DARWIN REVIEW

Recent advances in ethylene research

Zhefeng Lin, Silin Zhong and Don Grierson*

Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

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Abstract

Ethylene regulates many aspects of the plant life cycle, including seed germination, root initiation, flower development, fruit ripening, senescence, and responses to biotic and abiotic stresses. It thus plays a key role in responses to the environment that have a direct bearing on a plant’s fitness for adaptation and reproduction. In recent years, there have been major advances in our understanding of the molecular mechanisms regulating ethylene synthesis and action. Screening for mutants of the triple response phenotype of etiolated Arabidopsis seedlings, together with map-based cloning and candidate gene characterization of natural mutants from other plant species, has led to the identification of many new genes for ethylene biosynthesis, signal transduction, and response pathways. The simple chemical nature of ethylene contrasts with its regulatory complexity. This is illustrated by the multiplicity of genes encoding the key ethylene biosynthesis enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, multiple ethylene receptors and signal transduction components, and the complexity of regulatory steps involving signalling relays and control of mRNA and protein synthesis and turnover. In addition, there are extensive interactions with other hormones. This review integrates knowledge from the model plant Arabidopsis and other plant species and focuses on key aspects of recent research on regulatory networks controlling ethylene synthesis and its role in flower development and fruit ripening.

Key words: ACC synthase, ACC oxidase, ethylene biosynthesis, ethylene cross-talk, ethylene signalling, flower development, fruit ripening, sex determination, ubiquitin-mediated degradation.

Introduction

In this review, original, or widely accepted, gene names have been used, and where synonyms exist this is indicated. Recently, tomato (formerly Lycopersicon esculentum) has been renamed Solanum lycopersicum. Tomato genes named before this change are prefixed Le and and those named since are prefixed Sl.

Ethylene, or ethene, the simplest olefin, exists in the gaseous state under ambient conditions. It regulates many aspects of the plant life cycle, including seed germination, root initiation, root hair development, flower development, sex determination, fruit ripening, senescence, and responses to biotic (such as pathogen attack) and abiotic (such as wounding, hypoxia, and chilling) stresses. It is biologically active in trace amounts and its effects are of great commercial importance. The discovery of the biological activity of ethylene came about in the 19th century as leaks in illuminating gas caused premature senescence and defoliation of plants in the greenhouse and of trees near gas lines (Abeles et al., 1992). In 1901, Neljubov identified the active component in illuminating gas as ethylene. It is now known that ethylene is produced by all cells during plant development, but rates vary, with the highest rates being associated with meristematic, stressed, or ripening tissues (Abeles et al., 1992). It has full biological activity at 1 ml l⁻¹, which corresponds to 6.5×10⁻⁹ M at 25 °C. Ethylene can dissolve in both lipid membranes and the aqueous phase of cells, but it is ~14 times more soluble in the lipids than in water, and the solubility is decreased by solutes (Abeles et al., 1992).

The triple response phenotype of etiolated dicotyledonous seedlings: inhibition of hypocotyl and root elongation, radial swelling of hypocotyl and root cells, and exaggerated
apical hook when treated with ethylene or its precursor, 1-aminoacyclopropane-1-carboxylic acid (ACC), has been used to screen for mutants that are defective in ethylene responses in Arabidopsis (Bleecker et al., 1988; Guzman and Ecker, 1990). A large number of genes that function in ethylene biosynthesis, signal transduction, and response pathways have been identified based on mutant screens, and a linear model involving these components has been established based on epistasis analysis. In addition, map-based cloning and candidate gene characterization of natural mutants that are defective in ethylene responses, together with protein–protein interactions, DNA–protein interactions, and analysis of gene function have also been used to identify new components that function in ethylene signalling in other plant species including tomato. This review focuses on key aspects of recent research on ethylene, flower development, and ripening, and integrates knowledge from the model plant Arabidopsis and other plant species, particularly Solanaceae, to give an up-to-date view on ethylene and development.

Ethylene biosynthesis and regulation

The biochemistry of ethylene biosynthesis was the subject of intensive study in plant hormone physiology in the second half of the 20th century. Because of the simple structure of ethylene, a number of compounds were proposed as the precursors, including linolenic acid, propanal, β-alanine, and methionine. Lieberman and Mapson first discovered that methionine was the precursor of ethylene in a chemical model system and showed that ethylene was derived from C3 and C4 of methionine (reviewed by Yang and Hoffman, 1984). Direct evidence in support of the role of methionine as an ethylene precursor in vivo was reported by Lieberman et al., who found that labelled methionine was efficiently converted to ethylene by apple fruit tissue. These findings were subsequently confirmed by other investigators with apple and other plant tissues. The major breakthroughs, however, were the establishment of S-adenosyl-L-methionine (S-AdoMet) and ACC as the precursors of ethylene in plants (see Yang and Hoffman, 1984). Methionine gives rise to ethylene via three key enzymatic reactions: (i) methionine is converted to S-AdoMet by S-AdoMet synthetase; (ii) ACC synthase (ACS) converts S-AdoMet to ACC; and (iii) ACC oxidase (ACO) degrades ACC to release ethylene (Fig. 1). The formation of ACC is often cited as the rate-limiting step in the pathway, but there are situations where ACC is absent and ACS and ACO are induced, for example by wounding and the ripening stimulus (Alexander and Grierson, 2002). The conversion of ACC to ethylene catalysed by ACO is oxygen dependent, and, under anaerobic conditions, ethylene formation is completely suppressed. In this reaction, Fe²⁺ and ascorbate are required as cofactors and a co-substrate, respectively. Apart from ACC, ACS also produces 5′-methylthioadenosine (MTA), which is utilized for the synthesis of new methionine, ensuring that high rates of ethylene biosynthesis can be maintained even when the pool of methionine is small (Fig. 1). It was the observation that O₂ is absolutely required for ethylene synthesis that led to the identification of ACC as the immediate precursor, since it accumulates in apple tissue under N₂ (see Yang and Hoffman, 1984). ACC can be translocated from flooded roots, to liberate ethylene in aerial parts of the plants, or metabolized to malonyl ACC, which is believed to be inactive. The poisonous gas hydrogen cyanide (HCN) formed from the decomposition of ACC is detoxified by β-cyanoalanine synthase (Fig. 1).

The ACS multigene family

ACS isoforms are encoded by a multigene family in all plants investigated, and these genes are differentially regulated at the level of transcription by developmental and environmental signals in response to both internal and external stimuli. In tomato (Solanum lycopersicum, syn. Lycopersicon esculentum), nine ACS genes from LeACS1A and B to LeACS8 have been cloned (Oeller et al., 1991; Rottmann et al., 1991; Yip et al., 1992) and their expression studied. Using an RNase protection assay, Barry et al. (2000) showed that LeACS1A, LeACS2, LeACS4, and LeACS6 were all transcribed in mature and ripening fruit, but with distinct expression patterns, whereas LeACS1B, LeACS3, LeACS5, and LeACS7 were undetectable in fruit tissue. These genes also responded differently to ethylene, with reduction in LeACS1A and LeACS6 mRNA accumulation and induction of LeACS2 mRNA. Nakatsuka et al. (1998) showed that the ACS family members displayed different responses to the ethylene inhibitor 1-methylcyclopropene (MCP). The expression of LeACS2 and 4 mRNAs in ripening fruit was largely prevented by MCP; LeACS6 transcripts normally accumulated in non-ripening fruit and were undetectable in ripening fruit, but MCP treatment induced its accumulation in ripening fruit. LeACS1A and LeACS3 were expressed constitutively throughout fruit development and ripening irrespective of MCP treatment, and Nakatsuka et al. (1998) suggested that ethylene produced by LeACS1A and 3 effectively negatively regulates LeACS6 prior to the expression of LeACS2 and 4.

The Arabidopsis genome encodes 12 ACS-like genes. ACS3 is a pseudogene with a short sequence, whereas ACS10 and 12 can complement the Escherichia coli aminotransferase mutant DL39 and are thus aminotransferases (Yamagami et al., 2003). Therefore, there are nine authentic ACS genes in the Arabidopsis genome and these can be divided into three groups (Fig. 2). All the members display distinct spatial and temporal expression patterns throughout growth and development, and in response to various stress conditions (Yamagami et al., 2003; Tsuchisaka and Theologis, 2004; Peng et al., 2005). Using promoter–β-glucuronidase (GUS) and promoter–green fluorescent protein (GFP) fusion reporters, Tsuchisaka and Theologis (2004) examined the expression of the Arabidopsis ACS genes of vegetative parts, reproductive organs, and vascular tissue. Overlapping expression of the ACS genes was observed in hypocotyls, roots, various floral parts, and in
the stigmatic and abscission zones of the siliques. However, unique expression patterns were also seen. For example, ACS11 was expressed in the trichomes of sepals and ACS1 in the replum. The *Arabidopsis* ACS genes displayed different responses to ethylene and auxin. In roots, for example, the expression of ACS2, ACS4, ACS5, ACS8, and ACS11, but not ACS1 and ACS9, was induced by indole acetic acid (IAA), whereas the expression of ACS7 was re-localized to different cells by IAA treatment. Different family members also displayed opposite responses to the ethylene-inducing signals including cold, heat, anaerobiosis, and Li⁺ ions. Peng *et al.* (2005) investigated the responses of *Arabidopsis* ACS genes to hypoxia stress and they found that ACS2, ACS6, ACS7, and ACS9 were specifically induced during hypoxia. Vandenbussche *et al.* (2003) reported that ACS8 transcript levels were controlled particularly by light and shade, and Thain *et al.* (2004) also found that this gene was controlled by the circadian clock.

Differences in expression patterns of the ACS transcripts have also been found in other plant species (Trebitsh *et al.*, 1997; Yamasaki *et al.*, 2001; Salman-Minkov *et al.*, 2008), and these will be discussed in the following sections of this review.

