Research review

The regulation of ethylene biosynthesis: a complex multilevel control circuitry

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Summary

The gaseous plant hormone ethylene is produced by a fairly simple two-step biosynthesis route. Despite this pathway's simplicity, recent molecular and genetic studies have revealed that the regulation of ethylene biosynthesis is far more complex and occurs at different layers. Ethylene production is intimately linked with the homeostasis of its general precursor \(S\)-adenosyl-L-methionine (SAM), which experiences transcriptional and posttranslational control of its synthesising enzymes (SAM synthetase), as well as the metabolic flux through the adjacent Yang cycle. Ethylene biosynthesis continues from SAM by two dedicated enzymes: 1-aminocyclopropane-1-carboxylic (ACC) synthase (ACS) and ACC oxidase (ACO). Although the transcriptional dynamics of ACS and ACO have been well documented, the first transcription factors that control ACS and ACO expression have only recently been discovered. Both ACS and ACO display a type-specific posttranslational regulation that controls protein stability and activity. The nonproteinogenic amino acid ACC also shows a tight level of control through conjugation and translocation. Different players in ACC conjugation and transport have been identified over the years, however their molecular regulation and biological significance is unclear, yet relevant, as ACC can also signal independently of ethylene. In this review, we bring together historical reports and the latest findings on the complex regulation of the ethylene biosynthesis pathway in plants.

Introduction to the ethylene biosynthesis pathway

The volatile hormone ethylene is a major regulator of many developmental and physiological responses in plants (Abeles et al., 1992). As a gas, ethylene quickly diffuses from sites of production, where it can be perceived, but is not modified nor metabolised. As such, precise regulation of ethylene biosynthesis is crucial. This is achieved by transcriptional and posttranslational regulation of the ethylene biosynthesis enzymes and the adjacent Yang cycle, and by transport and conjugation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). Although ACC was first identified in plants in the 1950s (Burroughs, 1957; Vahatalo & Virtanen, 1957), only recently new evidence has been gathered which showed that ACC also acts as a signalling molecule in plants, independently of ethylene. ACC has been shown to regulate cell wall metabolism (Xu et al., 2008; Tsang et al., 2011), guard cell differentiation (Yin et al., 2019), vegetative development (Tsuchisaka et al., 2009; Vanderstraeten et al., 2019) and pollen tube attraction (Mou et al., 2020) in Arabidopsis (Arabidopsis thaliana). These new insights add weight to the importance of a careful regulation of ACC homeostasis due to its dual role in controlling ethylene biosynthesis and ACC signalling.

The ethylene biosynthesis pathway is relatively simple, taking place via only two committed enzymatic reactions (Fig. 1). In the first step, the substrate \(S\)-adenosyl-L-methionine (SAM) is converted to ACC and 5'-methylthioadenosine (MTA) by the enzyme ACC synthase (ACS) (Adams & Yang, 1977, 1979; Boller et al., 1979). In the second step, ACC is converted to ethylene, CO\(_2\) and cyanide, by the enzyme ACC oxidase (ACO) (Hamilton et al., 1991; Ververidis & John, 1991). The toxicity of the cyanide by-product is rapidly dealt with by conversion to \(\beta\)-cyanoalanine (Yip & Yang, 1988) by a group of \(\beta\)-cyanoalanine synthases (Hatzfeld

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et al., 2000). Because ACS and ACO are the only two enzymes dedicated to ethylene biosynthesis, much of the regulation of overall ethylene production occurs by manipulating transcription, translation and protein stability of these two enzymes. Additionally, metabolic regulation of ethylene production is achieved by ACC homeostasis, which encompasses ACC biosynthesis, transport and conjugation.

**Regulation of the dedicated ethylene biosynthesis enzymes ACS and ACO**

ACS and ACO exclusively operate in the ethylene biosynthesis pathway. Both enzymes appear to operate in the cytosol (Boller et al., 1979; Reinhardt et al., 1994; Chung et al., 2002; Hudgins et al., 2006), however other studies have suggested that ACO could also be associated with the plasma membrane (Rombaldi et al., 1994; Ramassamy et al., 1998). ACS belongs to the family of pyridoxal-5'-phosphate (PLP) dependent aminotransferases, which require vitamin B6 as a co-factor for activity. ACO is a member of the 2-oxoglutarate-dependent dioxygenase (2OGD) superfamily, which require ferrous iron (Fe²⁺) as the active-site co-factor, and 2OG and molecular oxygen as co-substrates for activity. ACO is unique within this family because it uses ascorbate, not 2OG, as a co-substrate (Kawai et al., 2014). Both ACS and ACO belong to multigene families in most plants (Booker & DeLong, 2015; Houben & Van de Poel, 2019) and their activity can be spatially and temporally separated, allowing precise control of ethylene production. Under normal basal levels of ethylene production, it is generally thought that ACS catalyses the rate limiting step in biosynthesis (Adams & Yang, 1979). However, under certain conditions ACO activity is the rate limiting step (Houben & Van de Poel, 2019). Furthermore, ACS and ACO show tissue specific expression and localisation patterns (Rodrigues-Pousada et al., 1993; Blume & Grierson, 1997; Wang et al., 2005; Datta et al., 2015; Park et al., 2018), indicating that both ACS and ACO are under tight regulatory control, which takes place at the transcriptional, posttranscriptional and posttranslational level. For more insights on the chemical control of ACS and ACO, we refer to a recent review of Depaepe & Van Der Straeten (2019).

**Transcriptional regulation of ACS**

Early work on the discovery of several ACS clones in different species (Sato & Theologis, 1989; Nakajima et al., 1990; Van Der Straeten et al., 1990), revealed that ACS is a multigene family which is differentially expressed in plants. For example, differential expression of four tomato (Solanum lycopersicum) ACS genes was found to be necessary for proper navigation of the transition from autoinhibitory (system I) to autocatalytic (system II) ethylene production during tomato fruit ripening (Barry et al., 2000). The first transcription factor identified to regulate expression of ACS was the MADS box transcription factor SIRIN, which directly enhances expression of some, but not all, of the tomato ACS (Ito et al., 2008). Many other transcription factors which regulate expression of ACS have been identified in recent years (summarised in Table 1). Both positive and negative regulators of ACS expression exist and regulate numerous developmental processes. There is also evidence for direct regulation of ACS transcription by ethylene. For example, the tomato ethylene response factor SIERF2/TERF2 was shown to interact with the promoter of NaACS3 when expressed in tobacco (Nicotiana tabacum), inducing its expression (Zhang et al., 2009). However, the indirect regulation of ACS by ethylene signalling may be more important. Auxins, cytokinins, brassinosteroids, jasmonates and abscisic acid are all known to regulate ethylene biosynthesis (Yang & Hoffman, 1984; Rivo et al., 1990; Vogel et al., 1998). While hormonal regulation of ethylene biosynthesis has been well documented for decades, the identification of the hormone regulated transcription factors which directly modulate ACS expression has been more challenging.

**Posttranslational regulation of ACS**

As well as the transcriptional, the posttranslational regulation of ACS is crucial for controlling ethylene production (Booker & DeLong, 2015). The regulation via posttranslational modifications depends on particular residues within the ACS proteins (Fig. 2). While the N terminus and catalytic core of different ACS isoforms are well conserved, there is more variability in the C terminus. Based on the presence of particular sequences at the C terminus, the ACS family can be divided into three major groups: type 1 has target sites for mitogen-activated and calcium-dependent protein kinases (MAPK and CDPK respectively), type 2 has target sites for CDPK and E3 ligases, and type 3 has no target sites (for review see Yoon, 2015). Studies from various species have identified kinases that stabilise type 1 ACS by phosphorylation, thereby promoting ethylene production (Tatsuki & Mori, 2001; Joo et al., 2008; Li et al., 2012; Meng et al., 2014). By contrast, dephosphorylation has been shown to promote degradation of ACS, although this is dependent on the ACS type (Skottke et al., 2011; Ludwików et al., 2020).
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Table 1 Transcription factors regulating expression of ACS.

