-insensitive (*ain*) mutants) or (b) constitutively display the triple response in the absence of hormone (constitutive triple response (*ctr1*) and ethylene-overproducing (eto)) were isolated [7, 23, 33, 49, 113].

Ethylene-insensitive mutants

Etiolated seedlings of the ethylene-insensitive mutants do not show the triple response in the presence of ethylene; they are tall with open hooks as compared to their wild-type counterparts which are thick and short. Eight distinct ethylene-insensitive mutants have been isolated so far [7, 23, 48, 113]. Three of them, *etr1*, *ein2*, and *ein3*, have been recently cloned [23]. The ETR1 locus is interesting since all of its alleles isolated so far are dominant, suggesting that the mutant proteins encoded by these alleles may either inhibit a complex that ETR1 is part of (dominant negative) or constitutively suppress the ethylene response by locking ETR1 into a particular conformational state [16]. Genetic data indicate that ETR1 acts very early in the ethylene signal transduction pathway, either as the ethylene receptor itself, or as a protein which interacts with the receptor [16]. A second ethylene-insensitive mutation known as *ein2* is recessive and not allelic to *etr1*. Strong alleles to *ein2* are pleiotropic and do not show all known ethylene responses [23, 33]. Additional Ein$^-$ mutants have been isolated that show weak triple response. A representative member of this class is the recessive mutation *ein3* [48]. The *ain* mutants are another class that was isolated during a screen for ACC insensitivity (i.e. long, etiolated plants among a short wild-type background of seedlings). Six of the alleles are due to a single recessive mutation and confer ethylene insensitivity in seedlings at concentrations of ethylene as high as 100 ppm. Interestingly, Ain$^-$ mutants retain their apical hook unlike the Ein$^-$ mutants. In light-grown adult plants, *ain* mutants show decreased ethylene sensitivity with respect to leaf senescence but show no differences compared to wild-type plants when exposed to biotic and abiotic stresses known to elicit ethylene production. Unlike the *ein* loci, the *ain1-1* allele produces three-fold less ethylene. This result suggests the AIN1 protein positively regulates ethylene production.

In tomato there is a ripening mutant *nr* (never ripen) which was isolated about 40 years ago [91] and has recently been found to be ethylene-insensitive. The *nr* mutation is semidominant and pleiotropic, blocking senescence and abscission of flowers, epinasty, fruit ripening, and the triple response in etiolated seedlings [54]. It has been recently shown that NR is the tomato homologue of ETR1 (M. Lanahan and H. Klee, personal communication).

Constitutive triple-response mutants

The second class of ethylene perception mutants has the opposite phenotype of their ethylene-insensitive counterparts; they show the triple response in the absence of exogenous ethylene. All these mutants are recessive and fall into a single complementation group called *ctr1*. The Ctr$^-$ phenotype is pleiotropic like other ethylene perception mutants and mimics wild-type plants

grown in 10 ppm ethylene. The apical hook remains closed longer when plants are transferred from dark to light. The leaves, inflorescence, and root system are much more compact than in wild-type plants, due to the smaller cell size in the Ctr$^-$ plants [49]. The *ctr1* mutation also lengthens the time needed for bolting [49]. The Ctr$^-$ phenotype is insensitive to inhibitors of ethylene biosynthesis and action, indicating that CTR1 is involved in ethylene perception [49]. The data suggest that CTR1 represses the ethylene signal transduction pathway which is constitutively active, and ethylene relieves the inhibition.

In tomato there is a semidominant mutation, *epi*, which confers a constitutive ethylene response (epinastic leaves, swelling of stem and petiolar cortex, and abundant lateral roots) [28, 29]. Treatment of Epi$^-$ seedlings with inhibitors of ethylene biosynthesis or action fail to normalize the Epi$^-$ phenotype [28]. The possibility exists that the EPI protein is a homologue of CTR1.

Genetic and biochemical model for the ethylene-sensing apparatus

Double-mutant analysis (epistasis) has established the following genetic model for the ethylene signal transduction pathway:

ETO1, ETO2, ETO3→ETR1→CTR1→EIN2→EIN3→triple response.

