J Plant Growth Regul (2007) 26:92–105
DOI: 10.1007/s00344-007-0013-5

J P G R
Journal of Plant Growth Regulation

Regulation of Ethylene Biosynthesis

Cristiana T. Argueso,† Maureen Hansen† and Joseph J. Kieber*

Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA

Abstract

The biosynthesis of the gaseous phytohormone ethylene is a highly regulated process. A major point of regulation occurs at the generally rate-limiting step in biosynthesis, catalyzed by the enzyme ACC synthase (ACS). ACS is encoded by a multigene family, and different members show distinct patterns of expression during growth and development, and in response to various external cues. In addition to this transcriptional control, the stability of the ACS protein is also highly regulated. Here we review these two distinct regulatory inputs that control the spatial and temporal patterns of ethylene biosynthesis.

Key words: Ethylene biosynthesis; ACC synthase; Protein stability; Transcription.

Introduction

The simple gas ethylene has been recognized as a plant hormone for almost a century (Neljubov 1901; Crocker and Knight 1908; Knight and others 1910; Funke and others 1938). It influences a diverse array of plant growth and developmental processes, including germination, leaf and flower senescence and abscission, cell elongation, fruit ripening, nodulation, and the response to a wide variety of stresses (Mattoo and Suttle 1991; Abeles and others 1992). To understand how ethylene or any signaling molecule affects development, one needs to consider not only how it is transported and perceived but also how its level is controlled. In this review, we discuss recent progress in understanding the mechanisms governing the production of ethylene.

The biosynthesis of ethylene occurs through a relatively simple metabolic pathway (Figure 1) that has been extensively studied and well documented in plants (reviewed in: Yang and Hoffman 1984; Kende 1993; Zarembinski and Theologis 1994). Ethylene is derived from the amino acid methionine, which is converted to *S*-adenosylmethionine (AdoMet) by *S*-adenosylmethionine synthetase. AdoMet is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5′-deoxy-5′methylthioadenosine (MTA) by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACS) (Adams and Yang 1979), which is the first committed and in most instances the rate-limiting step in ethylene biosynthesis. Methylthioadenine is recycled to methionine through the Yang cycle, which allows high rates of ethylene production without depletion of the endogenous methionine pool (Miyazaki and Yang 1987). ACC is converted to ethylene, CO_2, and cyanide by ACC oxidase (ACO). The cyanide produced by this reaction is detoxified into β-cyanoalanine by the enzyme β-cyanoalanine synthase, preventing toxicity to plants in conditions of high ethylene biosynthesis.

ACC Synthase: the key Enzyme in the Pathway

ACS belongs to a family of proteins that require pyrodoxal-5′-phosphate (PLP) as cofactors, known

Received: 10 January 2007; accepted: 15 January 2007; Online publication: 10 May 2007
*Corresponding author; e-mail: jkieber@unc.edu
†These authors contributed equally to this work.

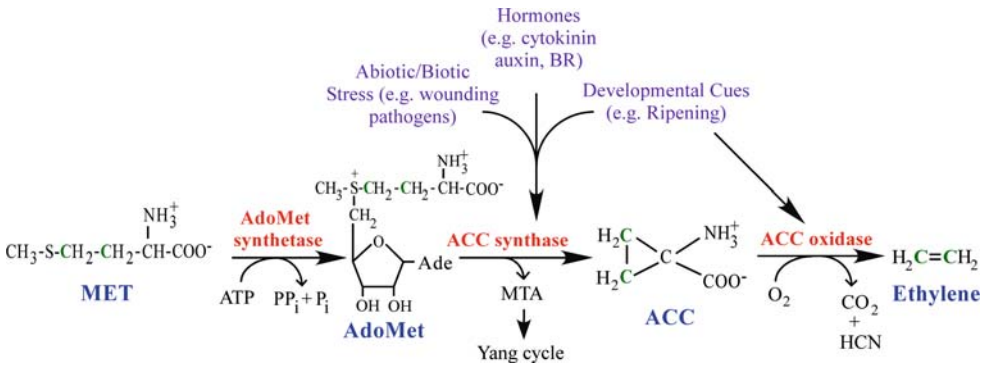

Figure 1. The ethylene biosynthetic pathway. The enzymes catalyzing each step are shown above the arrows. AdoMet: S-adenosyl-methionine; Met: methionine; ACC: 1-aminocyclopropane-1-carboxylic acid; MTA: methylthioadenine. Inputs that regulate the enzymes are shown above the pathway, either via a transcriptional or post-transcriptional mechanism. See text for further details.

as PLP-dependent enzymes. These enzymes are involved in the transamination, deamination, carboxylation, and elimination or replacement of β and γ carbons in a variety of amino acids. Although diverse in sequence and catalytic activities, the crystal structure of PLP-dependent enzymes reveals a striking conservation of structure in the catalytic core, indicating a common mechanism of catalysis. ACS converts AdoMet into ACC through a β,γ carbon elimination reaction (Li and others 2005). Sequence alignments of ACS and other PLP-dependent enzymes revealed that ACS is most similar to aspartate aminotransferases and tyrosine aminotransferases (Christen and Metzler 1985; Alexander and others 1994). Moreover, the crystal structure of apple ACS revealed that the overall folds and catalytic site of this enzyme are very similar to aspartate aminotransferases (Capitani and others 1999).

In most plant species, ACS is encoded by multigene families, which are differentially regulated by various environmental and developmental factors. In *Arabidopsis*, there are eight genes encoding active ACSs, and an additional gene encoding a catalytically inactive enzyme, *ACS1* (Liang and others 1992; Liang and others 1995; Yamagami and others 2003). ACS proteins in *Arabidopsis* can be divided into three main groups, based on their C-terminal sequences (Figure 2): (1) type 1 proteins have extended C-termini containing three conserved Ser residues that are targets for phosphorylation by mitogen-activated protein kinase 6 (MPK6) (Liu and Zhang 2004), as well as a conserved Ser residue that is a phosphorylation site for calcium-dependent protein kinase (CDPK) (Tatsuki and Mori 2001; Sebastià and others 2004) (see below); (2) type 2 proteins have shorter C-termini that harbor only the CDPK site; (3) type 3 proteins have a very short C-terminal extension that lacks both phosphorylation sites.

The active site of ACS was identified using labeled C^{14}-AdoMet (Yip and others 1990), and critical amino acids in the active site were identified through random and site-directed mutagenesis of the ACS protein (White and others 1994; Tarun and others 1998; Tarun and Theologis 1998). In LE-ACS2, mutations in residues Tyr92 and Lys278 greatly reduce enzymatic activity, indicating a role for these amino acids in the catalytic activity (Tarun and others 1998). Crystallography of recombinant apple ACS revealed that the amino acids Tyr85, Thr121, Asn202, Asp230, Tyr233, Ser270, Lys273, Arg281, and Arg497 are in the active sites and in contact with the substrate AdoMet (Capitani and others 1999).

This quaternary structure of ACS enzyme has been somewhat controversial (White and others 1994), but recent studies have elegantly demonstrated that these enzymes act as homo- or heterodimeric proteins, similar to other PLP-dependent enzymes. The first recent line of evidence came from the deduced crystal structure of apple ACS, which indicated that the active site of the enzyme formed at the interface of a dimer and was comprised of shared residues from each monomer. Further evidence that ACS enzymes work as dimers came from co-expression experiments in *E. coli* (Tarun and Theologis 1998). Expression of either of two different single mutant versions of LE-ACS2 resulted in inactive enzymes, but enzymatic activity was partially restored when the two different mutants were co-expressed. This provides compelling evidence that ACS proteins can heterodimerize and form active enzymes. Likewise, by means of this intermolecular complementation approach in