**Dimerization of ACS**

ACSs are evolutionarily related to aminotransferases (Theologis, 1992). Sequence analysis indicates that ACS isoforms share 15% sequence identity with aspartate aminotransferase (AATase) and that essential active site components conserved in AATase are also found in ACS. ACS is a pyridoxal phosphate (PLP)-dependent enzyme and it requires PLP as cofactor. Its activity in plant tissue is enhanced by factors that promote ethylene formation, including wounding and ripening (Yang and Hoffman, 1984). Most PLP-dependent enzymes have a lysine residue in their active site. The Lys278 present in the tomato ACS is conserved in other ACS isoforms, and this residue serves as the PLP-binding site by reducing the double bond between PLP and the enzyme itself (Yip *et al.*, 1990). Li and Mattoo

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**Fig. 1.** Ethylene biosynthetic pathway. The formation of S-AdoMet (S-adenosyl methionine) from methionine is catalysed by SAM synthetase at the expense of one molecule of ATP per molecule of S-AdoMet synthesized (i). A rate-limiting step of ethylene synthesis is the conversion of S-AdoMet to ACC by ACC synthase (ii). MTA (methylthioadenosine) is the by-product generated, along with ACC, by ACC synthase. Recycling of MTA back to methionine conserves the methylthio group and is able to maintain a constant concentration of methionine in cells even when ethylene is rapidly synthesized and the methionine pool is small. Malonylation of ACC to malonyl-ACC (MACC) depletes the ACC pool and reduces ethylene production. ACC oxidase catalyses the final step of ethylene synthesis using ACC as substrate and generates carbon dioxide and cyanide (iii). Cyanide is metabolized by β-cyanoalanine synthase to produce non-toxic substances. Transcriptional regulation of both ACC synthase and ACC oxidase by homeotic proteins and developmental and environmental cues is indicated by dashed arrows.
(1994) showed by gel filtration that the tomato ACS expressed in E. coli was a dimer. Crystal structure analysis of the apple ACS (Capitani et al., 1999) revealed that the amino acid residues at the active site are Tyr85, Thr121, Asn202, Asp230, Tyr233, Ser270, Lys273, Arg281, and Arg407, which are identical to those of the chicken mitochondrial AATase. These essential residues serve as the PLP-binding and homodimer interface for the enzyme. Yamagami et al. (2003) demonstrated that each Arabidopsis ACS could form a homodimer with shared active sites, and these homodimers have functional activity with an optimum pH, ranging between 7.3 and 8.2. Their $K_m$ values for S-AdoMet range from 8.3 mM to 45 mM, whereas their $k_{cat}$ values vary from 0.19 s$^{-1}$ to 4.82 s$^{-1}$ per monomer. Their $K_i$ values for AVG (aminoethoxyvinyglycine, an inhibitor of PLP-requiring enzymes) vary from 0.019 mM to 0.80 mM and from 0.15 mM to 12 mM, respectively. This has been taken to indicate that the ACS isoforms are biochemically distinct and function in unique cellular environments for the biosynthesis of ethylene (Yamagami et al., 2003).

Tsuchisaka and Theologis (2004) further analysed the formation of functional heterodimers of Arabidopsis ACS isoforms in E. coli using intermolecular complementation, revealing that all isozymes could form heterodimers; however, enzymatically active heterodimers were (with one exception) only formed among polypeptides that belong to the same phylogenetic branch, suggesting that the shared active sites formed between the heterodimeric subunits of the same branch are structurally similar to those of the corresponding homodimers and dissimilar to those from different branches. Interestingly, ACS1, which is non-functional as a homodimer, can also form functional heterodimers with members of its phylogenetic branch if the partners provide the wild-type K278 residue. Protein

Fig. 2. The ACC synthase gene family from Arabidopsis and tomato. (A) Alignment of the C-terminal sequences of the ACS proteins, with amino acids numbered. (B) Phylogenetic tree analysis used the sequences from (A) showing three distinct types. The melon CmACS7 protein, which corresponds to the a locus (see text), is also included.
analysis showed that certain heterodimers are inactive, due not to the absence of heterodimerization but rather to structural restraints that prevent the shared active sites from being functional. The ACS gene family members are potentially able to form 45 homo- and heterodimers, of which 25 are functional. It was proposed that the homo- and heterodimerization enhances the diversity of the ACS gene family and provides physiological versatility enabling members to operate in a broad gradient of S-AdoMet concentration in various cells/tissues during plant growth and development.

**Post-translational regulation of ACS**

The conclusion that ACS is labile and has a rapid turnover rate was developed from the early studies of auxin-induced ethylene production (Konze and Kende, 1979; Yu and Yang, 1979). It was found that the increase in ACS activity induced by IAA treatment was severely inhibited by the application of cycloheximide, actinomycin D, and z-amanitin, and the half-life of ACS in IAA-treated mung bean hypocotyls was estimated to be 25 min based on the decay kinetics of the enzyme in the presence of cycloheximide. This value was similar to that obtained from wound-induced ACS in tomato fruit tissue (Konze et al., 1980). Li and Mattoo (1994) showed that, in E. coli, deletion of the C-terminus of the tomato ACS protein (i.e. LeACS2) through Arg429 resulted in complete inactivation of the enzyme, while deletion of 46-52 amino acids from the C-terminus resulted in an enzyme that had nine times higher affinity for the substrate S-AdoMet than the wild-type enzyme. They also found that the highly efficient truncated ACS was a monomer of 52±1.8 kDa in gel filtration, whereas the wild-type ACS was a dimer, indicating that the non-conserved C-terminus of ACS affects its enzymatic function as well as dimerization. Tatsuki and Mori (2001) reported that the LeACS2 and LeACS4 isoforms had different phosphorylation patterns. Using site-directed mutagenesis they showed that LeACS2 was phosphorylated at Ser460, while LeACS4 did not show phosphorylation.

**The ACO gene family**

The final step in ethylene biosynthesis in plants is catalysed by ACO. This reaction converts ACC to ethylene, CO2, and HCN, and has a requirement for ascorbate and oxygen (Fig. 1). The enzyme had defied early attempts at purification, probably because it was wrongly assumed to be a membrane-bound enzyme. A systematic search identified a candidate tomato cDNA called TOM13, which was induced by wounding, and during senescence and ripening, which are all situations when ethylene synthesis increases (Smith et al., 1986; Holdsworth et al., 1987a, b; Davies and Grierson, 1989). Work by Holdsworth et al. (1987a, b, 1988) identified three tomato genes related to TOM13. Hamilton et al. (1990, 1991) first identified TOM13 as encoding the protein ACO1 (named previously as ethylene-forming enzyme), and showed that antisense silencing of this gene in tomato plants inhibited ethylene production (Hamilton et al., 1990) and that when the yeast Saccharomyces cerevisiae was transformed with ACO1, it was able to convert ACC to ethylene (Hamilton et al., 1991). The predicted structure of the protein indicated that it was likely to be soluble, rather than membrane bound, and also
indicated the nature of cofactors it might require, thus giving vital clues that led to the first purification of the enzyme (John, 1991).

ACO is a member of a superfamily of non-haem iron oxygenases and oxidases, most of which utilize Fe (II) as a cofactor and 2-oxoglutarate (2OG) as a co-substrate (John et al., 1991; Bidonde et al., 1998; Ryle and Hausinger, 2002), although ACO uses ascorbate not 2OG as a cofactor (Schofield and Zhang, 1999). No significant differences in optimum pH (6.8–7.2), temperature, and the absolute dependence on Fe²⁺ were found for these ACO isoforms, but there were differences in apparent Km and P1 (Bidonde et al., 1998). Crystal structure studies of the Petunia hybrida ACO have revealed that it forms a complex with Fe(II) and Cu(I) under anaerobic conditions and its active site contains a single Fe(II) coordinated by three residues (His177, Asp179, and His234) (Zhang et al., 2004). The side chains of Arg175 and Arg244 are proposed to be involved in binding bicarbonate, which leads to the activation of the ACO enzyme (Zhang et al., 2004). The bicarbonate-dependent two-electron oxidation of ACC occurs concomitantly with the reduction of dioxygen and oxidation of a reducing agent, probably ascorbate, to produce ethylene, CO₂, cyanide, and two water molecules (Bassan et al., 2006). In the absence of bicarbonate, ACO undergoes rapid inactivation as it is unable to oxidize ACC to ethylene efficiently.

ACO is encoded by a multigene family in all plant species studied. The Arabidopsis genome encodes five ACO genes. In tomato, six ACO genes (LeACO1–LeACO6) exist in the databases, although the catalytic activity of only three of these has been studied in detail (Bidonde et al., 1998). The sequences are highly conserved throughout the protein-coding regions but do show a degree of sequence divergence within the 3‘-untranslated regions. Expression analysis reveals that the ACO genes display a high degree of differential expression in tissues at various stages of the life cycle. For example, tomato ACO1 is the main gene required during fruit ripening (Blume and Grierson, 1997), and ACO1, 2, and 3 transcripts accumulate during the senescence of leaves, fruit, and flowers (Barry et al., 1996). In addition, ACO1 mRNA is wound inducible in leaves (Smith et al., 1986; Holdsworth et al., 1987a, b; Blume and Grierson, 1997). Four ACO genes are expressed during flower development, with each showing a temporally and spatially distinct pattern of accumulation (Barry et al., 1996; Llop-Tous et al., 2000). ACO1 is predominantly expressed in the petals and the stigma and style, ACO2 expression is mainly restricted to tissues associated with the anther cone, whereas ACO3 transcripts accumulate in all of the floral organs examined apart from the sepal (Barry et al., 1996; Llop-Tous et al., 2000).

The levels of ACO transcripts have been shown to be regulated by ethylene itself and other phytohormones. Chae et al. (2000) showed that OsACO2 transcripts in etiolated rice (Oryza sativa L.) seedlings were markedly increased by IAA treatment, whereas OsACO3 mRNA accumulated in response to ethylene treatment. The auxin-induced OsACO2 expression was partially inhibited by ethylene, while ethylene induction of OsACO3 transcription was completely blocked by auxin, indicating that the two genes are regulated by complex hormonal networks in a gene-specific manner. Furthermore, post-translational modification was also suggested as they found that okadaic acid, a potent inhibitor of protein phosphatase, effectively suppressed the IAA induction of OsACO2 expression. The expression of ACO genes has also been reported to be regulated by gibberellic acid (GA) (Calvo et al., 2004) (see below), and it has been known for a long time that ACO1 can also be rapidly induced by wounding (Smith et al., 1986; Holdsworth et al., 1987b).