The ETR1 and EIN2 are placed in the same pathway rather than in separate pathways because the effects of the *etr1* and *ein2* mutations are not additive [23]. The precise position of *ein2* in this pathway has been recently determined to be downstream of CTR1 (G. Roman and J. Ecker, personal communication). Recently, the cloning of ETR1 [16] and CTR1 [49] has indicated that some of the components of the pathway are protein kinases suggesting that plants sense ethylene via a kinase cascade.

The nature of the ETR1 protein

The amino acid sequence of ETR shows striking similarity to a superfamily of prokaryotic proteins which are components of a basic communication module known as the two-component system. There are as many as fifty different types of two-component systems within a prokaryotic cell [103]. Each two-component system consists of two separate proteins: a sensor and an associated response regulator [83, 103]. The sensor has two domains: an extracellular input and a cytoplasmic histidine kinase domain. The response regulator is composed of a receiver module and typically an output domain (transcriptional activation). This arrangement offers a highly efficient mechanism by which prokaryotes respond to changes in their environment, such as nitrogen availability, chemical signals, osmotic stress, and oxygen tension.

ETR1 shares highest similarity to a subset of the bacterial two-component proteins that contain both histidine kinase (sensor) and receiver (response regulator) domains on the same polypeptide (Fig. 4; [16]). The N-terminus of ETR1 contains three putative transmembrane domains (Fig. 4). ETR1 lacks the variable carboxyl-terminal domain which is present in the response regulator of most two-component members and

Fig. 4. Amino acid sequence alignments of ETR1 with all known eukaryotic two-component system homologues and various bacterial two-component sensors containing both a histidine kinase and receiver domain. ETR1 [16], ARCB [44], BARA [75], RCSC [104], BVGS [3], LEMA [40], SLN1 [81], SSK1 [65], 282, 80 [101]. 282 and 80 represent two consensus amino acid domains for prokaryotic sensor and response regulators obtained from the ProDom protein domain database [101]. Residues conserved between ETR1 and other two component proteins are shaded in blue. The dots correspond to the highly conserved amino acids involved in phosphotransfer in all two-component systems. Motifs and residues conserved between all histidine kinase and receiver domains are shaded in orange [84, 103]. The three hydrophobic regions that compose the putative transmembrane domain are shaded in red. The thick lines designate residues which form the hydrophobic core of the response regulator, CheY [103]. The open boxes denote the motif characteristic of ATP-binding proteins [81]. The numbering is with respect to the ETR1 amino acid sequence.

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      1                                               50                                               100                                              150
ETR1  ..MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKKSAVFPYRWVLVQFGAFIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT.......
ARCB  ..............................................MKQIRLLAQYYVDLMMKLGLVRFSMLLALALVVLAIVVQMAVTMVLHGQVESIDVIRSIFFGLLITP.........WAVYFLSVVVEQ..LEESRQRLSRLVQKLEEMRE.......RDLSLNVQLKDNIAQLN......
BARA  ...................................................MTNYSLRARMMILILAPTVLIGLLLSIFFVVHRYNDLQRQLEDAGASIIEPLAVSTEYGMSL...........QNRESIGQLISV..LHRRHSDIVRAISVYDENNRLFVTSNFHLDPSSMQLGSNVPFPR.......
RCSC  FSAAYDLNRVFLIGSDNLCHANFGLRDMPVERDTALKALHERINKYRNAPQDDSGSNLYWISEGPRPGVGYFYALTPVYLANRLQALLGVEQTIRMENFFLPGTLPMGV......TILDENGHTLIS..LTGPESKIKGDPRWMQERSWFGYTEGFRELVLKKNLPPSS..LS........
BVGS  IGVDTVEELIAKLRSGEADMAGALFVNSARESFLSFSRPYVRNGMVIVTRQDPDAPVDADHLDGRTVALVRNSAAIPLLQRRYPQAKVVTADNPSEAMLMVANGQADAVVQTQISASYYVNRYFAGKLRIASALDLPPAEIALATTRGQTELMSILNKALYSISNDELASIISRWRGSDGDPRT
LEMA  ..........................................MLLLTILPASLMAMLGGYFTWMQLSELQSQLLQRGEMIAQDLAPLAANALGR.........KDKVLLSRIAT....QTLEQTDVRAVSFLDTDRTVLAHAGPTMISPS.PIGSGSQL.........
SLN1  ALEHSTIAIISAVYNSQGKASGYHFVPPYGSRSDLPQKVFSIKNDTFISSAFENGKGG.SLKQTNILSTRNTALGYSPCSFNLMNWVAIVSQPESVFLSPATKLAKIITGTVIAIGVFVILLTLPLAHWAVQPIVRLQKATELITEGRGLRPSTPRTISRASSFKRGFSSGFAVPSS..........
SSK1  ..............................................................................................................................................................................
292   IGVDTVEELIAKLRSGEADMAGALFVNAARESFLSFSRPYVRNGMVIVTRQDPDAPVDADHLDGRTIAMVRNSAAIPLLQRRYPQAKVVTADNPTEAMLMVADGQADAVVQTQISASYYVNRYFAGKLRIASALDLPPAEIALATTRGQTELMSILNKALYSISNDELASIISRWRGSDGDPRT
80    ..............................................................................................................................................................................