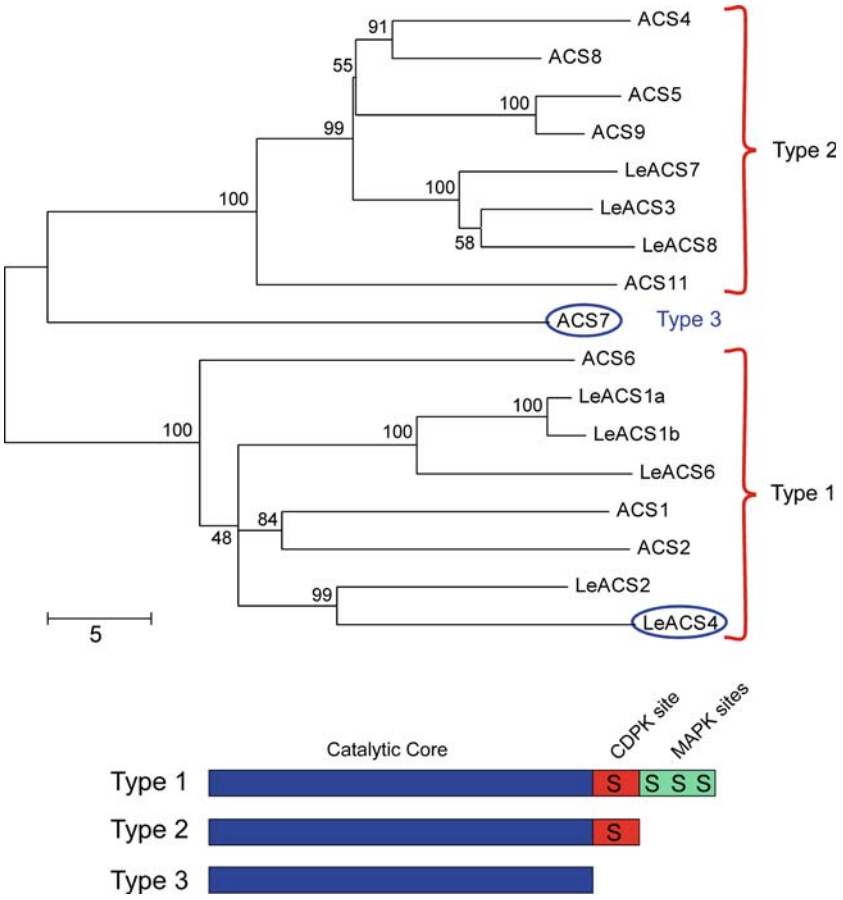

Figure 2. Top: Phylogeny of *Arabidopsis* and *Lycopersicon esculentum* (tomato) ACS proteins. Phylogeny constructed with the catalytic core of each ACS protein using MEGA version 3.1 (Kumar and others 2004). Type 1 and type 2 ACS are represented by the corresponding brackets, whereas type 3 ACS proteins are circled. Bootstrap values are listed, and the branch lengths are proportional to the number of substitutions per hundred residues as represented by the scale bar. Bottom: Cartoon representing type 1, 2, and 3 ACS proteins. The conserved catalytic core, present in all ACS proteins, is shaded, whereas putative CDPK and MAP kinase phosphorylation sites are represented by ''S'' in Type 1 and 2 ACS proteins.

Arabidopsis, members of the ACS family of proteins were also shown to heterodimerize in *E. coli*; also *in planta*, bimolecular fluorescence complementation (Tsuchisaka and Theologis 2004) provided confirmation that ACS enzymes are active as dimers *in vivo*. The presence of eight functionally active ACS enzymes in *Arabidopsis*, and their ability to form active heterodimers might act to increase the versatility of ethylene responses, enhancing the capacity to regulate ethylene production after different developmental and environmental stimuli.

ACC Oxidase: the Ethylene-forming Enzyme

ACC oxidase catalyzes the final step in ethylene biosynthesis, converting ACC into ethylene, CO_2, and cyanide (Figure 1). In conditions of high ethylene production, such as ripening fruit, ACO is often the rate-limiting step in biosynthesis. ACO belongs to a family of mononuclear, non-heme iron enzymes that are characterized by a 2-histidine-1-carboxylic acid iron-binding motif (reviewed in Hegg and Que 1997). Enzymes in this class are able to catalyze a variety of reactions such as hydroxylations, oxidative ring closure, ring expansions, and desaturations. In the case of ACO, ACC is converted to ethylene by a modification of carbons C-2 and C-3 of ACC, whereas C-1 is converted to cyanide, and the carboxyl group is converted into carbon dioxide (Peiser and others 1984).

The identification of the ''ethylene-forming enzyme'' (EFE), which is now known as ACC oxidase, (ACO) was initially very difficult. Because osmotic and cold shock, as well as treatment with detergents, inhibited the conversion of ACC to ethylene, it was then postulated that ACO was an integral membrane protein (Apelbaum and others 1981; Mayne and Kende 1986), implying that the difficulties in purification of this enzyme were due to its subcellular localization. It is now thought that the initial difficulties in its purification were in fact due to procedures that stripped the preparations of Fe^{2+}, an important cofactor for ACO. Transgenic tomato plants designed to express an antisense version of pTOM13, a gene identified as induced during tomato fruit ripening (Smith and others 1986),

displayed reduced ethylene production during ripening or after tissue wounding, suggesting that the protein encoded by pTOM13 was the ethylene-forming enzyme (Hamilton and others 1990). Additional evidence came from studies in yeast cells overexpressing pTOM13, where it was shown that the protein expressed by this clone had ethylene-forming activity (Hamilton and others 1991). Moreover, *Xenopus* cells transformed with RNA from cultured tomato cells, gained the ability to convert ethylene, but that ability was abolished by expression of an antisense pTOM13 clone (Spanu and others 1991).

The cloning of ACO allowed for the identification of similar enzymes in other plant species. The subcellular localization of ACO is still a matter of debate. Although these enzymes lack any N-terminal consensus sequences for plasma membrane localization, some reports using monoclonal antibodies raised against ACO have shown that ACO localizes at the cell wall in the pericarp of ripening tomato and climacteric apple (Rombaldi and others 1994), but this enzyme has been found to be cytosolic in apple (Chung and others 2002) and tomato (Reinhardt and others 1994).

Ethylene Biosynthesis Is Highly Regulated

Almost all plant tissues have the capacity to make ethylene, although in most cases the amount of ethylene produced is very low. Ethylene production increases dramatically during a number of developmental events such as germination, leaf and flower senescence and abscission, and fruit ripening (Yang and Hoffman 1984; Mattoo and Suttle 1991; Abeles and others 1992). There is a diverse group of stimuli that can increase the level of ethylene biosynthesis. Application of other plant hormones, such as auxin, brassinosteroids, and cytokinin can affect ethylene production (Yang and Hoffman 1984; Mattoo and Suttle 1991; Abeles and others 1992; Vogel and others 1998; Woeste and others 1999), and ethylene can affect its own biosynthesis, either increasing (autostimulation) or decreasing (autoinhibition) its rate of production. Light also affects the level of ethylene biosynthesis in many plant tissues (Goeschl and others 1967; Jiao and others 1987). Finally, a wide variety of stresses including wounding, pathogen attack, flooding, drought, hypoxia, temperature shifts, physical loads and noxious chemicals such as ozone and sulfur dioxide can induce ethylene production (Yang and Hoffman 1984; Abeles and others 1992; Bleecker and Kende 2000).

Regulation of Ethylene Biosynthesis: Abiotic Stress

One of the most studied abiotic stimuli involving stress-ethylene responses is wounding. The plant hormone jasmonic acid (JA) is a major regulator of wounding responses (Wasternack and others 2006), and ethylene seems to play an essential role (O'Donnel and others 1996). After mechanical or herbivory wounding, ethylene levels in plants increase and the expression of ethylene biosynthetic genes is altered. In *Arabidopsis*, the expression of multiple *ACS* genes increases after wounding (Tsuchisaka and Theologis 2004). The expression of the JA-inducible and pathogen- and wound-responsive gene *PDF1.2* is also regulated by ethylene (Penninckx and others 1998). Intriguingly, JA has been found to be conjugated to ACC in *Arabidopsis* plants, suggesting that JA–ACC conjugates could be involved in the co-regulation and crosstalk between JA- and ethylene-dependent pathways in plants (Staswick and Tiryaki 2004).

Although the role of ethylene in developmentally regulated senescence has been extensively studied (Grbic and Bleecker 1995; John and others 1995), its role in the regulation of drought-induced leaf senescence is less well understood. Under drought stress, ethylene emission increases (Apelbaum and others 1981; McKeon and others 1982). Inhibition of ethylene synthesis in wheat inhibits chlorophyll loss associated with drought-induced senescence (Beltrano and others 1999). In maize, *Mu*-insertion mutants in the *ACS* genes *ZmACS2* and *ZmACS6* produce less ethylene than wild-type plants and also show delayed drought-induced senescence (Young and others 2004). Interestingly, *ZmACS6* mutants also show increased chlorophyll, Rubisco, soluble protein, even in leaves not undergoing senescence, implicating ethylene in the regulation of leaf performance throughout the life cycle of the leaf, and not only when under senescence-promoting conditions. The plant hormone abscisic acid (ABA) plays an important role in drought stress in plants through the regulation of stomata closure. ACC application or the use of the *Arabidopsis* ethylene overproducing mutant *eto1* leads to decreased stomata closure after ABA application, indicating that ethylene inhibits ABA-induced stomatal closure (Tanaka and others 2006).