E8, encoding a 2OG-Fe(II) oxygenase, was reported to modulate ACO expression and ethylene production in tomato. E8 and ACO share 34% amino acid sequence identity, and sense suppression (gene silencing) of the expression of E8 in tomato resulted in increased levels of ACC and ethylene. This was associated with an ~7-fold increase in expression of ACO mRNA and a 2.5-fold increase in LeACS2 mRNA in fruit (Kneissl and Deikman, 1996). It has been suggested that the product of the E8 reaction participates in feedback regulation of ethylene biosynthesis during fruit ripening, but the mechanism remains to be elucidated.

**Homeotic proteins transcriptionally regulate ACS and ACO genes**

Transcriptional regulation of both ACS and ACO in response to various developmental and environmental factors, including floral organ development, ripening, senescence, and stresses such as wounding, pathogens, ozone, and UV-B, has been observed in all plant species studied (Fig. 1). Identification of the corresponding transcriptional regulators, however, has proved difficult despite the presence of numerous putative cis-elements in the promoter sequences of ACO and ACS genes. For example, the LeACO1 promoter sequences contain ethylene-responsive elements (EREs), core motifs of transcription factors, such as GT-1 and DoF1 that have been shown to be important for light-regulated genes, and putative homeobox protein-binding sites (Z Lin and D Grierson, unpublished data). Nevertheless, two recent publications have reported that ethylene biosynthetic genes are transcriptionally regulated by homeotic proteins (Ito et al., 2008; Lin et al., 2008a).

Lin et al. (2008a) identified a leucine zipper homeobox protein, LeHB-1, that transcriptionally modulates the expression of tomato LeACO1. The LeHB-1 protein contains 285 amino acid residues with a conserved homeo-domain (HD) and a leucine zipper domain (Zip), belonging to the class-I HD-Zip proteins. In vitro gel retardation assays showed that LeHB-1 binds to the LeACO1 promoter sequences that contain a 9 bp AATA(A)TATT or 10 bp AATA(A)ATT sequence with dyad symmetry and similarity to the putative homeobox protein-binding sites (Fig. 5B). Using virus-induced gene silencing (VIGS) to down-regulate LeHB-1 in tomato resulted in reduced
expression of LeACO1 mRNA and delayed ripening. These results indicated that LeHB-1 is a transcriptional activator that controls LeACO1 expression in vivo (Lin et al., 2008a). Other effects of altering LeHB-1 expression are discussed below.

Ito et al. (2008) reported that a tomato MADS-box protein LeMADS-RIN (RIN) binds specifically to the CaR box site of the LeACS2 promoter in chromatin immunoprecipitation PCR assays, but not the CaR box site found in LeACO1 and NR promoter sequences. The recessive rin mutation blocks the ripening process in tomato and results in fruit that fail either to produce high endogenous ethylene or to respond to exogenous ethylene and ripen (Vrebalov et al., 2002). RIN encodes a MADS-box transcription factor of the SEPALLATA clade, which controls the identity of floral organs in Arabidopsis. Although rin mutant fruit are responsive to ethylene (indicated by monitoring ethylene-inducible genes), the non-ripening phenotype is not changed by supplying exogenous ethylene (Knapp et al., 1989). Barry et al. (2000) showed that both LeACS2 and 4 transcripts were absent in the rin fruit. Based on those observations, it has been suggested that the RIN protein lies upstream of crucial ripening regulators (including ethylene production) and functions independently from ethylene (Giovannoni, 2007). The finding that the RIN protein binds to the LeACS2 promoter provides evidence that RIN directly controls ethylene biosynthesis in fruit.

There is mounting evidence that homeobox proteins are involved in a range of hormone-regulated processes (Scofield and Murray, 2006; Ariel et al., 2007). The KNOX protein and related proteins are known to be involved in transcriptional control of the synthesis of gibberellins and cytokinins (Sakamoto et al., 2001; Jasinski et al., 2002), and these factors regulate shoot apical meristem maintenance and organ initiation (reviewed by Baurle and Laux, 2003). The HD-ZIP proteins have also been reported to be involved in hormone and light regulation, and developmental process affecting shoot polarity and floral meristem activity (McConnell et al., 2001; Johannesson et al., 2003; Mallory et al., 2004; Saddic et al., 2006; Manavella et al., 2006). The HD-Zip proteins bind to specific DNA sequences as dimers (Sessa et al., 1993), and interactions between MADS-domain proteins are essential for MADS-box protein functions (Leseberg et al., 2008). Thus these homeotic regulatory proteins have the potential to form a large number of interacting complexes affecting hormone-related and developmental functions in vivo. The transcriptional regulation of ethylene biosynthesis by LeHB-1 and LeMADS-RIN provides new insight into floral organ promotion and perhaps sex determination (see discussions in later sections). Interestingly, the ethylene response factors (ERFs) have also recently been reported to regulate ethylene biosynthesis in tomato and tobacco (Nicotiana tabacum). Zhang et al. (2009) found that LeERF2/TERF2, which binds promoter elements in NtACS3 and LeACO3, was ethylene inducible, and ethylene production was suppressed in antisense LeERF2/TERF2 transgenic tomatoes, suggesting that LeERF2/TERF2 functions as a positive regulator in the feedback loop of ethylene induction.

**Ethylene signalling**

**Ethylene signalling pathway**

Although discovering the biosynthesis pathway of a signal molecule such as ethylene is a big step forward in understanding how it functions, it is also of critical importance to work out the molecular mechanism of the signal transduction pathway. Progress was slow for more than a quarter of a century until the establishment of Arabidopsis as the model plant and the development of molecular genetics technologies to identify ethylene mutants. The simplicity of the triple response to ethylene makes it ideal for quickly identifying ethylene signalling mutants, and the first breakthrough came with the isolation of a dominant ethylene-insensitive mutant, ethylene response 1 (ctr1), which encodes a two-component histidine kinase-like receptor that, in the mutated form, fails to bind ethylene (Bleecker et al., 1988; Chang et al., 1993; Schaller and Bleecker, 1995). Subsequently, the first tomato ethylene receptor, NR, was identified (Wilkinson et al., 1995). A second component of the pathway was also identified from a recessive mutant ctr1 (constitutive triple response 1) that displayed constitutive activation of ethylene responses in the absence of exogenous ethylene. Epistasis analyses have positioned CTR1 genetically downstream of the ethylene receptors (Fig. 3A), and CTR1 encodes a Raf-like serine/threonine kinase that can physically interact with the receptors (Kieber et al., 1993; Clark et al., 1998). Downstream of the receptors–CTR1 complex is EIN2 (ETHYLENE INSENSITIVE2), which is a positive regulator of the signalling pathway (Alonso et al., 1999). The N-terminus of EIN2 shares sequence homology with NRAMP ion transporters, but how EIN2 functions in ethylene signalling is not fully understood. Qiao et al. (2009) recently reported that EIN2 undergoes a rapid proteasome-mediated protein turnover and its accumulation is positively regulated by ethylene. The C-terminal domain of EIN2 interacts with two F-box proteins, ETP1 and ETP2 (EIN2 TARGETING PROTEIN), targeting it for degradation. A complex interplay between ethylene, ETP1 and 2, and degradation of EIN2 is suggested to trigger ethylene responses in Arabidopsis (Qiao et al., 2009). Kim et al. (2009) recently reported that EIN2, together with a NAC transcription factor ORE1 (Oresara means long-living in Korean) and the microRNA (miRNA) miR164 regulates ageing-induced programmed cell death in Arabidopsis. Within the nucleus, ethylene responses are amplified by a transcription factor cascade that includes ETHYLENE INSENSITIVE3 (EIN3), ETHYLENE RESPONSE FACTOR 1 (ERF1), and their homologues (Solano et al., 1998). Counterparts of these signal transduction components have now been identified in many other plants, but the complexity and subtlety of signalling mechanisms are still being established.
With these ethylene mutants at hand, a series of elegant genetic analyses have reconstructed a largely linear model (Fig. 3A) for the ethylene perception and signalling network that transduces the signal from the endomembrane receptor to the nuclear effector (Roman et al., 1995; Schaller and Kieber, 2002). Essentially, the ethylene receptors negatively regulate ethylene responses through CTR1. In brief, the receptors activate CTR1, which signals downstream to repress the ethylene response in the absence of ethylene. Ethylene binding leads to inactivation of the receptors and the inhibitory signal from CTR1 is then switched off. Evidence for a receptor inhibition model of ripening has also been demonstrated for the tomato ethylene receptor NR, indicating that negative regulation is a general mechanism for controlling ethylene responses (Hackett et al., 2000; see later). Upon inactivation of CTR1, EIN2 activates the ethylene response through downstream transcription factors such as EIN3 and other EIN3-like proteins (EILs), which subsequently regulate other ethylene-responsive genes in the transcription cascade. It has been suggested that the three tomato EILs are functionally redundant (Tieman et al., 2001), but Chen et al. (2004) showed that overexpressing LeEIL1 in the ethylene-insensitive ripening mutant Nr only restored expression of some ethylene-regulated genes, raising the possibility of distinct roles for specific EILs. Recent studies have revealed the complex and elaborate post-transcriptional regulatory mechanisms governing the EILs and EIN3 proteins in response to the ethylene signal (reviewed by Yoo et al., 2009). It has been shown that the EILs and EIN3 proteins are controlled by two F-box proteins (EBF1/2), which are Skp–Cullin–F-box (SCF) E3 ligases targeting EIN3 and EILs for 26S proteasome degradation (Guo and Ecker, 2003). Interestingly, the fates of the EBF1/2 mRNAs are also subjected to post-transcriptional regulation by a 5′ to 3′ exoribonuclease XRN4 (Olmedo et al., 2006). In addition, EIN3 protein stability can be further regulated by MAPK phosphorylation. Also, Yoo et al. (2008) have shown that the MAPK9–MAPK3/6 cascade phosphorylates the T174 and T592 sites in EIN3 and thus affects its stability. They have suggested that the simultaneous activation of the MAPKK9 cascade and the inhibition of the CTR1 pathway specify and quantitify control of EIN3 levels (see review by Yoo et al., 2009).

