



Something old, something new: Conservation of the ethylene precursor 1-amino-cyclopropane-1-carboxylic acid as a signaling molecule

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Abstract

In seed plants, 1-amino-cyclopropane-1-carboxylic acid (ACC) is the well-known precursor of the plant hormone ethylene. In nonseed plants, the current view is that ACC is produced but is inefficiently converted to ethylene. Distinct responses to ACC that are uncoupled from ethylene biosynthesis have been discovered in diverse aspects of growth and development in liverworts and angiosperms, indicating that ACC itself can function as a signal. Evolutionarily, ACC may have served as a signal before acquiring its role as the ethylene precursor in seed plants. These findings pave the way for unraveling a potentially conserved ACC signaling pathway in plants and have ramifications for the use of ACC as a substitute for ethylene treatment in seed plants.

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Keywords

Ethylene, Precursor, ACC, Biosynthesis, Signaling, Hormone, Evolution, Liverwort, Marchantia, Arabidopsis.

Introduction

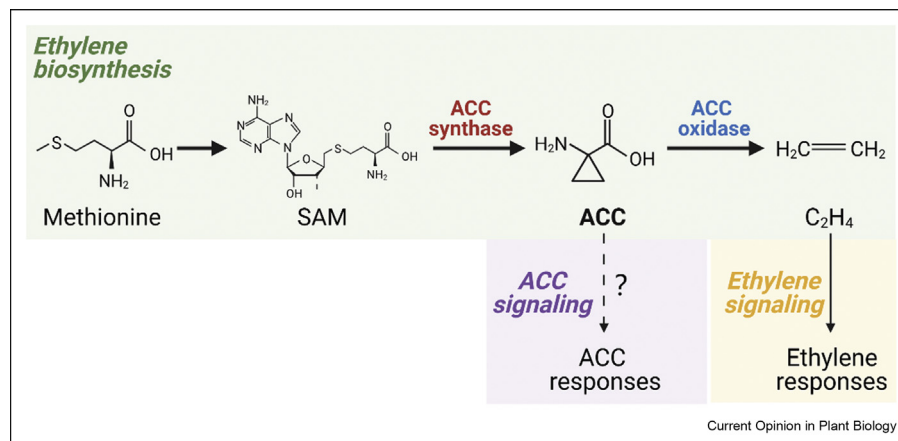
The plant hormone ethylene (C₂H₄) regulates well-known aspects of growth and development, such as

fruit ripening, senescence, abscission, and responses to stress, and controls diverse, lesser-known processes in many plants ranging from algae to angiosperms [1]. The ethylene signaling pathway, which was mainly elucidated in *Arabidopsis thaliana*, was likely functional at least 450 million years ago, as evidenced by conservation of the pathway in a Charophycean alga [2]. Ethylene signaling may have evolved before the ability to synthesize ethylene, given that the ethylene receptor likely originated in cyanobacteria [3] and because environmental ethylene is produced by microorganisms as well as by chemical breakdown of organic matter.

The ethylene biosynthesis pathway in angiosperms was uncovered in the late 1970s. A nonproteinogenic amino acid, 1-aminocyclopropane-1-carboxylic acid (ACC), is the immediate precursor of ethylene [4]. In the first committed step of ethylene biosynthesis, the aminotransferase enzyme ACC synthase (ACS) makes ACC from the universal methyl donor S-adenosyl-L-methionine; ACC is then converted to ethylene gas by ACC oxidase (ACO), a dioxygenase that requires oxygen (Figure 1 [4]). ACS synthesis is regulated transcriptionally and post-translationally in response to a wide range of external and internal stimuli [4,5]. The efficient uptake and conversion of ACC to ethylene by seed plants have enabled plant biologists to use ACC treatment as a substitute for ethylene gas to induce ethylene responses. However, as described in the next section, even though nonseed plants produce ethylene, nonseed plants that take up exogenous ACC produce little or no additional ethylene. Moreover, ACO homologs are absent in the available genome sequences of nonseed plants (Figure 2 [6]). In contrast, ACS homologs are widely conserved in land plants (Figure 2 [6]), raising the possibility that ACC synthesis evolutionarily preceded the efficient conversion of ACC to ethylene in angiosperms and gymnosperms. If indeed this was the case, what was the original role of ACC biosynthesis in nonseed plants?