The role of ethylene in osmotic stress is not well understood. ACS activity in tomato cells is increased after osmotic shock (Felix and others 2000). Expression of the ethylene receptor *ETR1* is reduced upon osmotic stress, and this change is also reflected at the protein level (Zhao and Schaller 2004).

Transgenic tobacco plants overexpressing the ethylene receptor *NTHK1* show increased salt sensitivity compared to wild-type plants, but early expression of the ACC oxidase gene *NtACO3* reduced the salt-inducible expression of the *ACS* gene *NtACS1* (Cao and others 2006). Overexpression of the tobacco transcription factor *ethylene-responsive factor NtERF1* leads to increased salt tolerance (Huang and others 2004).

Ethylene may also be involved in the regulation of plant responses to low oxygen conditions, or hypoxia. The mRNA levels of *ACS2*, *ACS6*, *ACS7*, and *ACS9* are upregulated in *Arabidopsis* plants under hypoxia. The regulation of *ACS9* is inhibited by amino-oxyacetic acid (AOA), an inhibitor of ethylene biosynthesis, and reduced in the ethylene signaling mutants *etr1-1* and *ein2-1* (Peng and others 2005). The mRNA levels of *ACS2* are decreased after AOA treatment, and the levels *ACS6* and *ACS7* are not affected, suggesting that the regulation of ethylene emission under hypoxia is under complex control (Peng and others 2005).

Ethylene is also involved in the responses to other abiotic stimuli. Lithium ion induces ACS activity in various plant species (Boller 1984), and expression of multiple ACS genes is altered in *Arabidopsis* following Li^+ application (Liang and others 1996; Tsuchisaka and Theologis 2004). Application of high levels of ozone to plants induces a burst of ethylene (Mehlhorn and Wellburn 1987), promoting ozone-induced cell death (Overmyer and others 2003) through a mechanism that might involve the biosynthesis and accumulation of salicylic acid (SA) (Ogawa and others 2005) and suppression of the cell-protective action of JA (Tuominen and others 2004). In *Arabidopsis*, ozone treatment elevates the steady-state level of the ACS6 gene (Vahala and others 1998). Suppression of ACS activity in plants increases tolerance to oxidative stress and diminishes the damage caused by ozone treatment (Nakajima and others 2002; Sinn and others 2004).

Regulation of Ethylene Biosynthesis: Biotic Stresses

The involvement of ethylene in response to pathogen attack has long been recognized (Boller 1991). An early ethylene burst is observed after plants are attacked by pathogens. The effect of ethylene in disease resistance studies is somewhat variable; results seem to vary depending on the pathosystem and the conditions employed, and the fact that many pathogens are also able to produce ethylene makes interpretation of the results even more difficult. In general, plant-derived ethylene seems to be mostly associated with resistance, whereas pathogen-derived ethylene seems to contribute to pathogen virulence (van Loon and others 2006).

Microbial ethylene biosynthesis occurs through a pathway different from the one used by plants. Instead of ACC, microbial pathogens can utilize 2-keto-4-methyl-thiobutyric acid (KMBA), a transaminated derivative of methionine, or 2-oxoglutarate as ethylene precursors (Nagahama and others 1992; Fukuda and others 1993). KMBA is oxidized to ethylene through a non-enzymatic oxidation by oxidizing agents generated by a NADH:-Fe(III)EDTA oxidoreductase. 2-oxoglutarate can be converted into ethylene and succinate through an L-arginine-dependent reaction catalyzed by a single protein, the ethylene-forming enzyme EFE (distinct from ACO in plants, which also was once referred to as EFE) (Fukuda and others 1992).

Strains of the bacterial pathogens *Pseudomonas syringae* pv. *glycinea* and *Pseudomonas syringae* pv. *phaseolicola* have been previously shown to be able to produce ethylene *in planta* (Weingart and Volksch 1997). *Pseudomonas syringae* pv. *glycinea*, which is unable to produce ethylene due to a mutation in the *EFE* gene, is defective in its ability to grow in soybean plants. The growth of the wild-type strain and the ethylene-defective strain in ethylene-insensitive soybean plants was found to be similar, indicating a requirement for ethylene in the pathogenesis of this pathogen (Weingart and others 2001). Some microorganisms, such as plant-growth-promoting rhizobacteria, are also able to modulate ethylene responses by altering the levels of ACC produced by plants. Through this reaction, catalyzed by the microbial-encoded enzyme ACC deaminase, ACC is hydrolyzed to α-ketobutyrate and ammonia, decreasing the levels of ACC that are available for ethylene production (reviewed in Glick 2005). Decreased levels of ethylene alleviate ethylene-induced root growth inhibition during stress conditions, important for the growth of both the bacteria and the plant.

The regulation of plant ethylene biosynthetic genes by pathogen infection has been demonstrated. For example, a gene coding for ACS in tobacco was shown to increase after tobacco mosaic virus (TMV) infection (Knoester and others 1995) and ethylene emission and *ACS* transcription is also increased after inoculation of citrus plants with the bacterial pathogen *Xanthomonas campestris* pv. *citri* (Dutta and Biggs 1991). Although in most cases the regulation of ethylene biosynthetic genes by pathogens is considered to be a defense reaction from the plant, there is mounting evidence that

pathogens can manipulate the expression of these genes for their own benefit. Infection of tomato plants by strains of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 expressing the effector proteins AvrPto and AvrPtoB were shown to induce host gene expression that includes upregulation of the genes coding for ACC oxidase *LeACO1* and *LeACO2* (Cohn and Martin 2005). Strains of *Pseudomonas syringae* pv. *tomato* DC3000 expressing either AvrPto or AvrPtoB were unable to trigger necrosis and caused fewer disease symptoms on leaves of transgenic tomato plants that are deficient in ethylene production due to the expression of the ACC deaminase gene (Klee and others 1991), indicating that the manipulation of plant ethylene biosynthesis by these pathogens is likely to enhance disease symptoms, and that ethylene is required for the full virulence activity of these effectors (Cohn and Martin 2005).

Ethylene is likely involved in the response to nodulation by nitrogen-fixing bacteria, playing mostly an inhibitory role (reviewed in (Ferguson and Mathesius 2003). An increase in ethylene production was observed in roots of alfalfa plants inoculated with *Rhizobium* (Ligero and others 1987), and this is associated with defense responses. Application of ethylene reduces the number of nodules formed in many plant species, including *Lotus japonicus* (Nukui and others 2000). The exact role of ethylene biosynthesis in nodulation is still unclear, but it has been demonstrated that the expression of ACC oxidase in peas is elevated in inner cortical cells located in front of the root phloem poles, near where nodules are usually formed (Heidstra and others 1997). Some nitrogen-fixing bacteria, such as *Bradyrhizobium elkanii*, are also able to produce rhizobitoxine, a structural analog of AVG, which acts as an inhibitor of ACS and decreases ethylene biosynthesis (Yasuta and others 1999).

Regulation of ACC synthase: Transcriptional Control

One mechanism that regulates the production of ethylene is the differential transcription of *ACS* genes during the course of development and in response to various external cues. As discussed above, various biotic and abiotic stresses can influence the transcription of different *ACS* genes. It has been proposed that the various *ACS* genes may be differentially regulated to perform specific functions as the various ACS proteins have different enzymatic properties (Yamagami and others 2003) and different inputs that regulate their protein turnover.

Analysis of the patterns of expression of *ACS* genes in *Arabidopsis* has revealed diverse patterns of expression of the various family members. In *Arabidopsis*, *ACS* transcripts have been detected in roots, leaves, flowers, siliques, stems, and etiolated seedlings (Yamagami and others 2003; Tsuchisaka and Theologis 2004; Wang and others 2005). The *Arabidopsis* flower is an example of an organ that displays distinct patterns of *ACS* gene expression. *ACS2, 4, 5, 6, 7,* and *8* are all expressed in the sepals, the filament, and the style (Tsuchisaka and Theologis 2004; Wang and others 2005); *ACS 2, 4, 5*, and *8* are expressed in the pedicel; *ACS 2, 7*, and *8* are in the anther, and *ACS5* and *9* are in the stigma. Finally, *ACS9* and *11* are expressed at very low levels in flowers, the former in the stigma, and the latter in sepal trichomes. Thus, there is a diversity in the expression patterns for the *ACS* gene family in *Arabidopsis* flowers, which is also observed in other tissues, and it is possible that certain ACS isozymes with distinct biochemical and regulatory properties are optimized for the different cellular environments and different levels of ethylene production that occur in these various floral tissues.