**Ethylene binding and the receptor signal output**

There are five ethylene receptors in *Arabidopsis*: ETR1, ERS1, ETR2, ERS2, and EIN4 (Schaller and Kieber, 2002), and six in tomato LeETR1–LeETR6 (Wilkinson et al., 1995; Lashbrook et al., 1998; Tieman and Klee, 1999) [LeETR3 is often called NEVER-RIPE (NR), for historical reasons]. All these receptors share a similar domain structure consisting of N-terminal transmembrane domains, followed by a GAF domain, and a C-terminal signal output domain related to the bacterial two-component histidine kinase. The ethylene binding activity of all receptors has been demonstrated through *in vitro* kinetic experiments. Expression of the N-terminal transmembrane domains of the receptor confirmed that they contained the ethylene-binding sites and that ethylene binding requires a copper cofactor (Schaller and Bleecker, 1995; O’Malley et al.,...
2005). Wang et al. (2006) reported that the ethylene-binding domain-like sequences are present in land plants, green algae Chlamydomonas spp., and some fungi and cyanobacterial species, and these species also possess ethylene binding activity, suggesting that ethylene perception is evolutionarily conserved. Burg and Burg (1967) had foreseen the presence of a copper ion in the ethylene receptor based on the metal affinity of compounds that have ethylene-like or ethylene antagonistic activities. Even before their molecular action was known, chemicals such as Ag(I), and later, 1-MCP (Reid and Staby, 2008) had been extensively used in horticulture and floriculture industries to block ethylene action (Abeles et al., 1992). Now, it has been shown that the ethylene receptor ETR1 dimer holds a single Cu(I) cofactor within the hydrophobic pocket formed by their N-terminal transmembrane domains, and the residues Cys65 and His69 are responsible for copper association (Rodriguez et al., 1999). Further insight was provided through characterization of the mutant responsive to antagonist1 (ran1), which is defective in a copper transporter with sequence homology to Menkes/Wilson P-type ATPase (Hirayama et al., 1999). This is supported by the observation that the loss-of-function (LOF) ranl-3 mutant (Woeste and Kieber, 2000) confers a constitutive ethylene response that can be partially rescued by supplying exogenous copper. However, the exact molecular mechanism of how RAN1 loads the copper cofactor and whether or not ethylene binding leads to a conformational change of the receptor remains largely unknown.

Additional ethylene receptor regulatory genes, the Arabidopsis REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1) and its tomato homologue GREEN-RIPE (GR) (Barry and Giovannoni, 2006; Resnick et al., 2006), have also been reported. The Arabidopsis RTE1 was initially discovered when screening for mutants that suppress etr1-2. The LOF mutant of rtel exhibited ethylene hypersensitivity, while overexpression of the functional RTE1 resulted in reduced ethylene sensitivity. In line with these observations, ectopic expression of GR inhibits tomato fruit ripening, which indicates that RTE1 and GR are negative regulators of ethylene signalling. Interestingly, a detailed genetic examination showed that rtel was only capable of suppressing a subset of etr1 alleles and there does not appear to be a direct correlation between rtel suppression and the ethylene binding ability of etr1 alleles (Resnick et al., 2008). Thus, it was proposed that RTE1 regulates a conformational switch of the receptors upon binding with ethylene, and this is independent of RAN1 function. Similarly, overexpression of tomato GR under control of the cauliflower mosaic virus (CaMV) 35S promoter led mainly to reduced ethylene responses in fruit and floral tissues, further suggesting that GR could function in ethylene signalling in a tissue-specific manner, or, alternatively, GR may be involved in modulating the signal output of specific ethylene receptors, which have tissue-specific expression or function (Barry and Giovannoni, 2006). The receptor tissue-specific model is supported by the fact that although the Arabidopsis ethylene receptors have been considered to be functionally redundant, the tomato ethylene receptors NR, LeETR4, and LeETR6 are preferentially expressed in fruit and have been suggested to have unique roles during ripening (Tieman et al., 2000; Kevany et al., 2007).

The role of histidine kinase in ethylene receptors

The C-terminal domains of the ethylene receptors show sequence homology to bacterial two-component system histidine kinases. In a classic two-component system, a membrane-associated sensor protein autophosphorylates its histidine kinase at a conserved histidine residue in response to environmental stimuli. The phosphoryl group is then transferred to an aspartate residue in the response regulator (Schaller et al., 2008). For example, phosphorylation can affect DNA binding activity of the response regulator to modulate downstream signalling. Sometimes a multistep phosphorylation is employed by using ‘hybrid histidine kinase’, which contains both histidine kinase and receiver domains. Based on their amino acid sequence similarity to the bacterial histidine kinase, the five Arabidopsis ethylene receptors can be classified into two subfamilies. Subfamily I receptors (ETR1 and ERS1) contain the conserved histidine kinase motifs, while subfamily II receptors (ETR2, ERS2, and EIN4) have a more diverged histidine kinase region lacking some canonical features required for histidine kinase activity (Schaller and Kieber, 2002). In addition, ETR1, ETR2, and EIN4 all possess an extra C-terminal receiver domain similar to the hybrid histidine kinase, while ERS1 and ERS2 lack receiver domains. It has been demonstrated that all receptors can autophosphorylate in vitro. However, only subfamily I receptors ETR1 and ERS1 can phosphorylate on the histidine residue, while the three subfamily II receptors and ERS1 have been reported to have serine/threonine kinase activity (Moussatche and Klee, 2004). In tomato, the subfamily I receptors LeETR1, LeETR2, and NR contain all the essential residues for histidine kinase function, whereas the subfamily II receptors, again, lack some residues that are normally conserved. Multiple LOF Arabidopsis ethylene receptor mutants have constitutive ethylene responses (Hua and Meyerowitz, 1998). However, gain-of-function (GOF) mutants of any individual ethylene receptors, including the subfamily II receptors that contain a degenerated histidine kinase domain, are insensitive to ethylene. This suggests that all receptors are capable of activating CTR1 to suppress downstream signalling. Therefore, the lack of a conserved histidine kinase motif and receiver domain in functional receptors may imply that canonical histidine kinase activity is not required for ethylene signalling (Hua and Meyerowitz, 1998; Wang et al., 2003). Additionally, it has been shown that transforming wild-type plants with a truncated ETR1-1 lacking the entire histidine kinase domain was sufficient to cause ethylene insensitivity (Gamble et al., 2002). This hypothesis was further supported by a cleverly designed experiment which used a point-mutated kinase-inactive form of ETR1 to rescue the double LOF etr1-7;ers1-2 mutant (Wang et al., 2003).
The histidine kinase domain of the ethylene receptors has been shown to be important for the association of the receptors with CTR1 (or CTR1-like proteins) from both Arabidopsis and tomato (Clark et al., 1998; Gao et al., 2003; Lin et al., 2008b). Qu and Schaller (2004) demonstrated that a truncated ETR1(1–349) lacking the histidine kinase and receiver domain failed to rescue a triple receptor LOF mutant (etr1-6;etr2-3;ein4-4), while the truncated ETR1(1–603) lacking only the receiver domain was able to restore normal growth of the triple mutant in air, but the plant then became hypersensitive to ethylene (Qu and Schaller, 2004). In addition, the kinase-inactive form of ETR1, which had been successfully used to rescue the double receptor LOF mutant by Wang et al. (2003), can only partially restore the triple receptor LOF mutant phenotype. Binder et al. (2004) showed that the receiver domain of the ethylene receptors plays a role in growth recovery after ethylene removal. They demonstrated that the LOF mutations in ETR1, ETR2, and EIN4 significantly prolonged the time for recovery of growth rate after ethylene was removed, while the ers1-2;ers2-3 double mutant had no effect on recovery rate. Cho and Yoo (2007) showed that the histidine kinase activity of the ETR1 ethylene receptor promotes plant growth. Together, these results suggest a very different model for receptor action in which both the histidine kinase and receiver domain are essential. The solution to this controversy could lie in the structure of the ers1-2 allele used to construct the double receptor LOF mutant (Wang et al., 2003). It contained a T-DNA insertion in the promoter region of ERS1, which turned out to be a hypomorphic allele (partial LOF) rather than a complete LOF (Qu et al., 2007). However, if the histidine kinase activity was essential, how could a truncated ETR1-1 lacking both a histidine kinase and receiver domain, referred to as ETR1-1(1–349), cause dominant ethylene insensitivity in the wild type? Xie et al. (2006) addressed this issue by comparing the dominant-negative effect of ETR1-1(1–349) in a two receptor double LOF mutant background: etr1-7;esr1-2 and etr1-7;ers1-3. Unlike the ers1-2 allele, ers1-3 contained a T-DNA insertion within the first exon of ERS1 and was a complete LOF mutant. The result showed that ETR1-1(1–349) was only functional in etr1-7;ers1-2 but not in etr1-7;ers1-3. This suggested that the truncated ETR1 without the histidine kinase domain must rely on the remaining ERS1, which has the histidine kinase activity, to repress ethylene signalling.

Evidence for a receptor heterodimerization model

The findings of Xie et al. (2006), however, raised another question as to whether ethylene receptors could interfere with each other’s signal output, because in the traditional ethylene signalling model, receptors form homodimers and interact with CTR1 through their histidine kinase and receiver domains to repress downstream signalling in the absence of ethylene. Moreover, it has previously been shown in both Arabidopsis and tomato that only subfamily I receptors were capable of interacting strongly with the downstream CTR1 and CTR1-like proteins in the yeast two-hybrid assay (Clark et al., 1998; Lin et al., 2008b; Zhong et al., 2008a). So was it possible that the truncated ETR1-1 and the subfamily II receptors could tap into the signalling output of the subfamily I receptors through direct protein–protein interaction?

Using a mating-based split-ubiquitin system, Grefen et al. (2007) first demonstrated that all ethylene receptors can form homo- and heterodimers in vitro in any combination, while the most crucial in vivo evidence supporting ethylene receptor heterodimerization came from co-immunoprecipitation assays using transgenic Arabidopsis expressing receptors fused to epitope tags (Gao et al., 2008). Interestingly, the interaction between ETR1 and the tagged ERS2 can be disrupted by SDS, which suggests that the ethylene receptors can exist as a higher order non-covalent complex. It was hypothesized that this interaction could be mediated by the GAF domain, since truncated receptors lacking this domain could not dimerize when expressed in yeast (Xie et al., 2006; Gao et al., 2008). At present, the exact function of the receptor histidine kinase domains and the role of the receptor heterodimer interaction in ethylene signalling are still open questions.