| Species      | Target gene(s) | Transcription factor | Up/downregulated | Biological process                  | Reference         |
|--------------|----------------|----------------------|------------------|-------------------------------------|-------------------|
| Arabidopsis  | AtACS2, ACS6   | AtWRKY33             | Up               | Biotic stress                       | Li et al. (2012)  |
|              | AtACS2         | AITCP5               |                  | Petal development                   | van Es et al. (2018) |
|              | AtACS7, 9, 11  | AIBE51, AIBZR1       | Down             | Hormonal crosstalk                  | Lv et al. (2018)  |
|              | AtACS2, 5      | ATERF11              | Down             | Hormonal crosstalk                  | T. Li et al. (2011) |
|              | AtACS4, 8      | AATBI4               | Down             | Hormonal crosstalk                  | Dong et al. (2016) |
| Tomato       | SIACS2         | SRIN                 | Up               | Fruit ripening                      | Ito et al. (2008) |
| Sugarcane    | ScACS2         | ScFBH1-1             | Up               | Internode maturation                | Alessio et al. (2018) |
| Apple        | MdACS1         | MdMYB10              | Up               | Fruit ripening                      | Espley et al. (2019) |
|              | MdACS1, MdACS3a| MdARF5               | Up               | Hormonal crosstalk                  | Yue et al. (2020) |
|              | MdACS1         | MdMYC2               | Up               | Hormonal crosstalk                  | T. Li et al. (2017) |
| Kiwi         | AdACS1         | AdNAC2, 3            | Up               | Hormonal crosstalk/fruit ripening   | Wu et al. (2020)  |
| Japanese plum| PsACS1         | PsAB15               | Down             | Hormonal crosstalk/fruit ripening   | Sadka et al. (2019) |

In some cases, phosphorylation can also destabilise ACS. This was demonstrated by the phosphorylation of Arabidopsis type 2 ACSs by Casein Kinase 1.8 (CK1.8), which promotes interaction with the E3 ubiquitin ligase ETO1 (Ethylene Overproducer 1) and EOL (ETO1-Like), leading to ubiquitination and consequent degradation of ACSs via the 26S proteasome (Chae et al., 2003; Wang et al., 2004; Tan & Xue, 2014). While the C-terminal domain of type 1 and type 2 ACS is certainly important for regulation of protein stability, a short region of the N-terminal domain of the Arabidopsis type 3 ACS7 fulfils this role, as it can be marked for degradation via ubiquitination by XB3 orthologue 2 in Arabidopsis (XBAT32; Lyzenga et al., 2012; Xiong et al., 2014). ACS7 can also be stabilised via the interaction with several members of the Protein Phosphatase 2C family (PPP2Cs; Marczak et al., 2020). Another mechanism of ACS stabilisation occurs via the interaction with 14-3-3 proteins that protects them from degradation (Yoon & Kieber, 2013). In general, ACS protein stability is controlled by developmental and hormonal regulators, including cytokinins, brassinosteroids, auxins, jasmonic acid, abscisic acid, salicylic acid and gibberellic acid (Lee et al., 2017).

In addition to posttranslational modifications of ACS, hetero- or homo-dimerisation of different ACS isoforms has been shown to influence enzyme activity. While ACS homodimers show enzymatic activity (Tarun & Theologis, 1998), only heterodimers composed of members from the same phylogenetic branch are active (Tsuschisaka & Theologis, 2004). Hetero-dimerisation also prolongs the half-life of some short-lived ACS isoforms (Lee et al., 2004; Tan & Xue, 2014). While ACS homodimers show enzyme activity, both by positive and negative ERF-mediated feedback mechanisms (see Table 2). Similar to transcriptional regulation of ACS, hormonal crosstalk by auxins, brassinosteroids, jasmonic acid and abscisic acid is involved in the regulation of ACS transcription. For transcriptional regulation, there is some evidence for posttranscriptional regulation of ACS, at the level of mRNA transcript stability. In trifoliate orange, miRNA396b is able to cleave PtrACO transcripts (Zhang et al., 2016), while in tomato several noncoding RNAs and miRNAs were found to target different ACO transcripts during ripening (Zuo et al., 2020).

Posttranslational regulation of ACO

The ACO protein family can be divided in three groups (Types 1–3; Fig. 2) based on amino acid sequence similarity of the dioxygenase-specific conserved RXS motif, important for catalytic activity (Houben & Van de Poel, 2019). While posttranslational regulation of ACS has been well described, information on the posttranslational regulation of ACO has been largely lacking. Dilley et al. (2013) identified putative sites for phosphorylation and glycosylation within the ACO protein sequences, although this was not confirmed experimentally. There is more evidence for redox-specific posttranslational modifications of dedicated ACO cysteine residues (Fig. 2). For example, Arabidopsis ACO1 was documented to be S-glutathionylated, although how this modification affects ACO1 activity remains unknown (Dixon et al., 2005; Datta et al., 2015). S-sulfhydration of ACO4 was also observed in Arabidopsis (Aroca et al., 2017), while in tomato, S-sulfhydration of SIACO1 and SIACO2, was shown to inhibit ACO enzyme activity (Jia et al., 2018). Another cysteine modification of ACO, namely S-nitrosylation, was observed in both Arabidopsis and tomato (Hu et al., 2015; Gong & Shi, 2019). Recently, two studies have confirmed the importance of redox-specific cysteine modifications to control ACO activity and structural stability, by

Transcriptional regulation of ACO

Several transcriptional regulators of ACO have been identified from various species. The tomato HD-ZIP transcription factor S1HB-1 was the first transcription factor identified to directly regulate expression of SIACO1 (Lin et al., 2008). It was later reported that the ripening regulator RIN can bind promoters and upregulate the expression of both HB-1 and SIACO4 (Martel et al., 2011; L. Li et al., 2017). Various other classes of transcription factor have been implicated in the direct regulation of ACO expression in many species, as covered in a recent review by Houben & Van de Poel (2019). Recently, new regulators of ACO expression have been identified and are listed in Table 2. Ethylene has also been shown to directly regulate its own biosynthesis by controlling ACO expression, both by positive and negative ERF-mediated feedback mechanisms (see Table 2). Similar to transcriptional regulation of ACS, hormonal crosstalk by auxins, brassinosteroids, jasmonic acid and abscisic acid is involved in the regulation of ACO transcription. For transcriptional regulation, there is some evidence for posttranscriptional regulation of ACO, at the level of mRNA transcript stability. In trifoliate orange, miRNA396b is able to cleave PtrACO transcripts (Zhang et al., 2016), while in tomato several noncoding RNAs and miRNAs were found to target different ACO transcripts during ripening (Zuo et al., 2020).
performing site-directed mutagenesis assays (Fournier et al., 2019; Tachon et al., 2019). Altogether, there is now a growing body of evidence that ACO is redox-controlled in plants. The exact biological function of these cysteine modifications with respect to ACO activity or stability remain elusive. Another posttranslational modification of ACO is achieved through protein-protein interactions. A single study in petunia reported that the protein PhGRL2 negatively regulates PhACO1 activity by direct binding with PhACO1 (Tan et al., 2014).

**SAM homeostasis influences ethylene production**

Both ACS and ACO rely on the steady supply of SAM as a general precursor, which is derived from methionine via the action of SAM synthetase (SAMS), also known as methionine adenosyltransferase (MAT). Other metabolic pathways also influence the SAM pool (Fig. 3), which pinpoints to a more complex regulation of SAM homeostasis. Aside from its role in ethylene biosynthesis, decarboxylated SAM is involved in the biosynthesis of higher polyamines (spermidine and spermine), molecules that are involved in many aspects of plant growth, development and stress responses (reviewed by Chen et al., 2019). Additionally, SAM serves as a universal methyl donor for the largest class of methyltransferases, enzymes which catalyse methylation of a wide range of substrates, such as histones, DNA and RNA to modify transcription and translation (reviewed by Lindermayr et al., 2020). Other SAM-dependent methyltransferases are involved in the metabolism of important specialised plant molecules, such as nicotinamides (Rahikainen et al., 2018). New insights also placed SAM homeostasis and transmethylation in relation to virus infections, during which ethylene is also important (Mäkinen & De, 2019). It has been suggested that these different pathways may compete for SAM (Moffatt & Weretilnyk, 2001). However, analysis of ethylene, polyamines and transmethylation levels during ripening of tomato fruit revealed that these pathways are able to function simultaneously, due to an augmentation of the SAM pool during ripening (Van de Poel et al., 2013).

SAMS genes were shown to be regulated at the transcriptional level by developmental cues and hormonal signalling (Peleman et al., 1989; Gómez-Gómez & Carrasco, 1998). Surprisingly, the effect of ethylene seems to be less important, because SAMS expression levels remained unaltered or dropped during developmental stages characterised by high rates of ethylene production such as, pollination of Australian tobacco (Nicotiana suaveolens), senescence of carnation (Dianthus caryophyllus) petals and climacteric fruit ripening of tomato and peach (Prunus persica) (Woodson et al., 1992; Roeder et al., 2009; Van de Poel et al., 2013; Zeng et al., 2020). Alternatively, other studies have described the differential regulation of SAMS expression in response to stress, in various species, which is often linked with an increase in ethylene production (Kim et al., 2015; He et al., 2019). Few transcription factors involved in regulating SAMS expression have been identified. In tomato, the Auxin Response Factor 6a (SIARF6a) directly inhibits expression of SISMS1, providing evidence of a direct link between auxin signalling and SAM biosynthesis (Yuan et al., 2019).