A well-studied case of transcriptional regulation of *ACS* is in tomato fruit development. The tomato *ACS* family consists of at least eight genes, and these are differentially regulated by various biotic and abiotic factors. In tomato and other climacteric plants, two systems of ethylene production have been proposed. System 1 operates during vegetative growth, during which ethylene inhibits its own biosynthesis, and system 2 occurs during ripening of climacteric fruit and senescence of petals in some species, in which ethylene biosynthesis is autocatalytic (Barry and others 2000; Giovannoni 2001; Alexander and Grierson 2002). This positive feedback loop for ethylene biosynthesis is proposed to integrate ripening of the entire fruit once it has commenced. *LE-ACS6* is the only *ACS* gene detected in mature green fruit, but it is not expressed after the transition to the breaker stage (Figure 3). *LE-ACS1A* displays a transient peak of expression during the breaker stage, but its expression is not detected earlier or after the breaker stage. *LE-ACS2* and *LE-ACS4* are the primary *ACS* genes expressed after the breaker stage, with *LE-ACS2* showing the highest steady-state level of RNA expression (Barry and others 2000). The *LE-ACS2* and *LE-ACS4* genes are expressed in ripening fruit (Olson and others 1991; Rottmann and others 1991; Yip and others 1992; Lincoln and others 1993; Barry and others 2000). Use of the ethylene-insensitive mutant Never-ripe (*Nr*) revealed that expression of *LE-ACS2*, but not the other LE-*ACS* genes, is dependent on ethylene.

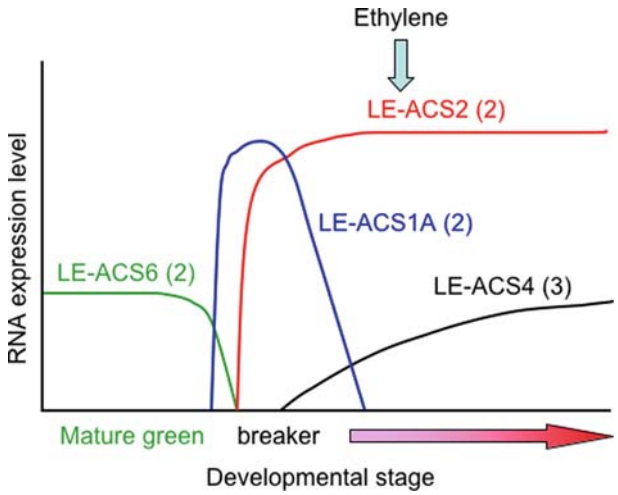

Figure 3. Cartoon representation of the expression of ACS genes during tomato fruit ripening. Based on data from Lincoln and others (1993) and Barry and others (2000). Note that the expression of only LE-ACS2 is elevated by ethylene. The class to which each ACS protein belongs (as defined in Figure 2) is shown in the parentheses. See text for additional details.

It has been proposed that *LE-ACS1A* and *LE-ACS6* are responsible for system 1 ethylene biosynthesis in green fruit, and upon induction of competence for ripening, *LE-ACS1A* expression increases and *LE-ACS4* is induced (Barry and others 2000). System 2 ethylene biosynthesis is initiated and maintained by the ethylene-dependent expression of *LE-ACS2*.Most *ACS* genes are transcriptionally induced in response to auxin, and in several auxin-regulated *ACS* genes, multiple, cis-acting AuxREs (auxin response elements) have been identified (Abel and others 1995; Ishiki and others 2000). However, induction of the *ACS* genes by auxin displays a complex pattern. In the root, although almost all *ACS* transcripts increase in response to IAA treatment, the spatial pattern of induction is different. For instance, *ACS8* expands expression into the second layer of lateral root cap cells, epidermis, and protoxylem, whereas *ACS11* expands into all cell types in the cell division zone of the root (Tsuchisaka and Theologis 2004). Thus, there is cell-type specificity for auxin induction of different *ACS* genes. Most of the *ACS* genes are also induced transcriptionally by cycloheximide, which implies the existence of a short-lived repressor protein that inhibits *ACS* transcription. Intriguing candidates for such a repressor are the Aux/IAA proteins, which have a very short half-life and negatively regulate auxin responses (Abel and others 1994). Consistent with this model, the only *ACS* gene that is not induced by auxin, *ACS1*, is also not induced by cycloheximide (Tsuchisaka and Theologis 2004).As already noted, in *Arabidopsis* various abiotic stresses often elevate ethylene biosynthesis via increased transcription of distinct subsets of *ACS* genes. *ACS6* transcript levels increase in response to ozone (Vahala and others 1998; Arteca and Arteca 1999). *ACS2*, *ACS6*, *ACS7*, and *ACS9* are elevated during hypoxia (Peng and others 2005), but anaerobic conditions result in reduced expression of all the *ACS* genes in *Arabidopsis* (Tsuchisaka and Theologis 2004). The transcript levels of distinct subsets of *ACS* genes increase after wounding and in response to osmotic stress, high temperatures, and drought conditions (Tsuchisaka and Theologis 2004; Wang and others 2005). These differences reflect distinct transcription responses of the *ACS* genes to these various inducers and may serve to optimize the response of the plant in these conditions.

Regulation of ACC Synthase: Control of Protein Turnover

ACS Protein Is Rapidly Degraded by the 26S Proteosome via a C-terminal-Dependent Mechanism. Although the regulation of *ACS* transcription clearly plays an important role in controlling the production of ethylene, recent studies have established that ACS protein turnover also is important in regulating production of this phytohormone. Various studies are consistent with a model in which the C-terminal region of ACS proteins plays a crucial role in regulating their turnover (reviewed in Chae and Kieber 2005).

Early studies on ACS stability in tomato revealed that the stability of ACS activity varied during fruit ripening. In particular, the half-life of ACS activity in green tomato pericarp tissue was shorter than that in ripening pericarp tissue (30–40 min versus 114 min) (Kende and Boller 1981). In suspension culture cells of parsley and tomato, the elevation of ACS activity observed in response to fungal elicitor was insensitive to inhibitors of RNA transcription (Chappell and others 1984; Felix and others 1991), suggesting that a post-transcriptional mechanism mediates this upregulation of ACS activity. Treatment of tomato suspension cells with elicitor resulted in the induction of ACS activity through a phosphorylation-dependent mechanism (Spanu and others 1990).

Evidence for the mechanism underlying the turnover of ACS stability has come from studies of the *Arabidopsis* ethylene overproducing (Eto) mutants (Chae and Kieber 2005). The Eto mutants produce 10- to 40-fold more ethylene in the dark as compared to wild-type seedlings and adopt a triple

response morphology (a morphology that etiolated seedlings adopt in the presence of ethylene) in the absence of exogenous application of ethylene (Guzman and Ecker 1990; Kieber and others 1993). The *eto2* and *eto3* mutants are dominant mutations in the C-terminus of ACS5 and ACS9, respectively (Vogel and others 1998; Chae and others 2003). The half-life of WT ACS5 protein is shorter than that of the *eto2* ACS5 protein, without any alteration in specific activity of the enzyme (Woeste and others 1999; Chae and others 2003). Likewise, the *eto3* mutation stabilizes the ACS9 protein (M. Hansen and J. Kieber, unpublished data).

One component regulating the turnover of ACS5, and possibly all type 2 ACS proteins, was identified by the cloning of the *ETO1* gene. The *eto1* mutation is a recessive mutation that elevates basal ethylene biosynthesis, especially in etiolated seedlings (Guzman and Ecker 1990). Cloning of *ETO1* revealed that it encodes an E3 ligase component, a BTB/TPR protein. ETO1 binds to type 2 ACS proteins, but not to type 1 or type 3 ACS proteins (Wang and others 2004; Yoshida and others 2005; Yoshida and others 2006). Disruption of *ETO1* resulted in increased stability of the ACS5 protein (Chae and others 2003) and consequently increased ethylene biosynthesis. There are two paralogs of ETO1 in *Arabidopsis*, called EOL1 and EOL2 (ETO1-like), which also interact with type 2 ACS proteins (Wang and others 2004). EOL genes have also been identified in tomato and in the monocot rice (Yoshida and others 2006). The C-terminal 14 amino acids from LE-ACS3 are sufficient to confer ETO/EOL-dependent rapid degradation to a fusion protein in cultured rice cells (Yoshida and others 2006), which, coupled with analysis of the *eto2* and *eto3* mutants, indicates that this region is necessary and sufficient for ETO1/EOL targeting. The ETO/EOL proteins are postulated to act as adaptors—which bind on one end to the substrate, in this case the ACS proteins, and on the other end to a CUL3/E3ligase—which then catalyze the addition of ubiquitin moieties on the ACS substrate. The ligase then ubiquitinates the substrate, thus targeting the protein for degradation by the 26S proteosome.