In this article, we review the evidence that ACC serves as a plant signaling molecule independent of its well-known role in ethylene biosynthesis. We also speculate on mechanisms of ACC signaling. By definition, a plant

Figure 1



Ethylene biosynthesis in seed plants and the dual roles of ACC. Ethylene biosynthesis is a two-step pathway from SAM, which is synthesized from methionine. In the first step, SAM is cleaved and converted to ACC by the enzyme ACS. In the second step, ACC is converted to ethylene by the enzyme ACO. ACC can also induce responses that are distinct from those induced by ethylene via an unidentified ACC signaling pathway (dotted line). This pathway is in contrast to the known conserved ethylene signaling pathway. This figure was created using BioRender.com. ACC, 1-amino-cyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; SAM, S-adenosyl-L-METHIONINE

signal operates at low concentrations, evokes local or distant physiological responses, and can be transported. ACC is known to be transported, and the evidence discussed as follows shows that ACC responses can be distinct from those induced by ethylene and occur even when ethylene signaling is blocked. In evolution, ACC may have served as a signal before acquiring its role as the ethylene precursor, yet ACC has unique signaling roles even in angiosperms.

Emerging views of 1-amino-cyclopropane-1-carboxylic acid function in nonseed plants

Do nonseed plants produce 1-amino-cyclopropane-1-carboxylic acid?

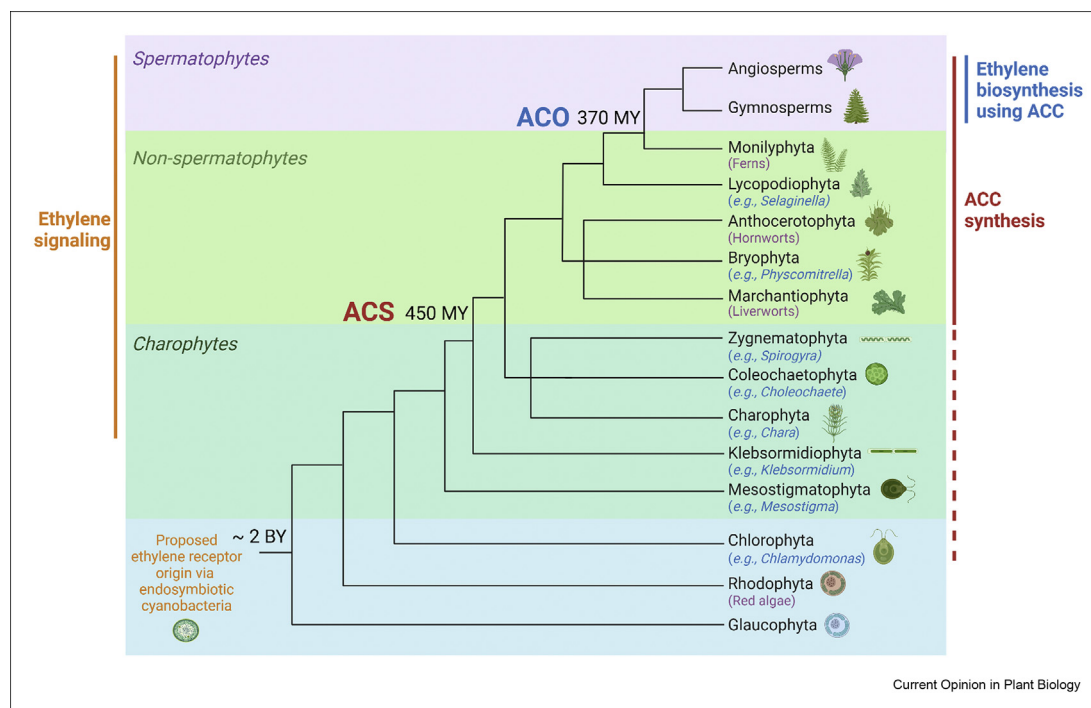
An essential question with respect to ACC signaling is whether a plant is capable of producing ACC. Consistent with the presence of ACS homologs in the genomes of nonseed plants, both ACS activity and ACC have been detected in the ferns *Regnellidium diphyllum* and *Marsilea quadrifolia* [7,8]. ACC was also measured in the liverwort *Riella helicophylla* [8] and the moss *Funaria hygrometrica* [9]. More recently, ACC was measured in the liverwort *Marchantia polymorpha*, and single and double knockout mutants were generated for the two *M. polymorpha* MpACS homologs [10••,11••]. Li et al. [10••] observed dramatically less ACC in the *Mpacs1Mpacs2* double knockout mutants than that of the wild type and, conversely, detected ACC produced by yeast heterologously expressing each MpACS gene. In contrast, using pooled lines of *Mpacs1Mpacs2* knockout mutants in a different *M. polymorpha* genetic background, Katayose et al. [11••] observed no change in