                        200                                               250                                                   300
ETR1  ........TLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAV.KISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSARQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAET
ARCB  .........QEIAVREKAEAELQETFGQLKIEIKEREETQIQLEQQSSFLRSFLDASPDLVFYRNEDK.EFSGCNRAMELLTGKSEKQLVHLKPADVYSPEAAAKVI....ETDEKVFRHNVSLTYEQWLDYPDGRKACF...EIRKVPYYDRVGK.....RHGLMGFGRDITERKRYQDALER
BARA  .........QLTVTRDGDIMILRTPIISESYSPDESPSSDA.KNSQNMLGYIALELDLKSVRLQQYKE.IFISSVMMLFCIGIALIFGWRLMRDVTGPIRNMVNTVDRIRRGQLDSRVEGFMLGELDMLKNGINSMAMSL...AAYHEEMQHNIDQATSDLRETLEQMEIQNVELDLAKKRAQE
RCSC  .........IVYSVPVDKVLERIRMVILNAILANVLAGAAL.FTLARMYERRIFIPAESDALRLEEHE.QFNRKIVASAPVGICI....LRTADGVNILSNELAHTYLNMLTHEDRQRLTQIICGQQVNFVDVLTSNNTNL...QISFVHSRYRNENVAICVLVDVSSRVKMEESLQEMAQAAEQ
BVGS  WYAYRNEIYLLIGLGLLSALLFLSWIVYLERQIRQRKRAERALNDQLEFMRVLIDGTPNPIYVRDKEGRMLLCHDAYLDTFGVTADAVLGKTIPEANVVGDPA..LAREMHEFLLTRVAAEREPRFEDRDVTLHGRTRHVYQWTIPYGDS.LGELKGIIGGWIDITERAELLRELHDAKESADA
LEMA  .........LSSTGTDATRYLLPVFGSQHLTSPIIPAEA....DTLLGWVELEISHNGTLLRGYRS.LFASLLLILTGLAFTATLAVRMSRTINGPMSQIKQAVSQLKDGNLETRLPPLGSRELDELASGINRMAATL...QNAQEELQLSIDQATEDVRQNLETIEIQNIELDLARKEALE
SLN1  ..........LLQFNTAEAGSTTSVSGHGGSGHGSGAAFSANSSMKSAINLGNEKMSPPEEENKIPNNHTDAKISMDGSLN.HDLLGPHSLRHNDTDRSSNRSHILTTSANLTEARLPDYRRLFSDELSDLTETFNTMTDALDQHYALLEERVRARTK...............QLEAAKIEAEA
SSK1  ..............................................................................................................................................................................
292   WYAYRNEIYLLIGLGLLSALLFLSWIVYLERQIRQRKRAERALNDQLEFMRVLIDGTPNPIYVRDKEGRMLLCHDAYLDTFGVTADAVLGKTIPEANVVGDPA..LAREMHEFLLTRMAAEREPRFEDRDVTLHGRTRHVYQWTIPYGDS.LGELKGIIGGWIDITERAEMLRELHDAKESADE
80    ..............................................................................................................................................................................