Further evidence of the involvement of the E3 ligase components in the regulation of ACS stability come from analysis of mutants in the E3 ligase pathway. The ubiquitination of target proteins requires an E1 activation enzyme to activate ubiquitin, an E2 conjugating enzyme, and finally the E3 ligase enzyme (Pintard and others 2004; Willems and others 2004). There are two general types of E3 ligases, HECT and RING; the former can carry a ubiquitin, whereas the latter associates with an E2-conjugating enzyme. The E3 ligase that has been implicated in type 2 ACS protein degradation is a RING ligase known as the BC_3B, or BTB ligase. The RING E3 ligase complex is composed of an E2-conjugating enzyme, a cullin component, an RBX1/ROC1/HRT1 protein (RBX1 in *Arabidopsis*), and an adaptor protein(s) that binds to a specific substrate. In BC_3B ligases, the cullin is CUL3a and 3b specifically, and the adaptor protein is a BTB protein (Dieterle and others 2005; Figueroa and others 2005; Gingerich and others 2005). In the case of ETO1, the BTB domain interacts with CUL3, whereas the TPR domain interacts with ACS5, and other type 2 ACS proteins to bring the substrate into contact with the E2 enzyme.

Further regulation of the E3 ligase function occurs via conjugation to a small peptide similar in sequence to ubiquitin, called RUB1 (Downes and Vierstra 2005). Attachment of RUB to an E3 ligase results in the activation of the ligase, as well as decreasing the stability of the cullin protein (Wu and others 2005). The *rce1* mutant, which is defective in a RUB1-conjugating enzyme, adopts a triple response in the dark as a result of elevated ethylene biosynthesis (Bostick and others 2004; Larsen and Cancel 2004).

An indication that the degradation machinery for ACS protein can be regulated, rather than simply acting constitutively, came from studies of *Arabidopsis* seedlings treated with cytokinin. Treatment of etiolated *Arabidopsis* seedlings with cytokinin elevates ethylene biosynthesis (Cary and others 1995; Vogel and others 1998; Vogel and others 1998; Woeste and others 1999). It was found that, in contrast to auxin and many other inducers of ethylene biosynthesis, cytokinin does not elevate ACS transcript levels (Vogel and others 1998), but rather decreases the rapid turnover of the ACS5 protein (Vogel and others 1998; Chae and others 2003). An additional example is the regulation of the turnover of type 1 ACS proteins by a stress- and pathogen-regulated MAP kinase (Liu and Zhang 2004), which is discussed below.

Role of Phosphorylation in Regulating ACS Protein Turnover

The stability of ACS proteins is regulated by protein phosphorylation. Treatment of tomato cells with protein kinase inhibitors K-252a and staurosporine leads to inhibition of elicitor-dependent induction of ACS and ethylene biosynthesis (Grosskopf and others 1990; Felix and others 1991) through a mechanism that most likely involves increased turnover of the ACS protein (Spanu and others 1994). In tomato cells, the ACS protein LE-ACS2

was shown to be phosphorylated by a CDPK from extracts of wounded tomato fruits (Tatsuki and Mori 2001). The target of CDPK phosphorylation was the conserved serine residue Ser-460 at the C-terminal region of the protein.

Additional evidence that CDPK phosphorylation may regulate ACS stability comes from *in vitro* phosphorylation studies, where a synthetic peptide based on the known CDPK phosphorylation site of LE-ACS2 was shown to be phosphorylated by maize extracts containing CDPK activity (Sebastià and others 2004). A novel CDPK phosphorylation motif was identified in the C-terminal domain of type 2 ACS proteins.

The current model is that phosphorylation of type 1 and type 2 ACS proteins blocks the ability of the ETO1/EOL proteins to bind, thus inhibiting the ubiquitination of these ACS proteins and thus their degradation by the 26S proteosome (Figure 4). Support for a role of calcium, and by inference the CDPKs, in regulating ACS protein stability has come from studies in which pea seedlings were treated with Ca^{2+} channel inhibitors and calmodulin-binding inhibitors. These treatments reduced the ethylene-induced expression of the ACO gene *ACO2* and *ACS2*, as well as ACO activity (Petruzzelli and others 2003). Similar results were observed on mung bean seedlings, where the expression of *Vr-ACS1* and *Vr-ACO1* after ethylene treatment, as well as the activity of Vr-ACO1, was reduced after treatment with Ca^{2+} inhibitors (Jung and others 2000).

Regulation of ethylene biosynthesis and ACS stability are also under control of MAP kinases. In tobacco, a stress-induced MAP kinase (SIPK) is involved in the response to different stresses, including pathogen- and ozone-induced ethylene biosynthesis. The expression of an activated form of NtMEK2, a tobacco kinase upstream of SIPK, leads to an increase in ethylene production, as well as an increase in ACS activity and *ACS*, *ACO*, and *ERF* gene induction, similar to the effect obtained after pathogen inoculation (Kim and others 2003). The closest homolog of SIPK in *Arabidopsis* is MPK6. To test the role of MPK6 in ethylene responses in *Arabidopsis*, the activated form of NtMEK2 was expressed in wild-type and *mpk6* mutant *Arabidopsis* plants, under the control of a DEX-inducible promoter (Liu and Zhang 2004). DEX application increased the amount of ethylene produced and ACS activity in wild-type plants, but not in *mpk6* plants, indicating a requirement for MPK6 in NtMEK2-induced ethylene biosynthesis (Liu and Zhang 2004). The same effect was observed after treatment with the pathogen elicitor flg22. MPK6 was shown to phosphorylate ACS2 and ACS6 *in vitro*, and transgenic plants overexpressing a phosphomimic-activated mutant version of ACS6 showed increased ethylene production. These results indicate that a pathway similar to the SIPK pathway in tobacco operates in *Arabidopsis*, and that MPK6 phosphorylates ACS proteins, thereby decreasing their turnover and increasing ethylene biosynthesis after pathogen stress.

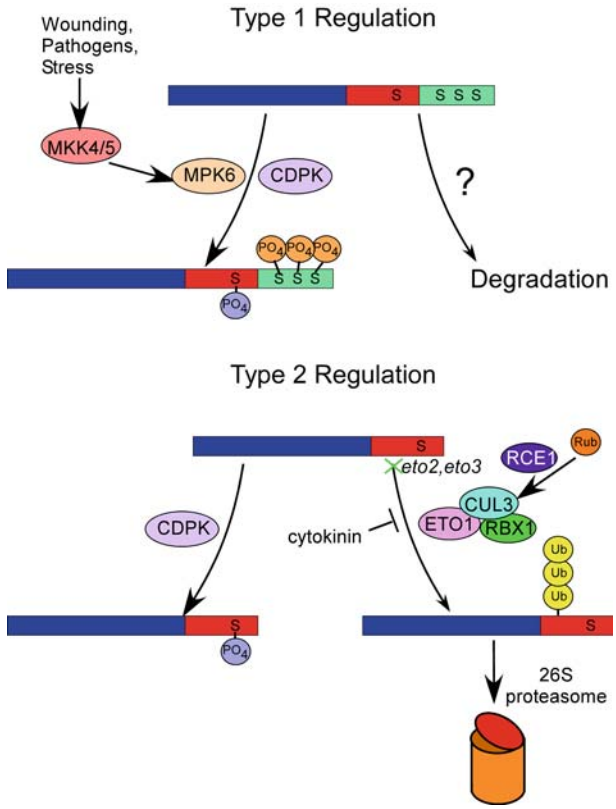

Figure 4. Model for the regulation of ethylene biosynthesis in *Arabidopsis*. Type 1 ACS proteins are phosphorylated in response to stresses at three serine residues in the C-terminal domain of type 1 ACS proteins by a MAP kinase called MPK6. In *Arabidopsis*, MPK6 is activated by the MAPKK MKK4/5. MPK6 phosphorylation is sufficient to stabilize type 1 ACS. Type 1 ACS proteins are also phosphorylated by a CDPK, although it is not known which particular CDPK phosphorylates ACS proteins *in vivo*. Phosphorylation at both sites is predicted to block the rapid degradation of the ACS proteins by the 26S proteosome. The ETO1 protein has been found to bind to the C-terminal domain of type 2 ACS proteins and thus target them for degradation by the 26S proteasome via polyubiqutination by a B_3B E_3 ligase complex. Cytokinin prevents the rapid degradation of type 2 proteins by an unknown mechanism. It is unknown what targets the type 1 proteins for rapid degradation. See text for further details.