While mechanistic insights into the transcriptional regulation of SAMS is limited, more progress has been made at the level of posttranslational regulation. In Arabidopsis, the gaseous signalling molecule nitric oxide (NO) was shown to inactivate SAMS1 by S-nitrosylation (Lindermayr et al., 2006). Interestingly SAMS2 and SAMS3 were not S-nitrosylated, suggesting only SAMS1 is responsible for NO mediated regulation of SAM biosynthesis. It is possible that S-nitrosylation affects ethylene biosynthesis in plants by both targeting SAMS1 and ACOs. In Arabidopsis, SAMS can also be phosphorylated by CPK28, which targets SAMS1-3 for degradation after phosphorylation (Jin et al., 2017). The cpk28 mutant displays less SAMS degradation leading to higher SAM levels and a higher ethylene production (Jin et al., 2017). Another kinase, the receptor-like kinase FERRONIA, was found to interact with SAMS1 and SAMS2 in Arabidopsis, potentially inhibiting its

**Fig. 2** Posttranslational modifications (PTMs) of ACS and ACO. (a) ACS can be divided into three phylogenetically related groups based on the presence of phosphorylation sites for CDPK and MPK (type I), CDPK only (type II) or no C-terminal regulatory sites (type III). Phosphorylation of type I ACS (CDPK and MPK) positively effects protein stability, while dephosphorylation (PP2A/C) negatively effects protein stability. Phosphorylation of type II ACS by CK1.8 enhances binding of ETO1/EOL, leading to protein degradation. This interaction is repressed by 14-3-3 proteins, which bind ACS and destabilises ETO1/EOL. XBAT32 directly binds type III ACS leading to protein degradation. Interactions depicted in green and red are positive and negative regulators of ACS activity, respectively. (b) ACO forms three phylogenetically related groups that can be separated based on the amino acids present in the RXS motif (red box). ACOs share two conserved cysteine residues which have been associated with posttranslational modifications (C63 and C168). Modifications at C63 include S-glutathionylation (SSG) and S-sulfhydration (SSH), whereas modifications at C168 include S-sulfenylation (SOH) and S-nitrosylation (SNO). So far, these cysteine modifications have only been described in type I ACO.
activity by phosphorylation, leading to a suppression of ethylene biosynthesis (Mao et al., 2015). Another protein that directly interacts with OsSAMS1 is Psn11, a protein encoded by the rice dwarf virus (Zhao et al., 2017). Psn11 binds and enhances activity of OsSAMS1, increasing ethylene biosynthesis, which aids viral infection (Mäkinen & De, 2019). These studies clearly show that posttranslational modifications of SAMS led to altered ethylene levels, revealing the intimate relationship between SAM homeostasis and ethylene biosynthesis.

The Yang cycle sustains ethylene biosynthesis

The Yang cycle mainly serves to recycle the precious sulfur moiety from MTA, a side-product from ACS activity, back to methionine (Fig. 3) (Murr & Yang, 1975; Adams & Yang, 1977; Pommernenig et al., 2011; Sauter et al., 2013). The plant Yang cycle shows many similarities, but also some discrepancies, to the methionine salvage cycles found in bacteria, fungi and animals (Murr & Yang, 1975; Adams & Yang, 1977; Sekowska et al., 2019).

The activity of the Yang cycle has been linked with the regulation of ethylene production. Early biochemical evidence hinted that the Yang cycle was ample to sustain high rates of ethylene production in ripening apple (Malus domestica) discs (Miyazaki & Yang, 1987). It has been shown that several Yang cycle genes are upregulated in plant organs that experience high ethylene levels, revealing the intimate relationship between SAM homeostasis and ethylene biosynthesis.

The regulation of ACC homeostasis through conjugation

ACC serves as the unique precursor of ethylene biosynthesis and recent insights pinpoint this small cyclopropane to be a signalling molecule independent of ethylene (reviewed by Van de Poel & Van Der Straeten, 2014; Polko & Kieber, 2019). This implies that the ACC pool is strictly regulated to serve both ACC signalling and ethylene biosynthesis. Plants have evolved an elegant mechanism to control the pool of active signalling molecules by derivatisation and catabolism. While other hormonal pathways are known to have several conjugates of their active compounds, ethylene has none, as gaseous modifications are unlikely to take place. Conjugation predominately occurs at the level of ACC and, so far, three derivatives have been identified: malonyl-ACC (MACC), glutamyl-ACC (GACC) and jasmonyl-ACC (JA-ACC) (Fig. 4). Compared with other hormonal pathways this is a rather low number of derivatives, so perhaps other ACC conjugates await to be discovered.
MACC: the most abundant ACC derivative

MACC was identified as a first ACC derivative in buckwheat (*Fagopyrum esculentum*) seedlings (Amrhein et al., 1981) and wheat (*Triticum aestivum*) leaves (Hoffman et al., 1982). MACC is formed by ACC-N-malonyl transferase (AMT), which transfers a malonyl group from malonyl-Co-A onto ACC releasing Co-A (Fig. 4). Besides malonyl-Co-A, a second acyl donor, namely succinyl-Co-A, is able to in vitro conjugate ACC, albeit less efficiently compared with malonyl-Co-A (Benichou et al., 1995; Martin & Saftner, 1995). MACC is typically present in a five- to 10-fold higher concentration compared with ACC (Liu et al., 1985a; Philosoph-Hadas et al., 1985; Sarquis et al., 1992; Van de Poel et al., 2012), making it the major conjugate of ACC (Liu et al., 1983; Hoffman et al., 1983a; Peiser & Yang, 1998). There is also evidence that plants can convert MACC back into ethylene (Yang et al., 1982; Hoffman et al., 1983a; Matern et al., 1984; Jiao et al., 1986; Hanley et al., 1989), which seems to be tissue specific and age dependent (Matern et al., 1984; Yin et al., 2019). The enzymatic nature of MACC catabolism and whether or not it operates via the conversion towards ACC, remain to be uncovered.

Shortly after the discovery of MACC, multiple research groups partially purified AMT from mung bean (*Vigna radiata*) hypocotyls (Kionka & Amrhein, 1984; Su et al., 1985; Guo et al., 1992; Benichou et al., 1995; Chick & Leung, 1997), tomato (Martin & Saftner, 1995) and chick-pea (*Cicer arietinum*) seeds (Martinez-Reina et al., 1996). Initially it was assumed that AMT was a single monomeric enzyme, however Table 3 shows that different studies reported different molecular masses and enzyme characteristics for AMT. This observation suggests that perhaps multiple isoforms of AMT exist. Despite all these efforts, the actual AMT genetic sequence remains unidentified and certainly blurs our insight into understanding the regulation of ethylene biosynthesis through ACC sequestering into MACC.

AMT activity seems to be controlled in part by ethylene itself. It was shown that ethylene stimulates MACC formation in nonnecressing tobacco leaf discs (Philosoph-Hadas et al., 1985) and preclimacteric tomato and grapefruit (Liu et al., 1985a; Martin & Saftner, 1995). We hypothesise that MACC formation is enhanced in certain tissues where high rates of ethylene production are not yet desirable by scavenging free ACC to prevent ethylene synthesis. Conversely, certain tissues that produce high amounts of ethylene also show high MACC levels. For example, MACC levels were found to increase during ripening of tomato and apple fruit (Peiser & Yang, 1998; Van de Poel et al., 2012), senescing of carnation flowers (Hanley et al., 1989), cocklebur (*Xanthium strumarium*) germination (Satoh & Esashi, 1984) and mechanical pressure (Sarquis et al., 1992). These observations allowed us to conclude that ethylene triggers MACC formation in conditions in which ethylene productions needs to be repressed or activated.

MACC is likely to be synthesised in the cytosol, and can accumulate in the vacuole (Bouzayen et al., 1988). These observations suggest that MACC is transported across the tonoplast, presumably requiring specific active (ATP-consuming)
transporters (Bouzayen et al., 1988). Once MACC has reached the vacuole, it probably remains there (Bouzayen et al., 1988), leading to the idea that MACC is an end product. However, the fate of cytosolic MACC is less sure, and perhaps MACC can migrate to other plant parts (Bouzayen et al., 1988). There is some evidence that MACC is transported over longer distances, as basipetal transport of MACC was suggested in wounded pea (Pisum sativum) plants (Fuhrer & Fuhrer-Fries, 1985) and MACC has been retrieved in the phloem of pumpkin (Cucurbita pepo) after an application with the synthetic auxin 1-naphthylacetic acid (Amrhein et al., 1982). However, another study in cotton (Gossypium hirsutum) plants only observed phloem-mediated ACC, and not MACC transport (Morris & Larcombe, 1995). Finlayson et al. (1991) retrieved MACC in the xylem of sunflower (Helianthus annuus) seedlings that were treated with exogenous ACC. Altogether, more research is needed to create clarity about long-distance MACC transport and the existence of dedicated transporters.