          350 ●                                 400                                               450
ETR1  AIRARNDFLAVMSHEMRTPMHAIIALSSLLQ..ETELTPEQRLMVETILKSSNLLATLMNDVLDLSRLEDGSLQLELGTFNLHTLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVV..GDEKRLMQIILNIVGNAVKFSK.QGSISVTALVTKSDTR.......
ARCB  ASRDKTTFISTISHELRTPLNGIVGLSRILL..DTELTAEQEKYLKTIHVSAVTLGNIFNDIIDMDKMERRKVQLDNQPVDFTSFLADLENLSALQAQQKGLRFNLEPTLPLPHQVI..TDGTRLRQILWNLISNAVKFTQ.QGQVTVRVRYDEG....D.......
BARA  AARIKSEFLANMSHEIRTPLNGVIGFTRLTL..KTELTPTQRDHLNTIERSANNLLAIINDVLDFSKLEAGKLILESIPFPLRSTLDEVVTLLAHSSHDKGLELTLNIKSDVPDNVI..GDPLRLQQIITNLVGNAIKFTE.NGNIDILVEKRALSNTKV......
RCSC  ASQSKSMFLATVSHELRTPLYGIIGNLDLLQ..TKELPKGVDRLVTAMNNSSSLLLKIISDILDFSKIESEQLKIEPREFSPREVMNHITANYLPLVVRKQLGLYCFIEPDVPVALN..GDPMRLQQVISNLLSNAIKFTD.TGCIVLHVRADGDY........
BVGS  ANRAKTTFLATMSHEIRTPMNAIIGMLELALLRPTDQEPDRQS.IQVAYDSARSLLELIGDILDIAKIEAGKFDLAPVRTALRVLPEGAIRVFDGLARQKGIELVLKTDIVGVDDVL..IDPLRMKQVLSNLVGNAIKFTT.EGQVVLAVTARPDGDA.A......
LEMA  ASRIKSEFLANMSHEIRTPLNGILGFTHLLQ..KSELTPRQFDYLGTIEKSADNLLSIINEILDFSKIEAGKLVLDNIPFNLRDLLQDTLTILAPAAHAKQLELVSLVYRDTPLALS..GDPLRLRQILTNLVSNAIKFTR.EGTIVARAMLEDETEEHA......
SLN1  ANEAKTVFIANISHELRTPLNGILGMTAISM..EETDVNKIRNSLKLIFRSGELLLHILTELLTFSKNVLQRTKLEKRDFCITDVALQIKSIFGKVAKDQRVRLSISLFPNLIRTMVLWGDSNRIIQIVMNLVSNALKFTPVDGTVDVRMKLLGEYDKELSEKKQYKEVYIKKGTEVTENLETT
SSK1  ..............................................................................................................................................................................
292   ANRAKTTFLATMSHELRTPMNGIIGMLQLLL..NTELTPEQQDKYLNTIHRSARSLLEIIGDILDFAKIEAGKFDLEPIPFALRALMEDVIRVFDGLARQKGLELVLNIDIVVPDDVI..IDPMRLKQVLSNLVNNAIKHTP.EGG...SITISVQADE.D.......
80    ..............................................................................................................................................................................

                                                                                                   500                                     550
ETR1  ............................................................................................AVKDSGAGINPQDIPKIFTKFAQTQ.SLATRSSGGSGLGLAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKL
ARCB  ............................................................................................MLHFEVEDSGIGIPQDELDKIFAMYYQVKDSHGGKPATGTGIGLAVSRRLAKNMGGDITVTSEQ.GKGSTFTLTIHA
BARA  ............................................................................................QIEVQIRDTGIGIPERDQSRLFQAFRQADAS.ISRRHGGTGLGLVITQKLVNEMGGDISFHSQP.NRGSTFWFHINL
RCSC  ............................................................................................LSIRVRDTGVGIPAKEVVRLFDPFFQVGTG.VQRNFQGTGLGLAICEKLISMMDGDISVDSEP.GMGSQF..TVRI
BVGS  ............................................................................................HVQFSVSDTGCGISEADQRQLFKPFSQVGGSAEAGPAPGTGLGLSISRRLVELMGGTLVMRSAP.GVGTTVSVDLRL
LEMA  ............................................................................................QLRISVQDTGIGLSSQDVRALFQAFSQADNS.LSRQPGGTGLGLVISKRLIEQMGGEIGVDSTP.GEGSEFWISLKL
SLN1  DKYDLPTLSNHRKSVDLESSATSLGSNRDTSTIQEEITKRNTVANESIYKKVNDREKASNDDVSSIVSTTTSSYDNAIFNSQFNKAPGSDDEEGGNLGRPIENPKTWVISIEVEDTGPGIDPSLQESVFHPFVQGDQT.LSRQYGGTGLGLSICRQLANMMHGTMKLESK.VGVGSKFTFTLPL
SSK1  ........................................................................................................................................................................
292   ............................................................................................NIQVEVSDSGIGIPEEQQDRLFEPFV.......TSKESGMGLGLSISRNIIDQHGGMITIGNRE.QGG...........
80    ........................................................................................................................................................................