A possible conversion on the CDPK- and MPK6-regulated pathways has been recently proposed

(Ludwig and others 2005). Tobacco plants transiently expressing an activated form of tobacco CDPK2 lacking the autoinhibitory and the calmodulin-like domains (CDPK-VK) show increased response to mild abiotic stress, as well as constitutive activation of some stress- and pathogen-responsive genes, and increased levels of SA, JA, and ACC biosynthesis, indicating a role for CDPK2 as a regulator of stress and pathogen responses in tobacco. Moreover, the activation of SIPK and WIPK by abiotic and biotic stresses, as observed in gel kinase assays, is compromised in the CDPK-VK plants. The increased ethylene production of CDPK-VK plants is abolished by the ethylene biosynthesis inhibitor AVG but not by silver thiosulfate, an inhibitor of ethylene perception, suggesting that ethylene perception is not necessary for CDPK2-regulated responses (Ludwig and others 2005).

The negative regulation of SIPK and WIPK by activated CDPK2 shows that both pathways are activated in defense response to pathogens, and they might exert regulatory effects on each other, allowing for the fine tuning of defense responses to plants. The increased ethylene phenotype, but decreased SIPK activation of CDPK-activated plants, is contradictory to the increased ethylene phenotype of MPK6-activated plants described by Liu and Zhang (Liu and Zhang 2004). These findings highlight the complexity of phosphorylation-regulated signaling and ethylene biosynthesis in plants in response to different stresses.

Conclusions

The biosynthesis of ethylene occurs via a simple, well-characterized biochemical pathway in which the two key enzymes, ACS and ACO, are both encoded by multigene families. The production of ethylene is highly regulated, and a key point of regulation involves controlling the level of active ACS protein, which is summarized in the model shown in Figure 4. Numerous studies have described how various factors regulate the transcription of distinct subsets of *ACS* genes. A major question is whether the different properties of the various ACS isoforms, combined with the distinct expression patterns, reflect an optimization of expression of a particular ACS for a particular cellular environment and for a particular function, such as the requirement for the level of ethylene production needed in a given tissue. Layered onto the transcriptional control is the regulation of the stability of the ACS proteins. Emerging evidence suggests that the different classes of ACS proteins are regulated by distinct regulatory inputs. Furthermore, that the ETO1/EOL proteins interact specifically with the type 2 ACS proteins suggests that distinct proteins are involved in targeting the type 2 and type 1/type 3 ACS proteins for degradation, and these distinct proteins remain to be identified. It is unclear how widespread the regulation of ACS protein stability is in different conditions of ethylene production, and what the relative contribution of each level of control is in various conditions. The regulation of ethylene production has a surprisingly complex circuitry, which we have only begun to understand.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Foundation to J.K. (MCB0541973).

REFERENCES

Abel S, Nguyen MD, Chow W, Theologis A. 1995. *ACS4*, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. J Biol Chem 270:19093–19099.

Abel S, Oeller PW, Theologis A. 1994. Early auxin-induced genes encode short-lived nuclear proteins. Proc Natl Acad Sci USA 91:326–330.

Abeles FB, Morgan PW, Saltveit ME Jr. 1992. Ethylene in Plant Biology. San Diego, CA, USA: Academic Press, Inc.

Adams DO, Yang SF. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76:170–174.

Alexander FW, Sandmeier E, Mehta PK, Chreisten P. 1994. Evolutionary relationships between pyrodoxal-5′-phosphate-dependent enzymes. Eur J Biochem 219:953–960.

Alexander L, Grierson D. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53:2039–2055.

Apelbaum A, Burgoon AC, Anderson JD, Lieberman M. 1981. Some characteristics of the system converting 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant Physiol 67:80–84.

Arteca J, Arteca R. 1999. A multi-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase (ACS6) in mature Arabidopsis leaves. Plant Mol Biol 39:209–219.

Barry CS, Llop-Tous MI, Grierson D. 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol 123:979–986.

Beltrano J, Ronco MG, Montaldi ER. 1999. Drought stress syndrome in wheat is provoked by ethylene evolution imbalance and reversed by rewatering, aminoethoxyvinylglycine, or sodium benzoate. J Plant Growth Regul 18:59–64.

Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18.

Boller T. 1984. Superinduction of ACC synthase in tomato pericarp by lithium ions In: Fuchs Y, Chalutz E, editors. Ethylene: Biochemical, Physiological and Applied Aspects. The Hague, The Netherlands: Junk Publishers. pp 87–88.

Boller T. 1991. Ethylene in pathogenesis and disease resistance In: Mattoo AK, Suttle JC, editors. The Plant Hormone Ethylene. Boca Raton, FL, USA: CRC Press. pp 293–314.

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 signaling, and ethylene production in Arabidopsis. Plant Cell 16:2418–2432.

Cao WH, Liu J, Zhou QY, Cao YR, Zheng SF, and others 2006. Expression of tobacco ethylene receptor NTHK1 alters plant responses to salt stress. Plant Cell Environ 29:1210–1219.

Capitani G, Hohenester E, Feng L, Storici P, Kirsch JF, and others 1999. Structure of 1-aminocyclopropane-1-carboxylate synthase, a key enzyme in the biosynthesis of the plant hormone ethylene. J Mol Biol 294:745–756.

Cary AJ, Liu W, Howell SH. 1995. Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. Plant Physiol 107:1075–1082.

Chae HS, Faure F, Kieber JJ. 2003. The *eto1*, *eto2* and *eto3* mutations and cytokinin treatment elevate ethylene biosynthesis in Arabidopsis by increasing the stability of the ACS5 protein. Plant Cell 15:545–559.

Chae HS, Kieber JJ. 2005. Eto Brute? The role of ACS turnover in regulating ethylene biosynthesis. Trend Plant Sci 10:291–296.

Chappell J, Hahlbrock K, Boller T. 1984. Rapid induction of ethylene biosynthesis in cultured parsley cells by fungal elicitor and its relationship to the induction of phenylalanine ammonia lyase. Planta 161:475–480.

Christen P, Metzler D. 1985. Transaminases. New York, NY, USA: Wiley

Chung MC, Chou SJ, Kuang LY, Charng YY, Yang SF. 2002. Subcellular localization of 1-aminocyclopropane-1-carboxylic acid oxidase in apple fruit. Plant Cell Physiol 43:549–554.

Cohn JR, Martin GB. 2005. *Pseudomonas syringae* pv. *tomato* type III effectors AvrPto and AvrPtoB promote ethylene-dependent cell death in tomato. Plant J 44:139–154.

Crocker W, Knight LI. 1908. Effect of illuminating gas and ethylene upon flowering carnation. Bot Gaz 46:259–276.

Dieterle M, Thomann A, Renou JP, Parmentier Y, Cognat V, and others 2005. Molecular and functional characterization of Arabidopsis Cullin 3A. Plant J 41:386–399.

Downes B, Vierstra RD. 2005. Post-translational regulation in plants employing a diverse set of polypeptide tags. Biochem Soc Trans 33:393–399.

Dutta S, Biggs RH. 1991. Regulation of ethylene biosynthesis in citrus leaves infected with *Xanthomonas-Campestris* Pv *Citri*. Physiol Plant 82:225–230.

Felix G, Grosskopf DG, Regenass M, Basse C, Boller T. 1991. Elicitor-induced ethylene biosynthesis in tomato cells. Characterization and use as a bioassay for elicitor action. Plant Physiol 97:19–25.

Felix G, Regenass M, Boller T. 2000. Sensing of osmotic pressure changes in tomato cells. Plant Physiol 124:1169–1180.

Ferguson BJ, Mathesius U. 2003. Signaling interactions during nodule development. J Plant Growth Regul 22:47–72.