endogenous ACC levels (but raising an interesting question concerning the source of the endogenous ACC). Moreover, both ACS homologs in the moss *Physcomitrella patens* exhibited C β -S lyase activity instead of ACS activity [12], bringing into question whether *P. patens* synthesizes ACC at all and whether ACS homologs of other plant species are C β -S lyases. C β -S lyases catalyze the cleavage of carbon–sulfur bonds of L-cystine and L-cysteine and are involved in amino acid metabolism. Further studies are required to obtain a clearer view of ACC synthesis and the functions of ACS homologs in nonseed plants.

1-amino-cyclopropane-1-carboxylic acid is only weakly converted to ethylene in nonseed plants

Consistent with the absence of ACO homologs in nonseed plant genomes, a number of reports have indicated that ACC is not the main ethylene precursor in nonseed plants. Studies in the 1990s concluded that there must be an alternative ethylene biosynthesis pathway for mosses, liverworts, and ferns [7,8,13]. In key experiments, treatment with radiolabeled [¹⁴C] ACC in the liverworts *R. helicophylla* and *M. polymorpha* [8] and in the ferns *R. diphyllum* and *M. quadrifolia* (ferns) [7,8] resulted in very little [¹⁴C] ethylene (e.g. 0.005%). In other studies (e.g. in a red alga [14], Chlorophycean algae [15–17], a Charophycean alga [2], and ferns [18]), treatment with nonradiolabeled ACC (ranging between 200 μ M and 10 mM) yielded only a fraction of the ethylene levels known to be produced by angiosperms treated with similar ACC concentrations. Assuming there was uptake of ACC in these experiments, such doses were likely above the physiological range. In more recent studies, *M. polymorpha* took up

Figure 2



Evolution of ACC and ethylene biosynthesis in relation to the phylogeny of the plant lineage. ACS homologs are present in genomes of essentially all sequenced land plants (including nonseed plants), whereas ACO homologs have been found only in the genomes of spermatophytes (gymnosperms and angiosperms), which arose about 370 MY ago. Thus, the synthesis of ACC by ACS is potentially more ancient than the efficient conversion of ACC to ethylene in seed plants. The solid red line representing ACC synthesis indicates the presence of supporting evidence, whereas the dotted red line indicates the lack of evidence. In terms of ethylene signaling, the ethylene receptor gene may have been acquired ~2 BY ago from an endosymbiotic cyanobacterium that became the plastid. The complete ethylene signaling pathway as characterized in angiosperms was likely assembled during the evolution of Charophytean algae, before the origin of land plants ~450 MY ago. This figure was created using BioRender.com. ACC, 1-amino-cyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase.

ACC from the growth medium [10●●] as shown previously for liverworts [8], but only the highest doses of ACC tested (500 μ M and 1 mM) gave a detectable increase in ethylene ([10●●,11●●], respectively). In *Mpacs1Mpacs2* double mutants, ethylene production was 60% that of the wild type in one study (suggesting ACC could have at least a partial role in ethylene biosynthesis) [10●●] but remained unchanged in another study [11●●]. It is worth mentioning that exogenous ACC taken up by the plant can potentially be converted to ethylene by nonenzymatic reactions or by unknown enzymes or conceivably trigger ethylene biosynthesis from a different precursor. Overall, the current opinion is that ACC is at best a weak ethylene precursor in nonseed plants.

The alternative pathways of ethylene production in nonseed plants have yet to be identified. Certain bacteria and fungi produce ethylene from 2-oxoglutarate using the ethylene-forming enzyme [19] or by a nonenzymatic conversion of α -keto γ -methylthiobutyric acid [20]. Still, other bacteria convert carbon monoxide or sulfur compounds to ethylene using vanadium

nitrogenase or methylthio-alkane reductase enzymes, respectively [21–23]. However, there is currently no evidence indicating that these particular mechanisms exist in plants.