GACC: apoplastic conjugation of ACC

A second derivative, GACC, was found to be present in much lower amounts compared with MACC levels in mature green tomato fruit (Martin et al., 1995). GACC is made from glutathione (γ-Glu-Cys-Gly) and ACC by γ-glutamyl transpeptidase (GGT) (Martin et al., 1995; Martin & Slovin, 2000). GGT has been purified from different species such as tomato, onion, radish and Arabidopsis (Martin & Slovin, 2000; Shaw et al., 2005; Nakano et al., 2006). In Arabidopsis, four GGT genes (GGT1-4) have been identified, of which only GGT1 and GGT2 appear to be catalytically active (Martin et al., 2007). GGT1 accounts for roughly all the activity to form GACC, while GGT2 activity is mainly restricted to seeds (Martin et al., 2007). GACC formation also influences ethylene-mediated responses, as ggt1 knockout mutants showed premature leaf senescence (Martin et al., 2007). A possible explanation for this premature senescence could be that the pool of ACC is replenished and more ACC is available to form ethylene. Suprisingly, both GGT1 and GGT2 seem to be localised extracellularly, suggesting that GACC is formed in the apoplast (Martin et al., 2007). This observation made us wonder about the exact apoplastic role of ACC conjugation into GACC. Perhaps extracellular GACC production serves to prevent apoplastic ACC to enter the cell, or to sequester ACC out of the cell, in order to regulate ethylene biosynthesis. Alternatively, GACC itself could be involved in cell wall signalling or sensing early stress responses, or it has a predominant role in the glutathione metabolism of plants.

JA-ACC: on the crossroads between ethylene and jasmonic acid signalling

In 2004, the newest derivative of ACC was identified as JA-ACC in Arabidopsis, which is made by JAR1 from jasmonic acid and ACC (Staswick & Tiryaki, 2004). JAR1 plays an important role in activating JA to JA-isoleucine and other JA-amido conjugates including the nonproteinogenic amino acid ACC (Staswick & Tiryaki, 2004). Suprisingly, JA-ACC levels were twice as high in jar1 mutants compared with wild-type plants, suggesting that JAR1 negatively regulates JA-ACC levels or other JA-ACC conjugating enzymes exist (Staswick & Tiryaki, 2004). The real function of JA-ACC remains elusive, but it has been shown to inhibit root growth in Arabidopsis (Staswick & Tiryaki, 2004). Using JA and ethylene insensitive mutants, coi1-35 and etr1-1 respectively, it was shown that the inhibitory effect of JA-ACC on root growth was accounted for by ethylene and not JA (Staswick & Tiryaki, 2004). Perhaps JA-ACC is involved in hormonal crosstalk during biotic stress, as both ethylene and JA play an important role in plant defence to necrotrophic pathogens (Li et al., 2019).

**Fig. 4** Structural representation of 1-aminocyclopropane-1-carboxylic acid (ACC) conjugates. Malonyl-1-aminocyclopropane-1-carboxylic acid (MACC), the most abundant ACC conjugate, is formed by ACC-N-malonyl transferase (AMT). Jasmonyl-1-aminocyclopropane-1-carboxylic acid (JA-ACC) is formed by Jasmonic Acid Resistance 1 (JAR1). γ-glutamyl-1-aminocyclopropane-1-carboxylic acid (GACC) is formed by γ-glutamyl transpeptidase (GGT).
The regulation of ACC homeostasis via ACC transport

In addition to conjugation, transport of ACC plays an important role in controlling the spatial distribution of ethylene biosynthesis, as described in recent reviews (Vanderstraeten & Van Der Straeten, 2017; Polko & Kieber, 2019). Because ethylene rapidly diffuses as a gas, dedicated ACC transport enables remote ethylene signalling within plants. Both short- and long-distance transport of ACC has been known for decades. Bradford & Yang (1980) observed xylem-mediated root-to-shoot transport of ACC during root hypoxia of waterlogged tomato plants. While the major transport route of ACC is likely to be mediated by the xylem, ACC transport via the phloem has also been observed (Amrhein et al., 1982; Morris & Larcombe, 1995). In addition to long-distance transport via vascular tissues, short distance transport of ACC was demonstrated in soybean (Glycine max) which showed a reduced ACC uptake when fed neutral amino acids (Lursen, 1981). These early observations suggested the existence of a dedicated amino acid transporter that can also mobilise ACC. Additionally, intracellular transport of ACC across the tonoplast into the vacuole has also been demonstrated (Tophof et al., 1989; Saftner & Martin, 1993). It was decades later that the first ACC transporter was identified by Shin et al. (2015), who discovered an Arabidopsis mutant that was insensitive to ACC, but showed a normal response to gaseous ethylene. This mutant, designated ACC-resistant2 (are2), harbours a functional disruption of the amino acid transporter LYSINE HISTIDINE TRANSPORTER1 (LHT1) (Shin et al., 2015). LHT1 was previously identified as a transporter for positively charged amino acids (histidine, lysine and arginine) in plant roots (Chen & Bush, 1997; Hirner et al., 2006), confirming older speculations that ACC and other amino acids would share a transporter (Lursen, 1981). Recently, a second ACC transporter (LHT2) was identified by complementation of the Arabidopsis lht1 ACC insensitive line (Choi et al., 2019). ACC transport activity of both LHT1 and LHT2 was confirmed by electrophysiological analysis of Xenopus oocytes expressing LHT1 and LHT2. As LHT2 is mainly expressed in floral organs, and higher order mutants of ACS are known to show floral organ defects unrelated to ethylene signalling (Tsuchisaka et al., 2009), the lack of these defects seen in the lht2 mutant led the authors to speculate that there may be other ACC transporters (Choi et al., 2019). The identification of new ACC transporters awaits new discoveries in ACC mobility and homeostasis.

Regulation of ethylene biosynthesis by D-amino acids

Early on, it was discovered that methionine is the general precursor of ethylene biosynthesis and that besides L-methionine, D-methionine can also stimulate ethylene production in apple discs (Lieberman et al., 1966). D-Amino acids are stereoisomers and often enantiomers of L-amino acids, which means they have very similar physical properties, however they can have different biological functions. D-Amino acids are frequently encountered in high concentrations in the rhizosphere due to microbiological decay, and are readily taken up and metabolised by plants (Vranova et al., 2012).

In general, L-amino acids inhibit the production of ethylene when exogenous ACC is fed (Lursen, 1981; Liu et al., 1983). This observation hinted at the competitive inhibition in uptake between ACC and other L-amino acids, which was confirmed, as LTH1 and LTH2 can both transport ACC and other L-amino acids such as L-proline, L-lysine, L-histidine and L-arginine (Hirner et al., 2006; Shin et al., 2015; Choi et al., 2019). By contrast, D-amino acids were shown to stimulate ethylene production in several plant species (Liu et al., 1983; Kionka & Amrhein, 1984). An older hypothesis suggested that D-amino acids compete with ACC for N-malonylation by AMT (Ogawa et al., 1973; Kawasaki et al., 1982). Several laboratories found that D-amino acids inhibit the formation of MACC when feeding exogenous ACC (Liu et al., 1983, 1984; Kionka & Amrhein, 1984; Su et al., 1985; Benichou et al., 1995; Martin & Saftner, 1995). As such, the presence of D-amino acids results in less MACC formation, consequently leading to higher ACC and ethylene levels. This concept is further corroborated by the observation that in vivo malonylation of D-amino acids was stimulated by ethylene treatment (Liu et al., 1985b), similar to how ethylene can stimulate MACC formation by AMT (Martin & Saftner, 1995). This might suggest that AMT has a prime role in D-amino acid metabolism and not in ACC sequestering. However, it is also possible that there are multiple specialised malonyltransferases, which have selected specificities towards D-amino acids and ACC. This hypothesis is supported by the observation that 18 different D-amino acids did not inhibit the activity of a partially purified AMT (Martin & Saftner, 1995). Furthermore, it was shown that ACC is not a substrate for other amino acid malonyltransferases (Wu et al., 1995). We believe that multiple specialised enzymes exist to process D-amino acids.