Subcellular localization of the ethylene receptor–CTR complex

The solubility in lipid and aqueous phases and the diffusible nature of ethylene gas makes it unnecessary for plants to restrict the ethylene receptors to a specific subcellular compartment to perceive the signal molecule. Chen et al. (2002) first showed that the Arabidopsis ethylene receptor ETR1 is localized to the endoplasmic reticulum (ER) membrane by a thorough study utilizing immunostaining electron microscopy and sucrose density gradient membrane fractionation. AtETR1 was also observed subsequently in the Golgi apparatus and co-localized with RTE1 in roots by immunostaining and fluorescence microscopy (Dong et al., 2008). On the other hand, the tobacco ethylene receptor NTHK1 was found to be in the plasma membrane (Xie et al., 2003), while melon CmERS1 and tomato NR, which are ERS1 type receptors lacking a receiver domain, have been found at the ER and the plasma membranes (Ma et al., 2006; Zhong et al., 2008a) and nuclear membranes (Lin et al., 2008c). Care should be taken, however, when interpreting the results of such experiments, which relied on overexpression of the target protein using a strong CaMV 3SS promoter. For example, transient overexpression of several fluorescent protein-tagged ethylene signalling components (LeEIN2, LeETR4, and AtRAN1) led to accumulation of fluorescence signals in the ER (Zhong et al., 2008b). To rule out the possibility that these observations were mislocalization artefacts caused by saturation of the endogenous secretion pathway, further studies are needed to investigate their localization under native conditions. The reports of multiple subcellular locations for ethylene receptors nevertheless raise the possibility of functional specialization and/or receptor movement in ethylene perception and signalling.
The N-termini of the ethylene receptors were predicted to be facing the extracytosolic space (the ER lumen) with the C-termini exposed to the cytosol, based on the topography of the melon CmERS1 ethylene receptor (Ma et al., 2006). The C-terminal domains of the Arabidopsis ethylene receptor ETR1 and ERS1 have direct protein–protein interaction with the N-terminus of the serine/threonine protein kinase CTR1, which is co-localized with the receptors to the ER membrane (Clark et al., 1998; Gao et al., 2003). Mutation of the conserved N-terminal CN motif in both the Arabidopsis CTR1 (Gao et al., 2003; Huang et al., 2003) and tomato CTR3 (Zhong et al., 2008a) can disrupt their interaction with the receptors and results in accumulation of free CTR proteins in the cytosol. In the case of the Arabidopsis ctrl-8, this point mutation within the CN-box can cause a constitutive ethylene response in air, a phenotype resembling the ctrl null mutation. This suggested that the interaction between ethylene receptors and CTR1, and possibly the subsequent recruitment of CTR1 to the ER membrane by this interaction, is essential for CTR1 function in repressing the downstream ethylene response. However, ethylene treatment does not change the association of CTR1 with the receptors, neither does it change its ER localization. In fact, ACC or ethylene treatment actually increases the amount of membrane-associated CTR1 (Gao et al., 2003), but the significance of this remains to be established. Thus the question as to how ethylene receptors regulate the activity of CTR1 remains unanswered. Knowing the importance of the histidine kinase activity of the ethylene receptors, one emerging hypothesis is that receptors might have multiple functions. First, receptors might form a stable complex with CTR1 irrespective of the ethylene binding status, and anchor CTR1 to a subcellular compartment(s), where CTR1’s downstream substrates are present, in a tissue-specific manner. Secondly, in the absence of ethylene, the receptors might utilize histidine kinase activity to phosphorylate CTR1, either directly or through a chaperone protein(s), which then activates CTR1 and suppresses the downstream ethylene response pathways. In this case, ethylene binding might induce a conformational change in the receptor, which diminishes its histidine kinase activity thereby relieving the repression of the downstream pathway.

The C-terminus of CTR1 has sequence homology to Raf-like kinases. It is well known that binding of phosphatidic acid (PA) to mammalian Raf-1 can lead to its translocation from the cytosol to the plasma membrane and that CTR1 can also bind to PA (Testerink et al., 2007). This new finding raised the question as to whether plant CTR proteins are regulated in a similar manner. Testerink et al. (2007) also showed that PA can interfere with the in vitro association between the receptor and CTR1 using purified recombinant protein fragments (ETR1129-729 and CTR1151-821). However, yeast two-hybrid experiments have previously shown that only the N-terminus of CTR1 can interact with the ethylene receptors (Clark et al., 1998); therefore, additional studies are needed to test the in vivo effects of PA on CTR1 activity and localization.

Tomato has multiple CTR1-like proteins and it has been shown that LeCTR1, 3, and 4 can partially or completely complement the Arabidopsis ctr1 mutant (Adams-Phillips et al., 2004), although it was not known which receptors bound to these multiple CT Rs. Using yeast two-hybrid assays, Zhong et al. (2008a) showed that these three tomato CTRs all interacted with the tomato subfamily I receptors, and they were also relocated to the ER when co-expressed with the NR receptor in onion epidermal cells. It is not yet clear, however, whether LeCTR1, 3 and 4 are functionally redundant or whether they have unique roles. A fourth sequence, LeCTR2, which has similarity to CTR1 and also to EDR1, an Arabidopsis protein involved in defence and stress responses, interacts preferentially with the subfamily I ETR1-type ethylene receptors LeETR1 and 2, but not with the ERS type, the NR receptor, or the subfamily II receptors (Lin et al., 2008b). Like all of the tomato CTR-like proteins, LeCTR2 has a conserved N-terminal motif and a C-terminal serine/threonine kinase domain that possesses kinase activity. Lin et al. (2008b) showed that overexpression of the LeCTR2 N-terminus in tomato resulted in altered growth habit, including reduced stature, loss of apical dominance, production of adventitious shoots from leaf rachis and rachillae, highly branched inflorescences and fruit trusses, and indeterminate shoots in place of determinate flowers (Fig. 4). The transgenic plants also displayed enhanced susceptibility or enhanced hypersensitive response to the fungal pathogen Botrytis cinerea (Fig. 4). Although there was no noticeable change in ethylene synthesis, levels of mRNAs from the ethylene-responsive genes E4 and chitinase B were higher than in the wild type, and pathogenesis-related (PR) gene transcripts also increased in abundance. Interestingly, Liu et al. (2002) using a VIGS system did not notice any effect of silencing LeCTR2, but this may have been related to their use of seedlings, whereas the main effects observed by Lin et al. (2008b) were in mature plants.

The binding of LeCTR2 to the ethylene receptors LeETR1 and LeETR2, plus the fact that overexpression of the N-terminus of LeCTR2 leads to enhanced accumulation of PR- and other ethylene-related mRNAs and altered hypersensitive response to infection, are all consistent with the conclusion that LeCTR2 is involved in aspects of ethylene signalling. The lack of any major effect on fruit ripening and triple response, however, indicates that the main role of LeCTR2 involves a specific branch of ethylene signalling (Lin et al., 2008b). It is not known whether the Arabidopsis disease resistance protein EDR1 binds ethylene receptors, but it has been suggested to function as a point of cross-talk between ethylene and salicylic acid signalling (Tang et al., 2005). Constitutive overexpression of the LeCTR2 N-terminus in tomato led to production of adventitious shoots from leaf rachis and rachillae and formation of leafy inflorescences, suggesting reduced auxin and enhanced cytokinin responses, in addition to the ethylene-related pathways. This may be due to the effects of persistent overexpression of the LeCTR2 N-terminus, and indicates that there is still much to be learned about
cross-talk between ethylene and other plant hormone bio-
synthesis and signalling networks (discussed further in the
following sections). It is noteworthy that overexpression of
a tomato tetra-tricopeptide repeat protein (SlTPR1), which
is believed to operate at the receptor level although it has
different receptor-binding characteristics compared with
LeCTR2, also produces striking but quite different effects
(Lin et al., 2008; Fig. 3B), suggesting it may predominantly
influence another part of the ethylene signalling network.

Post-translational regulation of ethylene receptors

In the current ethylene signalling model, receptors are
negative regulators. Therefore, ethylene sensitivity could be
increased if the receptors were eliminated or down-regu-
lated. This was supported by an early observation that
transgenic tomato with reduced ethylene receptor levels
ripened early, which was caused by increased ethylene
sensitivity (Tieman et al., 2000). Klee and his co-workers
demonstrated that tomato plants could use receptor degra-
dation to modulate ethylene responses (Kevany et al., 2007).
They also showed that receptor degradation requires
ethylene binding, since pre-treatment with the competitive
inhibitor 1-MCP could stabilize the receptors. Receptor
degradation can also be blocked by MG132, a peptide
aldehyde, which inhibits the chymotrypsin-like activity of
the 26S proteasome (Kevany et al., 2007), hinting that
ubiquitination is involved in receptor degradation. The

Fig. 4. Overexpression of the LeCTR2 N-terminus in tomato resulted in developmental abnormality and enhanced hypersensitive
response to Botrytis cinerea infection. (A) Production of adventitious shoots from leaf rachis and rachillae of plants overexpressing the
LeCTR2 N-terminus. (B) Formation of highly branched leafy fruit trusses resulting from overexpression of the LeCTR2 N-terminus. (C)
Increased hypersensitive response to B. cinerea infection of transgenic plants overexpressing the LeCTR1 N-terminus.
Arabidopsis subfamily II receptor ETR2 has also been shown to be degraded following treatment with 10 ppm ethylene (Chen et al., 2007). Protein ubiquitin-26S proteasome-associated degradation has already been shown to regulate ethylene biosynthesis (see previous sections). Interestingly, a tomato TPR protein SlTPR1 has been recently identified and shown to interact with the ethylene receptor NR and LeETR1 in yeast and in vitro (Lin et al., 2008c). Over-expression of this gene in plants causes a variety of phenotypes suggesting altered hormone responses. A related protein has been identified in Arabidopsis, and shown by in vivo co-immunoprecipitation to bind receptor ERS1 dimers in the cell membrane (Z Lin et al., unpublished data). SlTPR1 shows sequence homology to mammalian TCC1, an adaptor for receptor degradation, leading to enhanced ethylene sensitivity (Fig. 3B, right). Alternatively, it is also possible that SlTPR1 functions as an adaptor for receptor degradation, leading to enhanced ethylene sensitivity (Fig. 3B, right).