                                                                                                                                                          600
ETR1  GISER....SNESKQS.............................................................................................................GIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLVHL
ARCB  PSVAE....EVDDAFDEDDMP........................................................................................................................LPALNVLLVEDIELNVIVARSVLEKL
BARA  DLNPN....IIIEGPSTQCLAGKRLAYVEPNSAAAQCTLDILSETPLEVV.....YSPTFSALPPAHYDMMLLGIAVTFREPLTMQHERLAKAVSMTDFL.....MLALPCHAQVNAEKLKQDGIGACLLKPLTPTRLLPALTEFCHHKQNTLLPVTDESKLAMTVMAVDDNPANLKLIGALLEDM
RCSC  PLYGA....QYPQKKGVEGLSGKRCWLAVRNASLCQFLETSLQRSGIVVTTYEGQEPTPEDV..............................LITDEVVSKKWQGRAVVTFCRRHIGIPLEEAPGEWVHSVAAPHELPALLARIYLIEMESDDPANALPSTDKAVSDNDDMMILVVDDHPINRRLLADQLGSL
BVGS  TMVEK....SVQAA...............................................................................................................PPAAATAATPSKPQVSLRVLVVDDHKPNLMLLRQQLDYL
LEMA  PKARE....DKEESLNIP.LGGLRAAVLEHHDLARQALEHQLEDCGLQTIVFNNLENLLNGVTAAHETPAAIDLAVLGVTALEISPERLRQHIWDLENLNCKVMVLCPTTEHALFQLAVHDVYTQLQAKPACTRKLQKALSELIAPRAVRADIGPPLSSRAPRVLCVDDNPANLLLVQTLLEDM
SLN1  NQTKEISFADMEFPFEDEFNPESRKNRRVKFSVAKSIKSRQSTSSVATPATNRSSLTNDVLPEVRSKGKHETKDVGNPNMGREEKNDNGGLEQLQEKNIKPSICLTGAEVNEQNSLSSKHRSRHEGLGSVNLDRPFLQSTGTATSSRNIPTVKDDDKNETSVKILVVEDNHVNQEVIKRMLNLE
SSK1  ...........................................................................................................................................FPKINVLIVEDNVINQAILGSFLRKH
292   ........................................................................................................................................................................
80    ..............................................................................................................................................DDDAAIRRVLQQMLENA