Table 3 Enzyme characteristics of partially purified AMT enzymes from different plant species.

| Species       | Molecular mass (kDa) | $K_m$ for ACC ($\mu$M) | $K_m$ for malonyl-CoA ($\mu$M) | Optimal $T$ (°C) | Optimal pH | Reference          |
|---------------|----------------------|------------------------|-------------------------------|------------------|------------|--------------------|
| Mung bean     | -                    | 170                    | 250                           | 40               | 8          | Kionka & Amrhein (1984) |
| Mung bean     | -                    | 150                    | 500                           | 35               | 8          | Su et al. (1985)      |
| Mung bean     | 50-55                | 500                    | 200                           | 50               | 8          | Guo et al. (1992)     |
| Mung bean     | 36                   | 68                     | 74                            | 8                | 8.5        | Benichou et al. (1995) |
| Tomato        | 38                   | 500                    | 100                           | -                | 8-8.5      | Martin & Saftner (1995) |
| Chick-pea     | 54                   | 400                    | 90                            | 40               | 7.5-8      | Martinez-Reina et al. (1996) |
| Mung bean     | 40                   | 66.7                   | 40                            | 45               | -          | Chick & Leung (1997)  |

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and ACC. This theory is supported by a recent observation in Arabidopsis, in which d-amino acid transaminase 1 (DAT1) was shown to have a specialised role in d-amino acid metabolism (Suarez et al., 2019). DAT1 is able to transaminate d-methionine into d-alanine, d-glutamic acid and L-methionine. The dat1 loss of function mutant showed an increased d-amino-acid-stimulated ethylene production compared with wild-type plants when fed d-methionine (Suarez et al., 2019). The authors also showed that this increase in ethylene production is not caused by a reduced malonylation of ACC, as both malonylated d-methionine (malonyl-L-methionine) and ACC (MACC) levels were higher in dat1 mutants (Suarez et al., 2019). In conclusion, it appears that there are specialised enzymes that metabolise d-amino acids and conjugate ACC, but also that there are still unknown biochemical links that could explain the d-amino acid stimulated ethylene production in plants.

Conclusions and outstanding questions

Despite the fact that the ethylene biosynthesis pathway is a simple two-step pathway and was discovered 4 decades ago, its regulation is less well understood. Recently the identity of several transcription factors that control ACS and ACO gene expression have been unravelled, with many more likely to be uncovered. Both enzymes also show a complex posttranslational regulation, of which the stability and dimerisation of ACS has been thoroughly studied. However, the role and biological significance of the redox-mediated posttranslational regulation of ACO remains elusive. At the metabolic level, it is clear that SAM levels and the activity of the Yang cycle play an important role in sustaining ethylene production. The pleiotropy of other SAM-demanding metabolic processes and the multistep recycling pathway of MTA, create many additional layers of complexity that influence the regulation of SAM homeostasis. On top of that, our knowledge of the regulation of the ACC pool is far from clear. Despite the identification of three ACC conjugates and two ACC transporters, the molecular regulation of these ACC-dependent steps are unknown and could advance the field. The emerging role of ACC as an ethylene-independent growth regulator only strengthens the belief that ACC levels are tightly controlled. The dual function of ACC in signalling and ethylene biosynthesis will certainly boost novel discoveries that will shed more light on ACC formation, conjugation, degradation and localisation.

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Author contributions

JP, JV-H and BVDP performed the literature research and wrote the paper. JP and JV-H contributed equally to this work.

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References

Abeles F, Morgan P, Saltveit MJ. 1992. Ethylene in plant biology, 2nd edn. San Diego, CA, USA: Academic Press.
Adams DO, Yang SF. 1977. Methionine metabolism in apple tissue. Plant Physiology 60: 892–896.
Adams DO, Yang SF. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Sciences, USA 76: 170–174.
Alessio VM, Cavaça na, Dantas LLDB, Lee N, Hotta CT, Imazumii T, Menossi M. 2018. The FBHII family of bHLH transcription factors controls ACC synthase expression in sugarcane. Journal of Experimental Botany 69: 2511–2525.
Amrhein N, Breuning F, Eberle J, Skorupka H, Tophof S. 1982. The metabolism of 1-aminocyclopropane-1-carboxylic acid. In: Wareing PF, ed. Plant growth substances. London, UK: Academic Press, 249–258.
Amrhein N, Schneebeck D, Skorupka H, Tophof S. 1981. Identification of a major metabolite of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid in higher plants. Naturwissenschaften 68: 619–620.
Arocá A, Benito JM, Gotor C, Romero LC. 2017. Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in Arabidopsis. Journal of Experimental Botany 68: 4915–4927.
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 Physiology 123: 979–986.
Benichou M, Martinez-Reina G, Romojaro F, Pech J, Latché A. 1995. Partial purification and properties of a 36-kDa 1-aminocyclopropane-1-carboxylate N-malonyltransferase from mung bean. Physiologia Plantarum 94: 629–634.
Blume B, Grierson D. 1997. Expression of ACC oxidase promoter-GUS fusions in tomato and Nicotiana plumaginiflora regulated by developmental and environmental stimuli. The Plant Journal 12: 731–746.
Boller T, Hanner RC, Kende H. 1979. Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145: 293–303.
Booker MA, DeLong A. 2015. Producing the ethylene signal: regulation and diversification of ethylene biosynthetic enzymes. Plant Physiology 169: 42–50.
Bouzayen M, Latché A, Albért G, Pech J-C. 1988. Intracellular sites of synthesis and storage of 1-(malonylamino)cyclopropane-1-carboxylic acid in Acer pseudoplatanus cells. Plant Physiology 88: 613–617.
Bradford KJ, Yang SF. 1980. Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. Plant Physiology 65: 322–326.
Burroughs L. 1957. 1-Aminocyclopropane-1-carboxylic acid: a new amino-acid in perry pears and cider apples. Nature 179: 360–361.
Bürstenbinder K, Rzewuski G, Wirtz M, Hell R, Sauter M. 2007. The role of methionine recycling for ethylene synthesis in Arabidopsis. The Plant Journal 49: 238–249.
Chae HS, Faure F, Kieber JJ. 2003. The eto1, eto2, and eto3 mutations and cytokinin treatment increase ethylene biosynthesis in Arabidopsis by increasing the stability of ACS protein. The Plant Cell 15: 545–559.
Chen D, Shao Q, Yin L, Younis A, Zheng B. 2019. Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Frontiers in Plant Science 9: 13.
Chen H, Sun J, Li S, Cui Q, Zhang H, Xin F, Wang H, Lin T, Gao D, Wang S et al. 2016. An ACC oxidase gene essential for cucumber carpel development. Molecular Plant 9: 1515–1527.
Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K, Saito K. 2000. Identification of a tomato gene for Activity of ageing carnation flower parts.

Guo L, Arteca RN, Phillips AT, Liu Y. 1992. Purification and characterization of Stress-induced protein S-

Dixon DP, Skipsey M, Grundy NM, Edwards R. 2005. Tools of the ethylene trade: a chemical kit to identifying S-nitrosylated proteins and unraveling S-

Fuhrer J, Fuhrer-Fries CB. 1985. Formation and transport of 1-

Chung MC, Chou SJ, Kuang LY, Charng YY, Yang SF. 2002. Identification of the ethylene-forming enzyme by expression in yeast.

Choi J, Eom S, Shin K, Lee R, Choi S, Lee J-H, Lee S, Soh M-S. 2019. Differential expression of the ethylene-forming enzyme by expression in yeast.

Lu W-J. 2016. Plant Physiology and Biochemistry: 141: 431–445.

Hiraner A, Ladwig F, Stransky H, Okumoto S, Keinath M, Harms A, Frommer WB, Koch W. 2006. Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. The Plant Cell 18: 1931–1946.

Hoffman NE, Fu J-R, Yang SF. 1983a. Identification and metabolism of 1-(malonylamino)cyclopropane-1-carboxylic acid in germinating peanut seeds. Plant Physiology 71: 197–199.

Hoffman NE, Yang SF, McKeon T. 1982. Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminoacyclopropane-1-carboxylic acid, an ethylene precursor in higher plants. Biochemical and Biophysical Research Communications 104: 765–770.

Houben M, Van de Poel B. 2019. 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. Frontiers in Plant Science 10: 15.

Huang J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J. 2015. Site-specific nitrosoproteomic identification of endogenously S-nitrosylated proteins in Arabidopsis. Plant Physiology 167: 1731–1746.

Huang S, Sawaki T, Takahashi A, Mizuno S, Takekawa K, Matsumura A, Yokotsuka M, Hirazawa Y, Sonoda M, Nakagawa H et al. 2010. Melon EIN3-like transcription factors (CmEIL1 and CmEIL2) are positive regulators of an ethylene- and ripening-induced 1-aminocyclopropane-1-carboxylic acid oxidase gene (CM-ACO1). Plant Science 178: 251–257.

Hudgins JW, Ralph SG, Franceschi VR, Bohlmann J. 2006. Ethylene induced cell wall defense: cDNA cloning, protein expression, and cellular and subcellular localization of 1-aminocyclopropane-1-carboxylic acid in resin duct and parenchyma cells. Plant 224: 865–877.