Ethylene and development

Ethylene has been reported to be involved in senescence (reviewed by Lim et al., 2007), abscission (reviewed by Lewis et al., 2006), root formation (Swarup et al., 2007; Ivanchenko et al., 2008; Negi et al., 2008), response to pathogens (reviewed by Kevin et al., 2002), and H₂O₂ production in response to ozone (Moeder et al., 2002), but a detailed consideration of all these processes is beyond the scope of this review, and this section focuses on regulation of ethylene in flower development, sex determination, and ripening.

Ethylene and flower development

The promotion of flowering by ethylene was first observed in pineapples in the 1930s, and it has become an important horticultural practice for production of pineapple and other bromeliads (Abeles et al., 1992). In Arabidopsis, mutants that either overproduce ethylene, such as eto1, 2, and 3, or constitutively switch on ethylene responses, such as ctr1, have reduced fertility (Bleecker et al., 1988; Guzman and Ecker, 1990; Kieber, 1993). In addition to being linked to flower senescence and abscission, it has been known for a long time that ethylene is also implicated in pollination and fertilization. In most species, pollination is accompanied by an increase in ethylene evolution in the stigma and style within hours after pollination and well before pollen germination, and also there is a burst of ethylene synthesis shortly after fertilization. These results are consistent with ethylene having a fundamental role in flower development, and many studies have indicated that ethylene, together with auxin, plays important roles in pollination-regulated developmental responses and participates in the inter-organ coordination of diverse components of pollination-regulated flower development (reviewed by O’Neill, 1997).

It has been proposed that flowering is triggered by a small burst of ethylene production in the meristem in response to environmental cues and, indeed, the pineapple (Ananas comosus) AcACS2 was shown to be induced in the meristem during induction of flowering (Trusov and Botella, 2006). In normal growth and development, the expression of ethylene biosynthesis genes appears to be related to development of particular floral organs. In Petunia (Petunia×hybrida), ACO3 and ACO4 were found to be specifically expressed in developing pistil tissue (Tang et al., 1994). In situ hybridization experiments revealed that ACO mRNAs were specifically localized to the secretory cells of the stigma and the connective tissue of the receptacle, including the nectaries. Treatment of young floral buds with ethylene led to the accumulation of ACO transcripts in cells surrounding the embryo sac of the ovules (Tang et al., 1994). Antisense suppression of the ethylene receptor PhETR2 in Petunia led to stomium degeneration and anther dehiscence before anthesis, indicating that PhETR2 regulates synchronization of anther dehiscence with flower opening (Wang and Kumar, 2007). In tomato, LeACS1A and LeACO1, 2, 3, and 4 genes were expressed in pistils with different patterns (Llop Tous et al., 2000). In tobacco, ACO transcripts were detected particularly in stigma, style, and ovary, but not in pollen and anthers, and during the early developmental stage, ACO expression occurred preferentially in the funiculus, the integument primordia, and the nucellus of the ovules (De Martinis and Mariani, 1999). The expression of mRNAs for specific ethylene receptors has also been shown to occur in particular cells. In Arabidopsis, the expression of the ethylene receptor ETR2 gene was higher in the inflorescence, floral meristems, and developing petals and ovules, suggesting a possible tissue-specific role for ETR2 (Sakai et al., 1998), and in tomato the NR receptor was developmentally regulated in floral ovaries and ripening fruit, with highly tissue-specific hormonal regulation (Payton et al., 1996; Lashbrook et al., 1998).

The precise timing of flowering can be controlled by multiple environmental and endogenous factors, and the formation and development of flowers involves a genetic network in shoot meristems that specifies floral identity. There is now increasing evidence, at least in some species, that ethylene is involved in this genetic network. At the centre of the network is the floral meristem identity gene LEAFY from Arabidopsis (FLORICAULA from Antirrhinum) (reviewed by Blazquez et al., 2006). LEAFY has been shown to bind specific sequences present in the promoters of the homeotic genes API and AGAMOUS (AG), which specify the identity of the two inner whorls in flowers. The tomato AG orthologue, TAG1, is carpel-specific and is expressed abundantly in ovules. Interestingly, ectopic expression of TAG1 in transgenic plants caused sepals to become fruit like (Pnueli et al., 1994), and the expression of TAG1 was shown to be suppressed by the ethylene inhibitor 1-MCP (Bartley and Ishida, 2007). Ando et al. (2001) showed that a MADS-box gene ERAF17 from cucumber was induced...
by treatment with ethephon, an ethylene-releasing compound, during the process of flower induction. ERAF17 belongs to the AG clade, with most similarity to TM8 from tomato. Thus the genetic network of floral organogenesis seems to involve ethylene (Fig. 7). Furthermore, Lin et al. (2008a) found that transient overexpression of LeHB-1 in tomato, which encodes a HD-Zip homeobox protein described above that binds the LeACO1 promoter, presumably via a 9 bp or 10 bp DNA sequence AATA (A/AA) TATT with dyad symmetry (Fig. 5A–C), resulted in production of carpels (or fruit-like structures) arising from the sepals (and occasionally the petioles), and multiple carpel formation within one sepal whorl (Fig. 5D). This is presumably because overexpressing LeHB-1 elevated ethylene production through its transcriptional activation of LeACO1, although it is likely that LeHB-1 also regulates other important genes.

Ethylene and sex determination

Ethylene-generating compounds such as ethrel [ethylene releaser (ethrel), also called ethephon] have been used to cause male sterility in wheat (Rowell and Miller, 1971; Hughes et al., 1974), and ethylene is known to play a critical role in sex determination of cucurbit species. In cucumber (Cucumis sativus), exogenous application of ethylene increases femaleans, and gynoecious genotypes (those that produce female flowers only) were reported to produce more ethylene (Iwahori et al., 1970). Two ACS genes, CsACS2 and CsACS1G, were correlated with female flower production (Trebitsh et al., 1997; Yamasaki et al., 2001). Kamachi et al. (1997) reported that both the timing and the levels of expression of the CsACS2 transcript were correlated with the development of female flowers. In gynoecious plants and under female inductive conditions, CsACS is expressed in pistil primordia, whereas in monococious plants (that have separate male and female flowers on the same plant), the CsACS transcripts are reduced and accumulate below the pistil primordia on the adaxial side of the petals (Kamachi et al., 1997). Furthermore, the timing of the induction of expression of the CsACS2 gene at the apex corresponded to the timing of the action of ethylene in the induction of the first female flower at the apex of individual gynoecious cucumber plants. Yamasaki et al. (2000) also reported that the mRNAs of cucumber ethylene receptors CsETR2 and CsERS accumulated more in the shoot apices of the gynoecious cucumber than those of the monococious type. Their expression patterns correlated with the expression of the CsACS2 gene and with ethylene evolution in the shoot apices of the two types of cucumber plants, and accumulation of CsETR2 and CsERS mRNA was

Fig. 5. LeHB-1 modulation of floral organogenesis in tomato via transcriptional regulation of LeACO1 expression. (A) A simplified structure of LeHB-1 protein with amino acids numbered. HD, homeodomain; Zip, leucine zipper domain. (B) Putative cis-element AATA(AA)TATT present in the F1-1 and F4-1 regions of the LeACO1 promoter, which bind LeHB-1 in vitro. (C) In vitro gel retardation assay showing that the recombinant GST-LeHB-1-HD-Zip polypeptide retarded migration of the F1-1 and F4-1 fragments (lanes 2 and 6), whereas the GST control did not (lanes 3 and 7). A 200-fold excess of unlabelled competitor was able to compete the binding (lanes 4 and 8). Lanes 1 and 5: free probe. (D) Developmental abnormalities caused by ectopic LeHB-1 expression including development of multiple flowers or double carpel-like structures within one sepal whorl (panels 1 and 2), formation of fruits from sepals (panels 3 and 4) and petioles (panel 5), compared with the control (panel Ctl). These effects were only observed with a full-length LeHB-1-coding sequence and were not seen using a construct with an engineered premature stop codon (Lin et al., 2008a).
significantly elevated by the application of ethrel to the shoot apices of monoeocious cucumber plants. Thus, there is a strong possibility that the induction of femaleness by ethylene in cucumber plants is related to regulatory effects of ethylene on expression of specific floral organ identity genes, although this remains to be determined.

In contrast to its feminizing effect in cucumber, in watermelon (Citrullus spp), ethylene promotes male flower development. Salman-Minkov et al. (2008) reported that among four ACS genes (CitACS1–CitACS4) from watermelon, CitACS1 and CitACS3 were expressed in floral tissue and CitACS1 was also expressed in vegetative tissue. Expression of CitACS1 was up-regulated by exogenous treatment with auxin, GA, or ACC. No discernible differential floral sex-dependent expression pattern was observed for this gene; in contrast, the CitACS3 gene was expressed in open flowers and in young staminate floral buds (male or hermaphrodite), but not in female flowers. CitACS3 was also up-regulated by ACC, and was thus thought likely to be involved in ethylene-regulated anther development.

Andromonoecy is a widespread sexual system characterized by plants carrying both male and bisexual flowers. In melon (Cucumis melo), this sexual form is controlled by the identity of the alleles at the andromonoecious (a) locus. Cloning of the a gene by Boualem et al. (2008) revealed that andromonoecy results from a single missense mutation, A57V, in the active site of CmACS-7. A57 is conserved in andromonoecy results from a single missense mutation, similar activity. At physiological PLP concentrations Boualem et al. (2008) reported that among 

\[ \text{expression level and pattern were not different between female and hermaphrodite flowers and because the loss of CmACS-7 activity accounts for the functional variation, they concluded that CmACS-7-mediated ethylene production in the carpel primordia affects the development of the stamens in female flowers but is not required for carpel development.} \]

Some bacteria and fungi also produce ethylene, although its role in these organisms is less well understood. It was recently reported that ethylene plays an important role in zygote formation in the cellular slime mould Dictostelium mucoroides (Amagai et al., 2007). Dictostelium mucoroides-7 (Dm7) and a mutant (MF1) derived from it shows developmental dimorphism, depending upon environmental conditions: macrocyst formation occurs during the sexual cycle, and sorocarp formation during the asexual process. Amagai et al. (2007) reported that exposure of cells to ethylene favours macrocyst formation, while exogenously added 3', 5'-cAMP induces sorocarp formation. Furthermore, AOA (arnimyox-acetic acid, an inhibitor of ethylene synthesis), was found to switch development of Dm7 and MF1 cells from macrocyst to sorocarp formation. This raises the intriguing question of whether ethylene has a primitive role in reproduction and sex determination that has been conserved through evolution.