Figueroa P, Gusmaroli G, Serino G, Habashi J, Ma L, and others 2005. Arabidopsis has two redundant Cullin3 proteins that are essential for embryo development and that interact with RBX1 and BTB proteins to form multisubunit E3 ubiquitin ligase complexes in vivo. Plant Cell 17:1180–1195.

Fukuda H, Ogawa T, Tanase S. 1993. Ethylene production by microorganisms. Adv Microb Physiol 35:275–306.

Fukuda H, Ogawa T, Tazaki M, Nagahama K, Fujii T, and others 1992. Two reactions are simultaneously catalyzed by a single enzyme—the arginine-dependent simultaneous formation of 2 products, ethylene and succinate, from 2-oxoglutarate by an enzyme from *Pseudomonas syringae*. Biochem Biophys Res Commun 188:483–489.

Funke GL, DeCoeyer F, DeDecker A, Maton J. 1938. The influence of the emanation of apples on several life phenomena of plants. Biologisch Jaarboek 5:335–381.

Gingerich DJ, Gagne JM, Salter DW, Hellmann H, Estelle M, and others 2005. Cullins 3a and 3b assemble with members of the broad complex/tramtrack/bric-a-brac (BTB) protein family to form essential ubiquitin-protein ligases (E3s) in Arabidopsis. J Biol Chem 280:18810–18821.

Giovannoni J. 2001. Molecular biology of fruit maturation and ripening. Annu Rev Plant Physiol Plant Mol Biol 52:725–749.

Glick BR. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7.

Goeschl JD, Pratt HK, Bonner BA. 1967. An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. Plant Physiol 42:1077–1080.

Grbic V, Bleecker AB. 1995. Ethylene regulates the timing of leaf senescence in Arabidopsis. Plant J 8:595–602.

Grosskopf DG, Felix G, Boller T. 1990. K-252a inhibits the response of tomato cells to fungal elicitors in vivo and their microsomal protein kinase in vitro. FEBS Lett 275:177–180.

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

Hamilton AJ, Bouzayen M, Grierson D. 1991. Identification of a tomato gene for the ethylene-forming enzyme by expression in yeast. Proc Natl Acad Sci USA 88:7434–7437.

Hamilton AJ, Lycett GW, Grierson D. 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 346:284–287.

Hegg EL, Que LJ. 1997. The 2-His-1-carboxylate facial triad. Eur J Biochem 250:625–629.

Heidstra R, Yang WC, Yalcin Y, Peck S, Emons AM, and others 1997. Ethylene provides positional information on cortical cell division but is not involved in Nod factor-induced root hair tip growth in Rhizobium–legume interaction. Development 124:1781–1787.

Huang ZJ, Zhang ZJ, Zhang XL, Zhang HB, Huang DF, and others 2004. Tomato TERF1 modulates ethylene response and enhances osmotic stress tolerance by activating expression of downstream genes. FEBS Lett 573:110–116.

Ishiki Y, Oda A, Yaegashi Y, Orihara Y, Arai T, and others 2000. Cloning of an auxin-responsive 1-aminocyclopropane-1-carboxylate synthase gene (CMe-ACS2) from melon and the expression of ACS genes in etiolated melon seedlings and melon fruits. Plant Sci 159:173–181.

Jiao X, Yip WK, Yang SF. 1987. The effect of light and phytochrome on 1-aminocyclopropane-1-carboxylic acid metabolism in etiolated wheat seedling leaves. Plant Physiol 85:643–647.

John I, Drake R, Fareel A, Cooper W, Lee P, and others 1995. Delayed leaf senescence in ethylene-deficient ACC-oxidase antisense tomato plants: molecular and physiological analysis. Plant J 7:483–490.

Jung T, Lee JH, Cho MH, Kim WT. 2000. Induction of 1-aminocyclopropane-1-carboxylate oxidase mRNA by ethylene in mung bean roots: possible involvement of Ca^{2+} and phosphoinositides in ethylene signalling. Plant Cell Environ 23:205–213.

Kende H. 1993. Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307.

Kende H, Boller T. 1981. Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit. Planta 151:476–481.

Kieber JJ, Rothenburg M, Roman G, Feldmann 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, and others 2003. Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. Plant Cell 15:2707–2718.

Klee HJ, Hayford MB, Kretzmer KA, Barry GF, Kishore GM. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. Plant Cell 3:1187–1193.

Knight LI, Rose RC, Crocker W. 1910. Effects of various gases and vapors upon etiolated seedlings of the sweet pea. Science 31:635–636.

Knoester M, Bol JF, Vanloon LC, Linthorst HJM. 1995. Virus-induced gene-expression for enzymes of ethylene biosynthesis in hypersensitively reacting tobacco. Mol Plant Microbe Interact 8:177–180.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163.

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 J 38:626–638.

Li J, Qu L, Li N. 2005. Tyr152 plays a central role in the catalysis of 1-aminocyclopropane-1-carboxylate synthase. J Exp Bot 56:2203–2210.

Liang X, Abel S, Keller JA, Shen NF, Theologis A. 1992. The 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 89:11046–11050.

Liang X, Oono Y, Shen NF, Köhler C, Li K, and others 1995. Characterization of two members (*ACS1* and *ACS3*) of the 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. Gene 167:17–24.

Liang X, Shen NF, Theologis A. 1996. Li^+-regulated 1-aminocyclopropane-1-carboxylate synthase gene expression in *Arabidopsis thaliana*. Plant J 10:1027–1036.

Ligero F, Lluch C, Olivares J. 1987. Evolution of ethylene from roots and nodulation rate of Alfalfa (*Medicago sativa* L.) plants inoculated with *Rhizobium meliloti* as affected by the presence of nitrate. J Plant Physiol 129:461–467.

Lincoln J, Campbell A, Oetiker J, Rottmann W, Oeller P, and others 1993. LE-ACS4, a fruit-ripening and wound-induced 1-aminocyclopropane-1-carboxylate synthase gene of tomato (*Lycopersicon esculentum*). Expression in *Escherichia coli*, structural characterization, expression characteristics, and phylogenetic analysis. J Biol Chem 268:19422–19430.

Liu Y, Zhang S. 2004. Phosphorylation of ACC synthase by MPK6, a stress-responsive MAPK, induces ethylene biosynthesis in Arabidopsis. Plant Cell 16:3386–3399.

Ludwig AA, Saitoh H, Felix G, Freymark G, Miersch O, and others 2005. Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. Proc Natl Acad Sci USA 102:10736–10741.

Mattoo AK, Suttle JC. 1991. The Plant Hormone Ethylene. Boca Raton, FL, USA: CRC Press

Mayne RG, Kende H. 1986. Ethylene biosynthesis in isolated vacuoles of *Vicia faba* L.— requirement for membrane integrity. Planta 167:159–165.

McKeon TA, Hoffman NE, Yang SF. 1982. The effect of plant-hormone pretreatments on ethylene production and synthesis of 1-aminocyclopropane-1-carboxylic acid in water-stressed wheat leaves. Planta 155:437–443.

Mehlhorn H, Wellburn AR. 1987. Stress ethylene formation determines plant sensitivity to ozone. Nature 327:417–418.

Miyazaki JH, Yang SF. 1987. The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. Physiol Plant 69:366–370.

Nagahama K, Ogawa T, Fujii T, Fukuda H. 1992. Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene. J Ferment Bioengineer 73:1–5.

Nakajima N, Itoh T, Takikawa S, Asai N, Tamaoki M, and others 2002. Improvement in ozone tolerance of tobacco plants with an antisense DNA for 1-aminocyclopropane-1-carboxylic acid synthase. Plant Cell Environ 25:727–736.

Neljubov D. 1901. Uber die horizontale Nutation der Stengel von Pisum sativum und einiger Anderer. Pflanzen Beih Bot Zentralb 10:128–139.

Nukui N, Ezura H, Yuhashi KI, Yasuta T, Minamisawa K. 2000. Effects of ethylene precursor and inhibitors for ethylene biosynthesis and perception on nodulation in *Lotus japonicus* and *Macroptilium atropurpureum*. Plant Cell Physiol 41:893–897.

O'Donnel PJ, Calvert C, Atzorn R, Waternack C, Leyser HMO, and others 1996. Ethylene as a signal mediating the wound response of tomato plants. Science 274:1914–1917.

Ogawa D, Nakajima N, Sano T, Tamaoki M, Aono M, and others 2005. Salicylic acid accumulation under O_3 exposure is regulated by ethylene in tobacco plants. Plant Cell Physiol 46:1062–1072.