Ito Y, Kitagawa M, Ishii N, Yabe K, Kimbara J, Yasuda J, Ito H, Inakuma T, Hiroi S, Kasumi T. 2008. DNA-binding specificity, transcriptional activation potential, and the rim mutation effect for the tomato fruit-ripening regulator RIN. The Plant Journal 55: 212–223.

Jia H, Chen S, Liu D, Liesche J, Shi C, Wang J, Ren M, Wang X, Yang J, Shi W et al. 2018. Ethylene-induced hydrogen sulfide negatively regulates ethylene biosynthesis by persulfidation of ACO in tomato under osmotic stress. Frontiers in Plant Science 9: 11.

Jiao X-Z, Philosoph-Hadas S, Su L-Y, Yang SF. 1986. The conversion of 1-(malonylamino)cyclopropane-1-carboxylic acid to 1-aminocyclopropane-1-carboxylic acid in plant tissues. Plant Physiology 81: 637–641.

Jin Y, Ye N, Zhu F, Li H, Wang J, Jiang L, Zhang J. 2017. Calcium-dependent protein kinase CPK28 targets the methionine adenosyltransferases for degradation by the 26S proteasome and affects ethylene biosynthesis and lignin deposition in Arabidopsis. The Plant Journal 90: 304–318.

Joo S, Liu Y, Lueth B, Zhang S. 2008. MAPK phosphorylation-induced stabilization of ACS6 protein is mediated by the non-catalytic C-terminal domain, which also contains the cis-determinant for rapid degradation by the 26S proteasome pathway. The Plant Journal 54: 129–140.

Kawai Y, Ono E, Mizutani M. 2014. Evolution and diversity of the 2-oxoglutarate-dependent dioxygenase superfamily in plants. The Plant Journal 78: 328–343.

Kawasaki Y, Ogawa T, Sasaoka K. 1982. Two pathways for formation of D-amino acid conjugates in pea seedlings. Agricultural and Biological Chemistry 46: 1–5.

Kim SH, Kim SH, Palaniyandi SA, Yang SH, Suh J-W. 2015. Expression of potato S-adenosyl-L-methionine synthase (SbSAMS) gene altered developmental characteristics and stress responses in transgenic Arabidopsis plants. Plant Physiology and Biochemistry 87: 84–91.

Kionka C, Amrein N. 1984. The enzymatic malonylation of 1-aminocyclopropane-1-carboxylic acid in homogenates of mung-bean hypocotyls. Planta 162: 226–235.

Kou X, Liu C, Han L, Wang S, Xue Z. 2016. NAC transcription factors play an important role in ethylene biosynthesis, reception and signaling of tomato fruit ripening. Molecular Genetics and Genomics 291: 1205–1217.

Kushad MM, Richardson DG, Ferro AJ. 1982. Calcium-dependent expansins play an important role in ethylene biosynthesis, reception and signaling of tomato fruit ripening. Molecular Genetics and Genomics 291: 1205–1217.

Lee HY, Chen Y-C, Kieber JJ, Yoon GM. 2017. Regulation of the turnover of ACO syntheses by phytohormones and heterodimerization in Arabidopsis. The Plant Journal 91: 491–504.

Li G, Meng X, Wang R, Mao G, Han L, Liu Y, Zhang S. 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream
WRKY transcription factor during ethylene induction in Arabidopsis. *PLoS Genetics* 8: 14.
Li L, Wang X, Zhang X, Guo M, Liu T. 2017. Unraveling the target genes of RIN transcription factor during tomato fruit ripening and softening. *Journal of the Science of Food and Agriculture* 97: 991–1000.
Li N, Han X, Feng D, Yuan D, Huang J. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonic in plant defense: do we understand what they are whispering? *International Journal of Molecular Science* 20: 15.
Li T, Xu Y, Zhang L, Ji Y, Tan D, Yuan H, Wang A. 2017. The jasmonate-activated transcription factor MdMYC2 regulates ETHYLENE RESPONSE FACTOR and ethylene biosynthetic genes to promote ethylene biosynthesis during apple fruit ripening. *The Plant Cell* 29: 1316–1334.
Li Z, Zhang L, Yu Y, Quan R, Zhang Z, Huang H. 2011. The ethylene response factor AtERF11 that is transcriptionally modulated by the bZIP transcription factor HYS is a crucial repressor for ethylene biosynthesis in *Arabidopsis*. *The Plant Journal* 68: 88–99.
Liang S, Xiong W, Yin C, Xie X, Jin Y, Zhang S, Yang B, Ye G, Chen S, Luw M. 2019. Overexpression of Os5RD1 improves submergence, drought, and salt tolerances of seedlings through the enhancement of ethylene synthesis in rice. *Frontiers in Plant Science* 10: 19.
Liebermann M, Kunishi A, Mapson L.W, Wardale DA. 1966. Stimulation of ethylene production in apple tissue slices by methionine. *Plant Physiology* 41: 376–381.
Lin Z, Hong Y, Yin M, Li C, Zhang K, Grierson D. 2008. A tomato HD-Zip homeobox protein, LeHB-1, plays an important role in floral organogenesis and ripening. *The Plant Journal* 55: 301–310.
Lindermayr C, Rudolf EE, Durner J, Groth M. 2020. ETHYLENE RESPONSE FACTOR MdMYC2 regulates 1-aminocyclopropane-1-carboxylic acid-conjugating activity in tomato fruit. *Plant Physiology* 159: 1971–1986.
Martin MN, Saladores PH, Lambert E, Hudson AO, Leustek T. 2007. Localization of members of the γ-glutamyl transpeptidase family identifies sites of glutathione and glutathione S-conjugate hydrolysis. *Plant Physiology* 144: 1715–1732.
Martin MN, Slavin JP. 2000. Purified γ-glutamyl transpeptidases from tomato exhibit high affinity for glutathione and glutathione S-conjugates. *Plant Physiology* 122: 1417–1426.
Martinez-Reina G, Matilla AJ, Martin-Remese C, Gallardo M, Muñoz de Rueda P. 1996. Biochemical properties of 1-aminocyclopropane-1-carboxylic acid N-malonyltransferase activity from early growing embryonic axes of chick-pea (*Cicer arietinum* L.) seeds. *Journal of Experimental Botany* 47: 1771–1788.
Matern U, Feser C, Heller W. 1984. N-Malonyltransf erase s from peanut. *Archives of Biochemistry and Biophysics* 235: 218–227.
Meng Y, Ma N, Zhang Q, You Q, Li N, Ali Khan M, Liu X, Wu L, Su Z, Gao J. 2014. Precise spatio-temporal modulation of ACC synthase by MPK6 cascade mediates the response of rose flowers to rehydration. *The Plant Journal* 79: 941–950.
Miyazaki HJ, Shang FY. 1987. Inhibition of the methionine cycle enzymes. *Phytochemistry* 26: 2655–2660.
Miyazaki HJ, Yang SF. 1987. Metabolism of 5-methylthioribose to methionine. *Plant Physiology* 84: 277–281.
Moffatt BA, Weretilnyk EA. 2001. Sustaining S-adenosyl-L-methionine-dependent methyltransferase activity in plant cells. *Physiologia Plantarum* 113: 435–442.
Moon J, Park YJ, Son S-H, Roh J, Yoon JH, Kim SY, Kim S-K. 2020. Brassinosteroids signaling via BZR1 down-regulates expression of ACC oxidase 4 to control growth of *Arabidopsis thaliana* seedlings. *Plant signaling & Behavior* 15: 6.
Morriss DA, Larcembe NJ. 1995. Inhibition transport and conjugation of foliar applied 1-aminocyclopropane-1-carboxylic acid in cotton (*Gossypium hirsutum* L.). *Journal of Plant Physiology* 146: 429–436.
Mou W, Yao Y-T, Michael E, Simon AA, Li D, Wedick MM, Lizzio MA, Feijo JA, Chang C. 2020. Ethylene-independent signaling by the ethylene precursor ACC in Arabidopsis ovular pollen tube attraction. *Nature Communications*, 11.
Murr DP, Yang SF. 1975. Conversion of 5-methylthioribose to methionine by apple tissue. *Phytochemistry* 14: 1291–1292.
Nakajima N, Morii H, Yamazaki K, Imaseki H. 1990. Molecular cloning and sequence of a complementary DNA encoding 1-aminocyclopropane-1-carboxylic acid synthase induced by tissue wounding. *Plant Cell Physiology* 31: 1021–1029.
Nakano Y, Okawa S, Yamauchi T, Koizumi Y, Sekiya J. 2006. Overexpression of a gene encoding 5-methylthioribose-1-phosphate kinase improves submergence, drought, and salt tolerances of *Arabidopsis thaliana*. *Journal of Biological Chemistry* 281: 329–335.
Liu J, Su L-Y, Yang SF. 1985b. Ethylene promotes the capability to malonylate 1-aminocyclopropane-1-carboxylic acid and α-amino acids in preclimacteric tomato fruits. *Plant Physiology* 77: 891–895.
Ludwikowski A, Ciesla A, Kasprowicz-Malinski A, Mitula F, Tajdel M, Galanski L, Ziołkowska PA, Kubial P, Malecka A, Piechala A et al. 2014. Arabidopsis protein phosphatase 2CA1B1 interacts with type 1 ACC synthases and is involved in the regulation of ozone-induced ethylene biosynthesis. *Molecular Plant* 7: 960–976.
Lurssen K. 1981. Interference of amino acids with the uptake of 1-aminocyclopropane-1-carboxylic acid in soybean leaf discs. *Plant Science Letters* 20: 365–370.
Lv B, Tian H, Zhang F, Liu J, Lu S, Bai M, Li C, Ding Z. 2018. Brassinosteroids regulate root growth by controlling reactive oxygen species homeostasis and dual effect on ethylene synthesis in *Arabidopsis*. *PLoS Genetics* 14: 26.
Lyenzhen WJ, Booth JK, Stone SL. 2012. The Arabidopsis RING-type E3 ligase XRAT32 mediates the proteasomal degradation of the ethylene biosynthetic enzyme, 1-aminocyclopropane-1-carboxylic synthase 7. *The Plant Journal* 71: 23–34.
Mäkinen K, De S. 2019. The significance of methionine cycle enzymes in plant virus infections. *Current Opinion in Plant Biology* 50: 67–75.
Mao D, Yu F, Li J, Van de Poel B, Tan D, Li J, Liu Y, Li X, Dong M, Chen L et al. 2015. FERONIA receptor kinase interacts with S-adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene biosynthesis in *Arabidopsis*. *Plant, Cell & Environment* 38: 2566–2574.
Marczek M, Ciesa A, Janicki M, Kasprowicz-Malinski A, Kubial P, Ludwikow A. 2020. Protein phosphatases type 2C A interact with and regulate the stability of ACC synthase 7 in *Arabidopsis*. *Cell* 9: 20.
Marter C, Vrebalo J, Tafelmeyer P, Giovannoni JJ. 2011. The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLORLESS NONRIPENING-dependent manner. *Plant Physiology* 157: 1568–1579.
Martin MN, Cohen JD, Saftner RA. 1995. A new 1-aminocyclopropane-1-carboxylic acid-conjugating activity in tomato fruit. *Plant Physiology* 109: 917–926.
Martin MN, Saftner RA. 1995. Purification and characterization of 1-aminocyclopropane-1-carboxylic acid N-malonyltransferase from tomato fruit. *Plant Physiology* 108: 1241–1249.
Martin MN, Saladores PH, Lambert E, Hudson AO, Leustek T. 2007. Arabidopsis RING-type E3 ligase. *New Phytologist* 1710: 2007–2014.
Silva JC, Santin FL, Sampaio AP, Ribeiro IM, Ferreira EA, Neves JT Jr. 2020. Identification of 1-aminocyclopropane-1-carboxylic acid N-malonyltransferase from tomato fruit. *Plant Physiology* 171: 2970–2981.
Miyazaki HJ, Yang SF. 1985. Intracellular localization of the γ-glutamyl transpeptidase family in *Arabidopsis*. *Plant Physiology* 77: 891–895.