                ●     650                                                       700
ETR1  GCE.VTTVSSNEECLRVVSH.....EH.KVVFMDVCMPGVENYQIALRIH.....................EKFTKQRHQRPL..LVALSGNTDKSTKEKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM.......................
ARCB  GNS.VDVAMTGKAALEMFKP.....GEYDLVLLDIQLPDMTGLDISRELT.....................KRYPREDLP..P..LVALTANVL.KDKQEYLNAGMDDVLSKPLSVPALTAMIKKFWDTQDDEESTVTTEENSKSEALLDIPMLEQY.....LELVGPKLITDGLAVFEK
BARA  VQH.VELCDSGHQAVERAKQ.....MPFDLILMDIQMPDMDGIRACELIH.....................QL...PHQQQTP..VIAVTAHAMAGQKEKLLGAGMSDYLAKPIEEERLHNLLLRYKPGSGISSRVVTPEVNEIVVNPNAT....LDWQLALRQAAGKTDLARDMLQMLLD
RCSC  GYQ.CKTANDGVDALNVLSK.....NHIDIVLSDVNMPNMDGYRLTQRTR.....................QLG...LTLP..VIGVTANALAEEKQRCLESGMDSCLSKPVTLDVIKQTLTVYAERVRKSRES.......................
BVGS  GQR.VIAADSGEAALALWRE.....HAFDVVITDCNMPGISGYELARRIR.....................AAEAAPGYGRTRCILFGFTASAQMDEAQACRAAGMDDCLFKPIGVDALRQRLNEAVARAAL..PTPPSPQAAAPATDDATPTAFSAESILALTQNDEALIRQLLEEVIR
LEMA  GAE.VVAVEGGYAAVNAVQQ.....EAFDLVLMDVQMPGMDGRQATEAIR.....................AWEAERNQSSLP..IVALTAHAMANEKRSLLQSGMDDYLTKPISERQLAQVVLKW...TGLALRNPAPERQHEALEVHVGPLVLDHEEGLRLAAGKADLAADMLAMLLA
SLN1  GIENIELACDGQEAFDKVKELTSKGENYNMIFMDVQMPKVDGLLSTKMIR.....................RDLGYTSPIVALTAFADDSNIKECLESGMNGFLSKPIKRPKLKTILTEFCAAYQGKKNNK..........................
SSK1  KIS.YKLAKNGQEAVNIWKE.....GGLHLIFMDLQLPVLSGIEAAKQIRDFEKQNGIGIQKSLNNSHSNLEKGTSKRFSQAPVIIVALTASNSQMDKRKALLSGCNDYLTKPVNLHWLSKKITEWGCMQALIDFDSWKQGESRMTDSVLVKSPQKPIAPSNPHSFKQATSMTPTHSPVR
292   ..................................................................................................................................................................
80    GYQ.VTAADDGQEALNLLQN.....NNPDVVIMDIHMPGMDGLELLRQLR.....................QQNP...MQQLP..IIIMTAHGDVADAVQALEAGADDYIAKPFDPDEL.......IARIR.......................
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serves as a transcriptional activation domain [103]. All four mutations in ETR1 are clustered in the putative transmembrane domains [16], suggesting that insertion of ETR1 into the plasma membrane is impaired in these mutants, or that the ethylene signal somehow cannot be relayed . from the N-terminal input domain to the histidine kinase domain due to conformational constraints. However, it has been recently shown that over-expression of the ETR1 polypeptide mutated at the critical His-353 and Asp-642 residues (see Fig. 4) confers ethylene insensitivity (C. Chang and E. Meyerowitz, personal communication). This result suggests that the *etr1* acts as dominant negative mutation.

By analogy to the bacterial two-component system, one can visualize ETR1 as the ethylene sensor. It may sense ethylene by an extracellular metal-containing input domain and transduces the signal through autophosphorylation of its histidine kinase domain. Subsequent phosphotransfer occurs first to the aspartate residue of the *cis* response regulator and then to the *trans* cognate response regulator domain to indirectly alter gene expression. Such phosphotransfer routes are seen in bacterial two-component systems in which the sensor has both a histidine kinase and a receiver module on the same protein [43, 112].

The nature of the CTR1 protein

The amino acid sequence of CTR1 shows that its carboxyl terminus shares significant similarity to the Raf family of serine/threonine protein kinases [49]. Raf was originally isolated as a key retroviral protein (v-raf) which tranforms embryo fibroblasts and epithelial cells in culture [32]. Since then, cellular homologues of v-raf have been isolated in mammals, *Drosophila*, and chicken [15, 32]. The Raf proteins mediate dramatic changes in cell growth and differentiation by transducing signals from cell-surface receptors to transcription factors [32]. Raf has been shown to affect dorsoventral patterning and R7 photoreceptor differentiation in *Drosophila* [11, 19], meiotic maturation and mesoderm development in *Xenopus* oocytes [25, 64], and vulval development in *C. elegans* [36]. CTR1 shares the same tridomain structure of all known Raf proteins [32]: Its carboxyl terminus shares high sequence similarity to the conserved kinase domain of Raf-1 [49]; The N-terminal half of CTR1 contains both the conserved cysteine motif and serine/threonine rich tract found in Raf proteins and probably acts to regulate the C-terminal kinase domain [49]. Interestingly, two point mutations found in the *CTR1* gene change invariant residues of the kinase domain, indicating that phosphorylation of downstream target proteins by the CTR1 suppresses ethylene's effects (Fig. 5).