Evolutionarily, aquatic species that subsequently gave rise to land plants would not have benefitted from the synthesis of high ethylene levels. This is because ethylene is removed only by diffusion and diffuses 1000 times slower in water than in air, thus precluding the rapid removal of cellular ethylene in aquatic organisms. We speculate that ethylene may have even served as a sensor of land versus water or dry versus wet times and that perhaps ACO activity was acquired as the earth's environment became markedly drier in the Permian period after the wet Carboniferous period when the earliest seed plants appeared. Interestingly, *ACO* genes have been lost in *Potamogeton pectinatus* [24], and both the ethylene biosynthesis and signaling pathways have been lost in *Zostera muelleri* [25] and *Zostera marina* [26]. These are all angiosperms that have (re)adapted to a fully submerged lifestyle, in contrast to angiosperms that grow on the surface of water, such as *Spirodela*

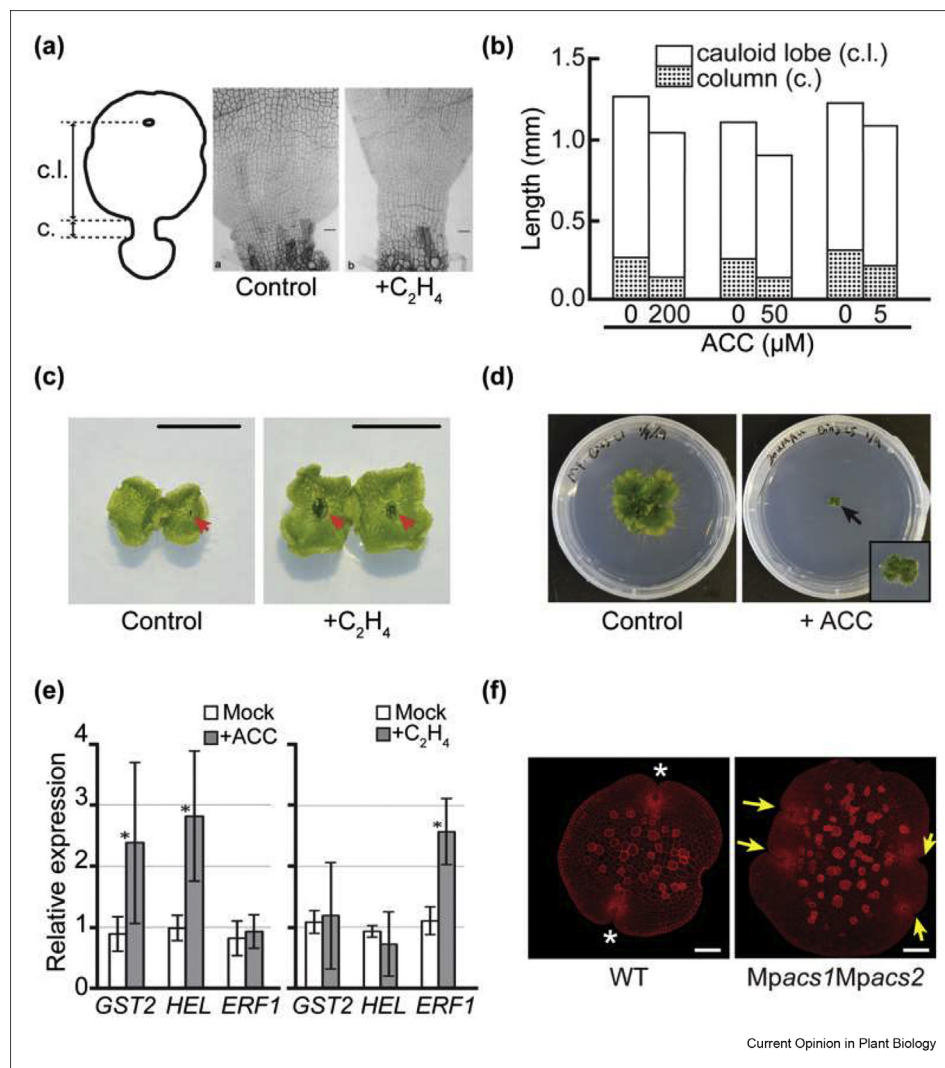
polyrhiza, which has retained the ethylene hormone pathways [27].