Peleman J, Saito K, Cotty B, Engler G, Seurinck J, Van Montagu M, Inzé D. 1989. Structure and expression analyses of the S-adenosylmethionine synthase gene family in Arabidopsis thaliana. Gene 84: 359–369.

Philosoph-Hadas S, Meir S, Athanori N. 1985. Autoinhibition of ethylene production in tobacco leaf discs: enhancement of 1-aminocyclopropane-1-carboxylic acid conjugation. *Physiologia Plantarum* 63: 431–437.

Polka JK, Kieber JJ. 2019. A-1-Aminocyclopropane-1-carboxylic acid and its emerging role as an ethylene-independent growth regulator. *Frontiers in Plant Science* 10: 9.

Pommerengen B, Feussner K, Zierer W, Rabinovich V, Klef I, Feussner I, Sauer N. 2011. Phloem-specific expression of xylogenic cycle genes and identification of novel xylogenic cycle enzymes in Plantago and Arabidopsis. *The Plant Cell* 23: 1904–1919.

Rahikainen M, Alegre S, Trotta A, Pascual J, Kangasjärvi M, Alegre J, Carretero-Paulet L. 2015. Immunocytolocalization of 1-aminocyclopropane-1-carboxylic acid oxidase in tomato cells. *Physiologia Plantarum* 159: 142–146.

Rivio J, Dagan E, Goren R, Yang SF. 1990. Characterization of absicic acid-induced ethylene production in citrus leaf and tomato fruit tissues. *Plant Physiology* 92: 48–53.

Rodrigues-Pousada RA, De Rycke R, Dedonder A, Van Caeneghem W, Engler G, Sauter M, Lorbiecke R, OuYang B, Pochapsky TC, Rzewuski G. 2005. Transport of 1-aminocyclopropane-1-carboxylic acid into isolated maize mesophyll vacuoles. *Physiologia Plantarum* 124: 650–662.

Sauter M, Lörcher M, Lorbiecke R, OuYang B, Pochapsky TC, Rzewuski G. 2005. The immediate-early ethylene response gene *OeRdR1* encodes an acrolein dioxygenase involved in recycling of the ethylene precursor S-adenosylmethionine. *The Plant Journal* 44: 718–729.

Sauter M, Moffatt B, Saehao MC, Bell R, Wirz M. 2013. Methionine salvage and S-adenosylmethionine: Essential links between sulfur, ethylene and polyamine biosynthesis. *Biochemical Journal* 451: 145–154.

Sekowska A, Ashida H, Danchin A. 2019. Revisiting the methionine salvage pathway and its paralogues. *Microbial Biotechnology* 12: 77–97.

Shaw ML, Pither-Joyce MD, McCallum JA. 2005. Purification and cloning of a γ-glutamyl transpeptidase from onion (*Allium cepa*). *Phytochemistry* 66: 515–522.

Shin K, Lee S, Song W-Y, Lee R-A, Lee I, Ha K, Koo J-C, Park S-K, Nam H-G, Lee Y et al. 2015. Genetic identification of ACC-RESISTANT2 reveals involvement of LYSINE HISTIDINE TRANSPORTER1 in the uptake of 1-aminocyclopropane-1-carboxylic acid in Arabidopsis thaliana. *Plant Cell Physiology* 56: 572–582.

Skottke KR, Yoon GM, Kieber JJ, DeLong A. 2011. Protein phosphatase 2A controls ethylene biosynthesis by differentially regulating the turnover of ACC synthase isoforms. *PLoS Genetics* 7: 13.

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

Su L-Y, Liu Y, Yang SF. 1985. Relationship between 1-aminocyclopropanecarboxylate malonyltransferase and D-amino acid malonyltransferase. *Phytochemistry* 24: 1141–1145.

Suarez J, Hener C, Lehnhardt V-A, Hummel S, Stahl M, Kolukisaoglu Ü. 2019. AtDAT1 is a key enzyme of D-amino acid stimulated ethylene production in Arabidopsis thaliana. *Frontiers in Plant Science* 10: 15.

Tachon S, Fourrier E, Decroos C, Manuelp E, Etienne E, Maresca M, Martinho M, Belle V, TRon T, Sama J. 2019. Chemical modification of 1-aminocyclopropane carboxylic acid (ACC) oxidase: Cysteine mutational analysis, characterization, and bioconjugation with a nitroxide spin label. *Molecular Biotechnology* 61: 650–662.

Tan S-T, Xue H-W. 2014. Casein kinase 1 regulates ethylene synthesis by phosphorylating and promoting the turnover of ACS5. *Cell Reports* 9: 1692–1702.

Tan Y, Liu J, Huang F, Guan J, Zhong S, Tang N, Zhao J, Yang W, Yu Y. 2014. PhGRL2 protein, interacting with PhACO1, is involved in flower senescence in the petunia. *Molecular Plant* 7: 1384–1387.

Taran AS, Theologis A. 1998. Complementation analysis of mutants of 1-aminocyclopropane-1-carboxylate synthase reveals the enzyme is a dimer with shared active sites. *The Journal of Biological Chemistry* 273: 12509–12514.

Tatsuki M, Mori H. 2001. Phosphorylation of tomato 1-aminocyclopropane-1-carboxylic acid synthase, LE-ACS2, at the C-terminal region. *The Journal of Biological Chemistry* 276: 28051–28057.