A model

One model that incorporates what we have learned from the genetics of ethylene signalling

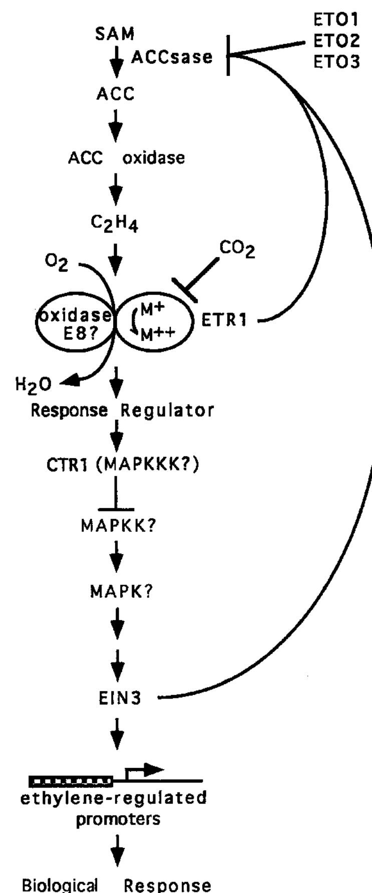

Fig. 1. A putative ethylene-sensing pathway.

and the putative nature of ETR1 and CTR1 proteins is presented in Fig. 5. Recently it has been shown in yeast that the homologue of ETR1, SLN1, has both a sensor domain and a response regulator domain on the same polypeptide [65, 81]. SLN1 is thought to phosphorylate a eukaryotic response regulator, SSK1, which in turn regulates a MAP kinase kinase kinase (MAPKKK) [42, 65]. This kinase then phosphorylates a MAPKK (PBS2 kinase) which regulates a MAPK (HOG1) which controls genes important for the osmolarity response [42, 65]. From work in mammalian cells, the CTR1 homologue, Raf-1, is a MAPKKK which directly phosphorylates and activates the MAPKK *in vitro* [32]. Since EIN3 is not a MAPKK or MAPK (J. Ecker, personal communication), it may act downstream of these kinases (Fig. 5). If CTR1 is in fact a Raf kinase, it is expected that two more classes of *ctr* mutations should be isolated: Class 1 would be lesions in the kinases downstream of CTR1 (such as MAPKK and MAPK), whereas Class 2 would be specific serine/threonine and tyrosine phosphatases which would neutralize the above kinases. Such classes of mutants have been isolated in an analogous yeast signal transduction pathway that senses osmolarity [42, 65].

According to the model presented in Fig. 5 which is based on the genetic evidence and our knowledge of signalling systems in other species, the ETR1 protein is a kinase which is active in the absence of ethylene, and inactive in its presence. The expectation would therefore be that if CTR1 is an indirect target of ETR1, it would be phosphorylated and active in the absence of ethylene, and dephosphorylated and inactive in its presence. Activated CTR1 may eventually phophorylate EIN3, which may be a transcription factor that is inactivated by phosphorylation and only active when the ETR1 and CTR1 kinases are switched off in the presence of ethylene.

In Fig. 5, the E8 is viewed as the putative oxidase of the ethylene receptor postulated by Stanley Burg [13]. This view is based on the observation that E8 shows sequence similarity to dioxygenases (Fig. 3) and its inactivation leads to ethylene overproduction [86]. We suggest that interference with the ethylene sensor that results in lower levels of reception is interpreted by the cell as an absence of the hormone leading to ethylene overproduction.