**Ethylene and fruit ripening**

The economic importance of fruit has served as an incentive to study ripening biochemistry, and an understanding of the physiological responses of ripening to ethylene was well established as early as the 1930s (Abeles et al., 1992). Increased respiration and a burst of ethylene biosynthesis were found in some fruit during ripening, such as tomato, avocado, apple, and banana, which were then classified as climacteric fruit. In contrast, some fruit showed no increase in respiration and ethylene production during ripening, such as strawberry, grape, and citrus, and were classified as non-climacteric fruit. In general, fruit with the highest respiration rates, such as banana and avocado, tend to ripen most rapidly, and for non-climacteric fruit the general correlation exists between high respiration and short shelf life (Tucker and Grierson, 1987; Tucker, 1993). The increase in ethylene production associated with the respiratory increase is autocatalytic and at least in some fruits there is clear evidence that ethylene causes the climacteric. McMurchie et al. (1972) explained this autocatalytic rise by proposing that two systems (system-1 and system-2) were involved in ethylene biosynthesis (reviewed by Leelievre et al., 1998). System-1 functions during normal vegetative growth, is autoinhibited by ethylene, and is responsible for producing the basal levels of ethylene synthesized by all plant tissues, including non-climacteric fruit. System-2 comes into play during the ripening of climacteric fruit and during petal senescence and, as we now know, this requires the induction of new ACS and ACO isoforms. Members of both gene families show ripening-related expression, and the accumulation of their mRNAs is stimulated by ethylene (Barry
Barry et al. (2000) proposed that system-1 ethylene is regulated by the expression of LeACS1A and 6, and that during the transition from system-1 to system-2 the RIN gene is involved in causing increased expression of LeACS1A and induction of LeACS4. The maintenance of system-2 ethylene production is due to the ethylene-dependent induction of LeACS2 (Barry et al., 2000), which Ito et al. (2008) subsequently showed is regulated by RIN (called LeMADS-RIN by Vrebalov et al., 2002). This autocatalytic ethylene synthesis initiates enhanced expression of a cascade of ripening genes that affect colour, flavour, texture, aroma, and taste (reviewed by Gray et al., 1994; Lelievre et al., 1998; Alexander and Grierson, 2002; Giovannoni, 2004).

Direct evidence for ethylene synthesis being essential for climacteric fruit ripening was established by experiments.
showing that reduction in the expression of tomato ACS2 and ACO1 in planta by antisense genes inhibited or delayed ripening (Oeller et al., 1991; Picton et al., 1993). Furthermore, it was also shown that the Nr mutant phenotype of tomato was caused by a dominant mutation in the NR ethylene receptor. This abolishes ethylene binding to the receptor and results in tomato plants that are insensitive to ethylene and produce non-ripening fruit (Wilkinson et al., 1995). Because the mutant NR receptor cannot bind ethylene, it continues to maintain an active dominant suppression of ethylene responses through its interaction with the tomato CTR proteins. Antisense inhibition of production of the mutant mRNA in the Nr mutant resulted in failure to synthesize the mutant receptor protein, and partially or completely restored ripening (Hackett et al., 2000), confirming that the receptor inhibition model of ethylene signalling applies to ripening as well as the triple response. In addition, reduction of the expression of the tomato ethylene receptors LeETR4 or LeETR6 elevated ethylene production and promoted ripening (Tieman et al., 2000; Kevany et al., 2007). Thus climacteric fruit ripening is also controlled at the receptor level.

Recently, the identification of several key ripening regulatory genes from tomato, such as MADS-box RIN (Vrebalov et al., 2002), SBP-box Colourless Non-Ripening (CNR) (Manning et al., 2006), and leucine zipper homeobox LeHB-1 (Lin et al., 2008a), has led to new insights into understanding of ethylene and ripening control mechanisms (see previous sections). Cnr is an epigenetic change that alters the promoter methylation of a SQUAMOSA promoter-binding (SPB) protein, resulting in a pleiotropic ripening inhibition phenotype and inhibited expression of ethylene-related genes ACO1, E8, and NR, and a range of ripening-related genes (Thompson et al., 1999). The MADS-box protein RIN and the homeobox protein LeHB-1 bind to the LeACS2 and LeACO1 promoters, respectively, and inhibition of their expression in tomato fruit results in delayed ripening (see previous sections).

Ripening control in non-climacteric fruit was originally thought to be independent of ethylene. Less is known about the mechanisms, but ethylene can affect non-climacteric fruit; for example, ethylene stimulates de-greening of citrus and there is recent evidence for small changes in ethylene biosynthesis genes and the involvement of ethylene in ripening of non-climacteric fruit. More recently, evidence has been accumulating for a common genetic regulatory mechanism that controls climacteric and non-climacteric ripening. Trainotti et al. (2005) reported that the non-climacteric fruit strawberries (Fragaria × ananassa) produced ethylene during ripening, although in limited amounts, and they also found a correlation between the expression of the strawberry FaACO1 and ethylene production, as found in climacteric fruit ripening. This challenges the concept that non-climacteric fruit ripening is independent of ethylene. Fei et al. (2004) compared the ripening gene profiling of climacteric tomato fruit and non-climacteric grapes and showed that the two species shared a subset of common ripening regulators, including members of the MADS-box, zinc finger, and bZIP transcription factor families, suggesting that these genes represent primitive functions that have been conserved through evolution for ripening control, and are active in both climacteric and non-climacteric fruits.

It is appropriate to consider ripening as the final phase in the continuous process of flower development and reproduction. Vriezen et al. (2008) studied the effects of hormones on the transcriptomic profile of tomato ovary after pollination/fertilization using two complementary approaches: cDNA-amplified fragment length polymorphism and microarray analysis. The results suggested that ethylene, together with other hormones, played an important part in fruit set and ovary development. Ishida et al. (1998) demonstrated that the floral organ identity genes, such as TAG1, were also involved in the ripening process. Sepals of the tomato cultivar VFNT Cherry, when cultured in vitro between 16 °C and 22 °C, changed their genetic programme to that of ripening fruit. This cool temperature-induced sepal morphogenesis process (referred to below as ‘ripening’ sepal) appeared to involve a number of regulatory genes, including TAG1, TDR4, a HD-Zip gene VaHOX1, and a ripening regulator NOR (NON-RIPENING), which is a NAC (No Apical Meristem-domain) transcription factor (Giovannoni, 2004). A mutation in this gene caused retardation in tomato fruit ripening similar to that found for the rin mutation. Although NOR has been suggested to function independently of ethylene in the tomato ripening process (Adams-Phillips et al., 2004; Giovannoni, 2004), Bartley and Ishida (2007) showed that the accumulation of NOR mRNA was affected by blocking ethylene perception with 1-MCP in cold-induced ‘ripening’ sepal. Sepals treated with 1-MCP produced only 10% ethylene compared with untreated sepal and did not undergo the expression of ripening genes; the transcripts of TAG1 and TDR4 in 1-MCP-treated sepal were also greatly repressed compared with untreated sepals, suggesting they may function downstream of ethylene (Bartley and Ishida, 2007). In contrast, the expression of VaHOX1 was not suppressed by 1-MCP, suggesting that it is either upstream of, or independent from, ethylene. As discussed above, another HD-Zip protein, LeHB-1, stimulates ethylene synthesis by activating transcription of LeACO1 whereas RIN activates LeACS2. Interestingly, the putative LeHB-1-binding site is also found in the promoter of RIN (Z Lin, unpublished data), although whether LeHB-1 transcriptionally regulates RIN in vivo requires investigation. It therefore seems that a genetic network involving ethylene and homeotic regulators may control plant reproduction, particularly floral sex determination, fruit development, and ripening (Fig. 7).

Ethylene cross-talk with other phytohormones

Ethylene regulates many aspects of plant developmental processes, and it is clear that the diversity of ethylene functions is achieved, at least in part, by its interactions.
production. NOR are known to regulate ripening, but it is not yet clear whether homeotic regulatory proteins, including LeHB-1, RIN, TAG1, and governing flower development and reproduction in tomato.

Curvature (Guzman and Ecker, 1990; Kieber and Theologis, 2004; Salman-Minkov species, auxin induces a subset of ACS genes (Tsuchisaka et al., 1995) increases the rate of ethylene production, for example in etiolated mung-beans (Grierson et al., 2002). Auxin can reduce the ability of ethylene to accelerate ageing-regulate the activities and levels of the other. For example, auxin [IAA, 2,4-D, and naphthaleneacetic acid (NAA)] also auxin can reduce the ability of ethylene to accelerate ageing-regulate the activities and levels of the other. For example, auxin increase (Stepanova et al., 2005). Ruzicka et al. (2007) also demonstrated that the effect of ethylene on root growth was largely mediated by the regulation of auxin biosynthesis and transport-dependent local auxin distribution. They found that in roots of intact seedlings treated with ACC, the activity of the synthetic auxin response peptide DR5 increased >4-fold compared with untreated seedlings. They also showed that ethylene modulated the capacity of basipetal auxin transport via auxin efflux and influx components, including PIN1, PIN2, PIN4, and AUX1.