Tohoph S, Martinoia E, Kaiser G, Hartung W, Amrhein N. 1989. Compartimentation and transport of 1-aminocyclopropane-1-carboxylic acid and N-malonyl-1-aminocyclopropane-1-carboxylic acid in barley and wheat mesophyll cells and protoplasts. *Physiologia Plantarum* 75: 333–339.

Tsuchisaka A, Yu G, Jin H, Alonso JM, Ecker JR, Zhang X, Gao S, Theologis A. 2013. A combinatorial interplay among the 1-aminocyclopropane-1-carboxylate synthase polypeptides encoded by the Arabidopsis gene family. *Proceedings of the National Academy of Sciences, USA* 110: 2275–2280.

Tsuchisaka A, Yu G, Jin H, Alonso JM, Ecker JR, Zhang X, Gao S, Theologis A. 2009. A combinatorial interplay among the 1-aminocyclopropane-1-carboxylate synthase isoforms regulates ethylene biosynthesis in Arabidopsis thaliana. *Genetics* 183: 979–1003.

Vahatalo ML, Virtanen A. 1957. A new cyclic α-aminocarboxylic acid in berries of cowberry. *Acta Chemica Scandinavica* 11: 741–743.

Van de Poel B, Bulens I, Markoula A, Hertog MLATM, Wirtz M, Van de Poel B, Bulens I, Oppermann Y, Keulemans J, Hell R et al. 2012. Targeted systems biology profiling of tomato fruit reveals coordination of the Yang cycle and a distinct regulation of ethylene biosynthesis during postclimacteric ripening. *Plant Physiology* 160: 1498–1514.

Van de Poel B, Bulens I, Oppermann Y, Hertog MLATM, Nicolai BM, Sauter M, Geeraerd AH. 2013. S-adenosly-1-methionine usage during climacteric ripening of tomato in relation to ethylene and polyamine biosynthesis and transmethylation capacity. *Physiologia Plantarum* 148: 176–188.

Van de Poel B, Van Der Straeten D. 2014. 1-Aminocyclopropane-1-carboxylic acid (ACC) in plants: More than just the precursor of ethylene! *Frontiers in Plant Science* 5: 11.

Van Der Straeten D, Van Wiemeersch L, Goodman HM, Van Montagu M. 1990. Cloning and sequence of two different cDNAs encoding 1-aminocyclopropane-1-
van Es SW, Silveira SR, Rocha DI, Bimbo A, Martinelli AP, Dornelas MC, Angenent GC, Immink RGH. 2018. Novel functions of the Arabidopsis transcription factor TCP5 in petal development and ethylene biosynthesis. The Plant Journal 94: 867–879.

Vanderstraeten L, Depaep T, Bertrand S, Van Der Straeten D. 2019. The ethylene precursor ACC affects early vegetative development independently of ethylene signaling. Frontiers in Plant Science 10: 1.

Vanderstraeten L, Van Der Straeten D. 2017. Accumulation and transport of 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: current status, considerations for future research and agronomic applications. Frontiers in Plant Science 8: 18.

Ververidis P, John P. 1991. Complete recovery in vitro of ethylene-forming enzyme activity. Physiologia Plantarum 30: 725–727.

Vogel JP, Woeste KE, Theologis A, Kieber JJ. 1998. Recessive and dominant mutations in the ethylene biosynthetic gene ACS5 of Arabidopsis confer cytokinin insensitivity and ethylene overproduction, respectively. Proceedings of the National Academy of Sciences, USA 95: 4766–4771.

Vranova V, Zahradnickova H, Janous D, Skene KR, Matharu AS, Rejsek K, Formanek P. 2012. The significance of D-amino acids in soil, fate and utilization by microbes and plants: review and identification of knowledge gaps. Plant and Soil 354: 21–39.

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 NN, Shih M-C, 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. Journal of Experimental Botany 56: 909–920.

Wang W, Wang J, Wu Y, Li D, Qian Y. 2020. Genome-wide analysis of coding and non-coding RNA reveals a conserved miR164-NAC regulatory pathway for fruit ripening. New Phytologist 225: 1618–1634.

Washington EJ, Mukhtar MS, Finkel OM, Wan L, Banfield MJ, Kieber JJ, Dangl JL. 2016. Pseudomonas syringae type III effector HopAF1 suppresses plant immunity by targeting methionine recycling to block ethylene induction. Proceedings of the National Academy of Sciences, USA 113: 3577–3586.

Woodson WR, Park KY, Droyer A, Larsen PB, Wang H. 1992. Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. Plant Physiology 99: 526–552.

Wu Y-Y, Liu X-F, Fu B-L, Zhang Q-Y, Tong Y, Wang J, Wang W, Grierson D, Yin X. 2020. Methyl jasmonate enhances ethylene synthesis in kiwifruit by inducing NAC genes that activate ACS1. Journal of Agricultural and Food Chemistry 68: 3267–3276.

Wu YM, Silverstone AL, Liu Y, Yang SF. 1995. Partial purification and characterization of tryptophan N-malonyltransferase from tomato leaves. Phytochemistry 40: 699–704.

Xiong L, Xiao D, Xu X, Guo Z, Wang NN. 2014. The non-catalytic N-terminal domain of ACS7 is involved in the post-translational regulation of this gene in Arabidopsis. Journal of Experimental Botany 65: 4397–4408.

Xu S, Rahman A, Baskin TJ, Kieber JJ. 2008. Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in Arabidopsis. The Plant Cell 20: 3065–3079.

Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35: 155–189.

Yang SF, Hoffman NE, McKeon T, Riar J, Kao CH, Yang KH. 1982. Mechanism and regulation of ethylene biosynthesis. In: Wareing PF, ed. Plant growth substances. London, UK: Academic Press, 239–248.

Yin J, Zhang X, Zhang G, Wen Y, Liang G, Chen X. 2019. Aminocyclopropane-1-carboxylic acid is a key regulator of guard mother cell terminal division in Arabidopsis thaliana. Journal of Experimental Botany 70: 897–907.

Yip W-K, Yang SF. 1988. Cyanide metabolism in relation to ethylene production in plant tissues. Plant Physiology 88: 473–476.

Yoon GM. 2015. New insights into the protein turnover regulation in ethylene biosynthesis. Molecules and Cells 38: 597–603.

Yoon GM, Kieber JJ. 2013. 14-3-3 regulates 1-aminocyclopropane-1-carboxylate synthase protein turnover in Arabidopsis. The Plant Cell 25: 1016–1028.

Yuan Y, Xu X, Gong Z, Tang Y, Wu M, Yan F, Zhang X, Zhang Q, Yang F, Hu X et al. 2019. Auxin response factor 6A regulates photosynthesis, sugar accumulation, and fruit development in tomato. Horticulture Research 6: 1.

Yue P, Lu Q, Liu Z, Lv T, Li X, Bu H, Liu W, Xu Y, Yuan H, Wang A. 2020. Auxin-activated MdARF5 induces the expression of ethylene biosynthetic genes to initiate apple fruit ripening. New Phytologist 226: 1781–1795.

Zeng W, Niu L, Wang Z, Wang X, Wang Y, Pan L, Lu Z, Cui G, Weng W, Wang M et al. 2020. Application of an antibody chip for screening differentially expressed proteins during peach ripening and identification of a metabolon in the SAM cycle to generate a peach ethylene biosynthesis model. Horticulture Research 7: 31.

Zhang X, Wang W, Wang M, Zhang H-Y, Liu J-H. 2016. The miR396b of Poncirus trifoliata functions in cold tolerance by regulating ACC oxidase gene expression and modulating ethylene-polyamine homeostasis. Plant and Cell Physiology 57: 1865–1878.

Zhang Z, Zhang H, Quan R, Wang X-C, Huang R. 2009. Transcriptional regulation of the ethylene response factor LeERF2 in the expression of ethylene biosynthesis genes controls ethylene production in tomato and tobacco. Plant Physiology 150: 365–377.

Zhou A, Hong W, Wu J, Wang Y, Ji S, Zhu S, Wei C, Zhang J, Li Y. 2017. A viral protein promotes host SAM51 activity and ethylene production for the benefit of virus infection. eLife 6: 22.

Zheng H, Dong L, Han X, Jin H, Yin C, Han Y, Li B, Qin H, Zhang J, Shen Q et al. 2020. The TuMYB46L-TuACO3 module regulates ethylene biosynthesis in einkorn wheat defense to powdery mildew. New Phytologist 225: 2526–2541.

Zuo J, Grierson D, Courtney LT, Wang Y, Gao L, Zhao X, Zhu B, Luo Y, Wang Q, Giovannoni JJ. 2020. Relationships between genome methylation, levels of noncoding RNAs, mRNAs and metabolites in ripening tomato fruit. The Plant Journal tpi.14778.