Ethylene-mediated changes in gene expression

Ethylene effects are believed to be mediated by transcriptional activation of a large set of genes [12, 24, 61]. Putative ethylene-regulated genes have been cloned and studied in order to understand ethylene-regulated processes such as fruit ripening [60, 68, 100], defense response to pathogens [12, 92], and senescence [88]. Unfortunately, there is a scarcity of information as to which *cis*-acting promoter elements in these genes confer ethylene inducibility. Deletion analysis has defined small (< 100 bp) promoter fragments conferring ethylene responsiveness in the bean chitinase 5B gene [12, 92], the tobacco PR-1B gene [24] and the tomato E4 gene [74]. While there is a 11 bp sequence conserved between the PR-1B gene and other ethylene-regulated pathogenesis-related (PR) genes [24], the E4 sequence is different [74], suggesting that there are at least two signal transduction pathways which transcriptionally activate ethylene-responsive genes, or that there is a promiscuous transcription factor which recognizes different *cis*-acting elements [73]. Several DNA-binding proteins have been detected that interact with these elements but none of them have been purified [24, 71, 74].

Ethylene and disease resistance

Pathogen attack typically has one of two outcomes on plants. If the bacteria contain an avirulence gene (i.e. avirulent bacteria) which corresponds to a particular plant resistance gene, a localized cell death patch occurs (hypersensitive response, HR) and bacteria fail to spread to other parts of the plant. This reaction is known as the resistant response. If such a match does not occur, the bacteria are considered virulent, cause necrotic lesions and spread systemically. This is

known as the susceptible response [6]. It has been proposed that ethylene plays a role in both responses [6] but genetic evidence is lacking. The question arises as to whether ethylene mediates the responses to the inducer (pathogen attack) or its production is a by-product of the defense response. Recent experimental evidence using the ethylene-insensitive mutants *etr1* and *ein2* and the ethylene overproducer *eto1* has indicated that ethylene is not essential. None of the mutants affect the resistant response in *Arabidopsis* and both the HR response and inhibition of bacterial growth occur when the mutants are infected with avirulent bacteria [6]. Interestingly, all mutants behave as wild-type plants when infected with virulent bacteria except for the *ein2* mutant. Ein2$^-$ shows far fewer necrotic lesions than its wild-type counterpart and no restriction in bacterial proliferation. The ethylene overproducer *eto1* neither confer greater disease resistance with avirulent bacteria nor greater disease susceptibility with virulent bacteria. These results indicate that EIN2 plays an essential role in causing necrotic lesions during pathogen attack [6].

Many plants respond to pathogen infection by inducing long-lasting, broad-spectrum resistance also called systemic acquired resistance (SAR) [95]. During this resistance response, numerous PR proteins are induced, one of which is known to confer increased tolerance to pathogen infection [2]. Using the ethylene-insensitive mutants *etr1* and *ein2*, it was shown that the development of SAR response does not require ethylene [56].

Conclusions and future directions

Studies on ethylene have led the way in advancing our understanding of the biosynthesis of a plant hormone at the biochemical and molecular level and they now lead our attempts to understand the biochemical machinery responsible for the perception of a plant hormone. Understanding the tissue and cell-specific expression of the ACC synthase and ACC oxidase multigene families during plant development will offer new knowledge of the role of ethylene as a signalling molecule. We must also understand the regulation of the ethylene signalling pathway at the biochemical level. It will require the isolation of all the components of the pathway and the development of the appropriate biochemical experimental system. The cloning of ethylene signalling homologues in yeast raises the possibility that this microorganism may become the system of choice for both biochemical and genetic analysis of the ethylene signal transduction pathway. We envision that not far in the future, it will be possible to construct an ethylene-sensing yeast strain with all or part of the *Arabidopsis* ethylene sensing components. The current knowledge also has the potential to elucidate the molecular details of the autocatalytic ethylene production. Finally, the spectacular advances in ethylene research and its applications to world agriculture offer the best example that fundamental research is the only tool for solving 'mission-oriented' and 'strategic importance' applied agronomical problems. This view is supported by the recent cloning of the *Arabidopsis* ethylene perception genes in tomato, indicating that the fundamental knowledge obtained from a weed can be effectively used to control senescense of agronomical important plants.

Acknowledgements

This work was supported by grants to A. T. from the NSF (DCB-8645952, 8819129, -8916286, and MCB-8316475), and the USDA (5835-21430-002-00D, 5335-21430-003-00D). We thank Alice Tarun and Claudia Kohler for reading the manuscript.

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