Olson DC, White JA, Edelman L, Harkins RN, Kende H. 1991. Differential expression of two genes for 1-aminocyclopropane-1-carboxylate synthase in tomato fruits. Proc Natl Acad Sci USA 88:5340–5344.

Overmyer K, Brosche M, Kangasjarvi J. 2003. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci 8:335–342.

Peiser GD, Wang T, Hoffman NE, Yang SF, Liu H, and others 1984. Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene. Proc Natl Acad Sci USA 81:3059–3063.

Peng HP, Lin TY, Wang NN, Shih MC. 2005. Differential expression of genes encoding 1-aminocyclopropane-1-carboxylate synthase in Arabidopsis during hypoxia. Plant Mol Biol 58:15–25.

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.

Petruzzelli L, Sturaro M, Mainieri D, Leubner-Metzger G. 2003. Calcium requirement for ethylene-dependent responses involving 1-aminocyclopropane-1-carboxylic acid oxidase in radicle tissues of germinated pea seeds. Plant Cell Environ 26:661–671.

Pintard L, Willems A, Peter M. 2004. Cullin-based ubiquitin ligases: Cul3–BTB complexes join the family. EMBO J 23:1681–1687.

Reinhardt D, Kende H, Boller T. 1994. Subcellular localization of 1-aminocyclopropane-1-carboxylate oxidase in tomato cells. Planta 195:142–146.

Rombaldi C, Lelievre JM, Latche A, Petitprez M, Bouzayen M, and others 1994. Immunocytolocalization of 1-aminocyclopropane-1-carboxylic acid oxidase in tomato and apple fruit. Planta 192:453–460.

Rottmann WH, Peter GF, Oeller PW, Keller JA, Shen NF, and others 1991. 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. J Mol Biol 222:937–961.

Sebastià CH, Hardin SC, Clouse SD, Kieber JJ, Huber SC. 2004. Identification of a new motif for CDPK phosphorylation in vitro that suggests ACC synthase may be a CDPK substrate. Arch Biochem Biophys 428:81–91.

Sinn JP, Schlagnhaufer CD, Arteca RN, Pell EJ. 2004. Ozone-induced ethylene and foliar injury responses are altered in 1-aminocyclopropane-1-carboxylate synthase antisense potato plants. New Phytol 164:267–277.

Smith CJS, Slater A, Grierson D. 1986. Rapid appearance of an mRNA correlated to ethylene synthesis encoding a protein of molecular weight of 35000. Planta 168:94–100.

Spanu P, Felix G, Boller T. 1990. Inactivation of stress induced 1-aminocyclopropane carboxylate synthase *in vivo* differs from substrate-dependent inactivation *in vitro*. Plant Physiol 93:1482–1485.

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 Physiol 106:529–535.

Spanu P, Reinhart D, Boller T. 1991. Analysis and cloning of the ethylene-forming enzyme from tomato by functional expression of its mRNA in *Xenopus laevis* oocytes. EMBO J 10:2007–2013.

Staswick PE, Tiryaki I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell 16:2117–2127.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, and others 2006. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis. J Exp Bot 57:2259–2266.

Tarun AS, Lee JS, Theologis A. 1998. Random mutagenesis of 1-aminocyclopropane-1-carboxylate synthase: a key enzyme in ethylene biosynthesis. Proc Natl Acad Sci USA 95:9796–9801.

Tarun AS, Theologis A. 1998. Complementation analysis of mutants of 1-aminocyclopropane-1-carboxylate synthase reveals the enzyme is a dimer with shared active sites. J Biol Chem 273:12509–12514.

Tatsuki M, Mori H. 2001. Phosphorylation of tomato 1-aminocyclopropane-1-carboxylic acid synthase, LE-ACS2, at the C-terminal region. J Biol Chem 276:28051–28057.

Tsuchisaka A, Theologis A. 2004. Heterodimeric interactions among the 1-amino-cyclopropane-1-carboxylate synthase polypeptides encoded by the Arabidopsis gene family. Proc Natl Acad Sci USA 101:2275–2280.

Tsuchisaka A, Theologis A. 2004. Unique and overlapping expression patterns among the Arabidopsis 1-amino-cyclopropane-1-carboxylate synthase gene family members. Plant Physiol 136:2982–3000.

Tuominen H, Overmyer K, Keinanen M, Kollist H, Kangasjarvi J. 2004. Mutual antagonism of ethylene and jasmonic acid regulates ozone-induced spreading cell death in Arabidopsis. Plant J 39:59–69.

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

van Loon LC, Geraats BJ, Linthorst HJM. 2006. Ethylene as a modulator of disease resistance in plants. Trend Plant Sci 11:184–191.

Vogel JP, Schuerman P, Woeste KW, Brandstatter I, Kieber JJ. 1998. Isolation and characterization of Arabidopsis mutants defective in induction of ethylene biosynthesis by cytokinin. Genetics 149:417–427.

Vogel JP, Woeste KW, Theologis A, Kieber JJ. 1998. Recessive and dominant mutations in the ethylene biosynthetic gene *ACS5* of *Arabidopsis* confer cytokinin insensitivity and ethylene overproduction, respectively. Proc Natl Acad Sci USA 95:4766–4771.

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

Wang N, Shih M, Li N. 2005. The GUS reporter-aided analysis of the promoter activities of Arabidopsis ACC synthase genes *AtACS4*, *AtACS5*, and *AtACS7* induced by hormones and stresses. J Exp Bot 56:909–920.

Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, and others 2006. The wound response in tomato—role of jasmonic acid. J Plant Physiol 163:297–306.

Weingart H, Ullrich H, Geider K, Volksch B. 2001. The role of ethylene production in virulence of *Pseudomonas syringae* pvs. *glycinea* and *phaseolicola*. Phytopathology 91:511–518.

Weingart H, Volksch B. 1997. Ethylene production by *Pseudomonas syringae* pathovars *in vitro* and *in planta*. Appl Environ Microbiol 63:156–161.

White MF, Vasquez J, Yang SF, Kirsch JF. 1994. Expression of apple 1-aminocyclopropane-1-carboxylate synthase in *Escherichia coli*: kinetic characterization of wild-type and active-site mutant forms. Proc Natl Acad Sci USA : 12428–12432.

Willems AR, Schwab M, Tyers M. 2004. A hitchhiker's guide to the cullin ubiquitin ligases: SCF and its kin. Biochim Biophys Acta 1695:133–170.

Woeste K, Vogel JP, Kieber JJ. 1999. Factors regulating ethylene biosynthesis in etiolated *Arabidopsis thaliana* seedlings. Physiol Plant 105:478–484.

Woeste K, Ye C, Kieber JJ. 1999. Two Arabidopsis mutants that overproduce ethylene are affected in the post-transcriptional regulation of ACC synthase. Plant Physiol 119:521–530.

Wu JT, Lin HC, Hu YC, Chien CT. 2005. Neddylation and deneddylation regulate Cul1 and Cul3 protein accumulation. Nat Cell Biol 7:1014–1020.

Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, and others 2003. Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the Arabidopsis gene family. J Biol Chem 278:49102–49112.

Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189.

Yasuta T, Satoh S, Minamisawa K. 1999. New assay for rhizobitoxine based on inhibition of 1-aminocyclopropane-1-carboxylate synthase. Appl Environ Microbiol 65:849–852.

Yip WK, Dong JG, Kenny JW, Thompson GA, Yang SF. 1990. Characterization and sequencing of the active site of 1-aminocyclopropane-1-carboxylate synthase. Proc Natl Acad Sci USA 87:7930–7934.

Yip WK, Moore T, Yang SF. 1992. Differential accumulation of transcripts for four tomato 1-aminocyclopropane-1-carboxylate synthase homologs under various conditions. Proc Natl Acad Sci USA 89:2475–2479.

Yoshida H, Nagata M, Saito K, Wang KL, Ecker JR. 2005. *Arabidopsis* ETO1 specifically interacts with and negatively regulates type 2 1-aminocyclopropane-1-carboxylate synthases. BMC Plant Biol 5:14 .

Yoshida H, Wang KL, Chang CM, Mori K, Uchida E, and others 2006. The ACC synthase TOE sequence is required for interaction with ETO1 family proteins and destabilization of target proteins. Plant Mol Biol 62:427–437.

Young TE, Meeley RB, Gallie DR. 2004. ACC synthase expression regulates leaf performance and drought tolerance in maize. Plant J 40:813–825.

Zarembinski TI, Theologis A. 1994. Ethylene biosynthesis and action: a case of conservation. Plant Mol Biol 26:1579–1597.

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