In tomato, cross-talk between ethylene and auxin was reported to be related to ethylene receptor levels. Whitelaw et al. (2002) showed that auxin movement in tomato plants was inhibited by the reduction in LeETRI transcript levels. This is consistent with the observation that enhanced ethylene responses hinder auxin transport. Also, Lin et al. (2008c) have suggested that in tomato, cross-talk between ethylene and auxin signalling involves SITPR1, which binds to tomato ethylene receptors NR and LeETR1 (Fig. 3B; see previous section). Overexpression of 35S::SITPR1 in vivo results in a range of developmental abnormalities related to ethylene and auxin, including dwarf plants, epinasty, degenerated flowers and infertility, parthenocarpic fruit, altered leaf and fruit morphology, and altered abscission (Lin et al., 2008b). Furthermore, overexpression of SITPR1 not only produced parthenocarpic fruit and altered morphology of fruit and leaves, but also altered the expression of early auxin response genes LeIAA9 and SISAURO-LIKE (Fig. 3B). The LeIAA9 mRNA is known to show reduced

Ethylene and auxin interactions

Early observations showed that ethylene and auxin can each regulate the activities and levels of the other. For example, auxin can reduce the ability of ethylene to accelerate age-dependent processes such as ripening and abscission. Auxin [IAA, 2,4-D, and naphthaleneacetic acid (NAA)] also increases the rate of ethylene production, for example in etiolated mung-beans (Grierson et al., 1982). In many plant species, auxin induces a subset of ACS genes (Tsuchisaka and Theologis, 2004; Salim-Minkov et al., 2008). Artieca and Artieca (2008) observed that different parts of Arabidopsis plants produced various levels of ethylene in response to IAA treatment, with the highest production by inflorescence stalks. Leaf age also had an effect on IAA-induced ethylene, with the youngest leaves showing the greatest stimulation. The highest amount of IAA-induced ethylene occurred in the root or inflorescence tip, with regions below this producing less, suggesting that ethylene and auxin synergistically regulate these developmental processes.

The apical hook of etiolated dicotyledonous seedlings results from asymmetric growth of its inner and outer sides, and this process is ethylene dependent (Raz and Ecker, 1999). Etiolated wild-type seedlings grown in ethylene, the ethylene overproduction mutant eto, and the constitutive ethylene response mutant ctrl all exhibit exaggerated hook curvature (Guzman and Ecker, 1990; Kieber et al., 1993). Auxin has also been shown to regulate seedling apical hook development. Wild-type Arabidopsis seedlings grown in the presence of auxin or the auxin transport inhibitor 1-naphthylphthalamic acid (NAP) display no hook, and the auxin transport mutant aux1 also disrupts hook formation (Roman et al., 1995). The Arabidopsis ‘hookless’ mutant phenotype is caused by a mutation in HOOKLESS1 (HLS1), which encodes an N-acetyltransferase (Lehman et al., 1996). Lehman et al. showed that the abundance of HLS1 mRNA was increased by treatment with ethylene and decreased in the ethylene-insensitive mutant ein2. Furthermore, the morphology of the hookless hypocotyl was phenocopied by inhibitors of auxin transport and by high levels of endogenous or exogenous auxin.

Interactions of ethylene signalling with the auxin biosynthesis pathway have been revealed by the characterization of two root-specific ethylene-insensitive mutants Weak Ethylene Insensitive2/Anthranilate Synthase alpha1 (WEI2/ASA1) and WEI7/Anthranilate-Late Synthase beta1 (ASB1) in Arabidopsis (Stepanova et al., 2005). Ethylene-triggered inhibition of root growth in Arabidopsis seedlings is mediated by the action of the WEI2 and WEI7 genes that encode α- and β-subunits of a rate-limiting enzyme of tryptophan biosynthesis, anthranilate synthase. Up-regulation of WEI2/ASA1 and WEI7/ASB1 by ethylene results in the accumulation of auxin in the tip of the primary root, whereas LOF mutations in these genes prevent the ethylene-mediated auxin increase (Stepanova et al., 2005). Ruzicka et al. (2007) also demonstrated that the effect of ethylene on root growth was largely mediated by the regulation of auxin biosynthesis and transport-dependent local auxin distribution. They found that in roots of intact seedlings treated with ACC, the activity of the synthetic auxin response peptide DR5 increased >4-fold compared with untreated seedlings. They also showed that ethylene modulated the capacity of basipetal auxin transport via auxin efflux and influx components, including PIN1, PIN2, PIN4, and AUX1.

Fig. 7. A potential genetic regulatory network centred on ethylene governing flower development and reproduction in tomato.

Ethylene-modulated flower/fruit development and ripening involves homeotic regulatory proteins, including LeHB-1, RIN, TAG1, and TDR4 (see text for discussion). The transcription factors CNR and NOR are known to regulate ripening, but it is not yet clear whether they are directly involved in modulating development or ethylene production.

with other hormone signalling pathways. The interactions between ethylene, jasmonic acid, and salicylic acid signalling have been reviewed elsewhere (Wang et al., 2002); the interactions with other hormones are discussed below, and have also been reviewed by Yoo et al. (2009).
expression in the presence of ethylene (Jones et al., 2002), and antisense inhibition of LeIAA9 results in altered leaf morphology and parthenocarpic fruit (Wang et al., 2005), as found in SMEPLR1-overexpressing tomato plants (Lin et al., 2008b).

Regulation of ACS by cytokinin and brassinosteroid

Other hormones are known to elevate ethylene biosynthesis, in addition to auxin, such as cytokinins and brassinosteroids (Woeste et al., 1999; Yi et al., 1999; Arteca and Arteca, 2008). Auxin treatment results in an increase in the level of several ACS transcripts, while cytokinin has been shown to increase ACS5 protein stability (Li et al., 1992; Abel et al., 1995; Vogel et al., 1998; Woeste et al., 1999; Chae et al., 2003; Yamagami et al., 2003; Tsuchisaka and Theologis, 2004; Wang et al., 2004). Hansen et al. (2009) showed that cytokinins increased the stability of a subset of ACS genes. The induction of ethylene by cytokinin requires the canonical cytokinin two-component response pathway, including histidine kinases, histidine phosphotransfer proteins, and response regulators. With cytokinin, brassinosteroid also acts post-transcriptionally by increasing the stability of ACS5 protein. In mung bean (Vigna radiata) hypocotyls, the brassinosteroid (BR) 2,4-epibrassinolide, specifically enhanced the expression of VrACS7, and BR also synergistically increased the IAA-induced VR-ACS6 and VrACS7 transcript levels (Yi et al., 1999). These data suggest that ACS is regulated by phytohormones through different regulatory inputs that probably act together to adjust ethylene biosynthesis continuously in various tissues and in response to various environmental conditions.

Ethylene cross-talk with GA signalling

GA is essential for plant growth and development processes. Calvo et al. (2004) reported that regulatory cross-talk involving ethylene and GA affects the transition from seed dormancy to germination in common beech (Fagus sylvatica L.) seeds. They observed a drastic increase in FsACO1 expression when seeds were treated with GA3 or ethephon, but the stimulatory effect of ethephon was reversed by paclobutrazol, a GA biosynthesis inhibitor, suggesting that GA positively regulated the expression of FsACO1. GA perception is mediated by the receptor GID1 (GA-INSENSITIVE DWARF1) and DELLA proteins. In Arabidopsis, the DELLA family comprises GAI, RGA (repressor of ga1-3), RGL1, RGL2, and RGL3. A key event in GA signalling is the degradation of DELLA proteins, which are negative regulators of the GA response that interact with GID1 in a GA-dependent manner. DELLA proteins also have roles in maintaining GA homeostasis and regulating cross-talk between GA and other plant hormones (Hirano et al., 2008). In the presence of GA, the DELLA proteins undergo degradation, which de-represses downstream responses. Acharnt et al. (2003) observed that ethylene delayed the GA-mediated disappearance of GFP–RGA from root cell nuclei via CTR1-dependent signalling, suggesting that ethylene stabilizes the DELLA proteins. Similarly, it has also been reported (Acharnt et al., 2007) that ethylene controls floral transition via DELLA-dependent regulation of floral meristem identity genes. Activation of ethylene signalling reduces bioactive GA levels, thus promoting the accumulation of DELLA proteins. DELLA accumulation in turn slows the plant life cycle and delays flowering via repression of the floral meristem identity genes LEAFY and SOC1 (SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1) (Acharnt et al., 2007).

Concluding remarks

Synchronizing flowering time, flower development, and seed dispersal with the environment are of great importance for plant competition and survival. Regulating appropriate responses to biotic and abiotic environmental stresses are also of critical importance. Ethylene is a key regulatory and signalling molecule in all of these processes and contributes to a plant’s fitness for survival. It is stimulating and challenging to consider, 150 years after the publication of Charles Darwin’s ‘On the origin of species’, and 200 years after his birth, that the time may not be far off when we are able to pinpoint the evolutionary origin of ethylene as regulator and hormone.

Ethylene mutants, epistasis analysis, and biochemical characterization have provided us with the knowledge to draw linear pictures of the ethylene biosynthesis and signalling pathways, their regulation, and interactions with other hormones. Arabidopsis has provided the key to unlocking the ethylene black box, but important insights into flower and fruit development and ripening have come from other species. That we are really dealing with a regulatory net, rather than a pathway, has been suspected for a long time, and this is now becoming clear. However, there are still many unresolved aspects of the network. First, at present we know about three transcriptional regulators for ACS and ACO genes (LeHB-1, RIN, and LeERF2) but there must surely be many more to discover that control the 15 or more members of these two gene families expressed in different tissues and organs and in response to various environmental and hormonal signals. Secondly, many details of the precise functions and interactions of the different ethylene receptors, and the multiple CTRs in species such as tomato, remain to be elucidated. Several lines of evidence point to the possible existence of separate ethylene signalling channels, based on the different receptors, multiple CTRs, other receptor-interacting proteins, and the differential expression of some of these components. The possibility that different protein complexes may regulate separate aspects of the ethylene response requires further investigation. Thirdly, it is clear that ethylene regulates flower organ development, as well as fruit ripening. A potential genetic network involving ethylene and homeotic proteins, including HD-Zip (such as LeHB-1 and TM1) and MADS-box proteins (such as RIN
TAG1), may regulate this fundamental developmental process, but the interactions between these parts of the network and other flower organ identity genes require further detailed study. Fourthly, although the regulatory networks are emerging, and we see roles for control of transcription, mRNA and protein degradation, and post-translational modification of proteins, it is not at all clear how the various parts of the network communicate with each other. Finally, ethylene a stress hormone, a developmental signal, or both, and is there an evolutionary link to reproduction as suggested by the results with Dictyostelium? Addressing these questions will bring us closer to understanding the function of this simple hormone with such a complex lifestyle.

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