Evidence for ethylene-independent 1-amino-cyclopropane-1-carboxylic acid signaling in liverworts

The evidence that nonseed plants can have detectable ACC, but do not necessarily rely on ACC for ethylene production, is compatible with ACC having unique

signaling functions independent from its role as the ethylene precursor. In 1989, contradictory effects were reported for ethylene and ACC treatments in the liverwort *R. helicophylla*, ethylene induced cell elongation in the column region of gemmalings (Figure 3a [28]), whereas ACC (5–200 μ M) inhibited cell division and reduced column length (Figure 3b [29]). Distinct ethylene and ACC responses were recently reported in another liverwort *M. polymorpha* [10••,11••]. Ethylene

Figure 3



Distinct ethylene and ACC responses in liverworts. (a) Ethylene (C₂H₄) treatment (10 ppm for 72 h) enhances column elongation in *R. helicophylla* gemmalings. Scale bars = 50 μ m. Diagram on the left shows the gemmaling cauloid lobe (c.l.) and the column (c.) regions. Photographs reproduced from Stange and Osborne [28] with permission. (b) ACC treatment inhibits the elongation growth of column cells in *R. helicophylla* gemmalings. The Y-axis indicates the lengths of both the cauloid lobe and the column. Modified from Stange and Osborne [29] with permission. (c) Ethylene treatment (100 ppm) increases thallus size and stimulates the formation of gemma cups (indicated by red arrows) in *M. polymorpha*. Reproduced from Li et al. [10••] with permission. Scale bars = 1 cm. (d) ACC treatment (20 μ M) inhibits thallus growth of the ethylene-insensitive mutant *Mpein3* in *M. polymorpha*. Reproduced from Li et al. [10••] with permission. (e) Treatment with ACC (100 μ M for 1 d) versus ethylene (50 ppm for 1 d) induces differential expression of three *M. polymorpha* genes (*GST2*, *HEL*, and *ERF1*). Adapted from Katayose et al. [11••] with permission. (f) A gemma of the *M. polymorpha* *Mpacs1Mpacs2* double mutant shows abnormal apical notches (meristems) in terms of their number, shape, and position (indicated by yellow arrows) compared with wild-type notches (indicated by white asterisks). Scale bars = 100 μ m. Reproduced from Li et al. [10••] with permission. ACC, 1-amino-cyclopropane-1-carboxylic acid.

treatment promoted thallus growth (Figure 3c [10••,11••]), which was also shown by analyzing knockout mutants of positive (*Mpein3*) and negative (*Mpctr1*) regulators in the ethylene signaling pathway [10••]. The phenotypes of these mutants additionally indicated that ethylene promotes both cell division and cell enlargement in the gemma epidermis [10••]. In contrast to ethylene, ACC treatment (100 μ M) suppressed thallus growth of the wild type [10••,11••]. Notably, this response was observed even in ethylene-insensitive *Mpein3* mutants using lower doses (e.g. 10 μ M ACC) (Figure 3d [10••]). In *Mpein3* gemmae, ACC treatment (100 μ M) resulted in significantly fewer newly generated epidermal cells with no difference in cell size, suggesting that ACC inhibits cell division but not cell expansion [10••]. By studying gene expression, Katayose et al. [11••] found that the ethylene-induced transcription factor gene, *MpERF1*, is not induced by ACC treatment (100 μ M) and conversely identified two genes (*MpGST2* and *MpHEL*) induced by ACC treatment but not ethylene (Figure 3e).

Single knockout mutants of each of the *MpACS* homologs produced less ACC and were larger in thallus size than those of the wild type. Notably, the single (*Mpacs2*) and double (*Mpacs1Mpacs2*) mutant gemmae displayed a higher frequency of abnormal apical notches (meristems), which was not seen in ethylene-insensitive mutants (Figure 3f [10••]). This provides evidence for endogenous ACC having a signaling role in liverworts, and together with the other findings in nonseed plants, suggests the possibility that, evolutionarily, ACC was a signal before serving as the ethylene biosynthesis precursor.

1-amino-cyclopropane-1-carboxylic acid signaling roles in angiosperms

ACC has various roles as an ethylene-independent signal even in *A. thaliana*, an angiosperm in which ACC is unquestionably the ethylene precursor. The untangling of responses that are induced by ACC versus ethylene in *A. thaliana* has relied on an array of genetic tools (such as well-characterized ethylene signaling mutants) and pharmacological tools (including chemical inhibitors of ethylene biosynthesis and ethylene perception) [30], with the caveat that chemical inhibitors can have unknown actions and side effects. In this section, all of the studies in which exogenous ACC was applied used concentrations that were likely to be in the physiological range.

ACC was first implicated as an ethylene-independent signal in *A. thaliana* in the regulation of root cell wall biosynthesis via the FEI pathway [31]. FEI1 and FEI2 are leucine-rich repeat receptor-like kinases, and the *fei1fei2* double mutant has shorter and swollen roots in

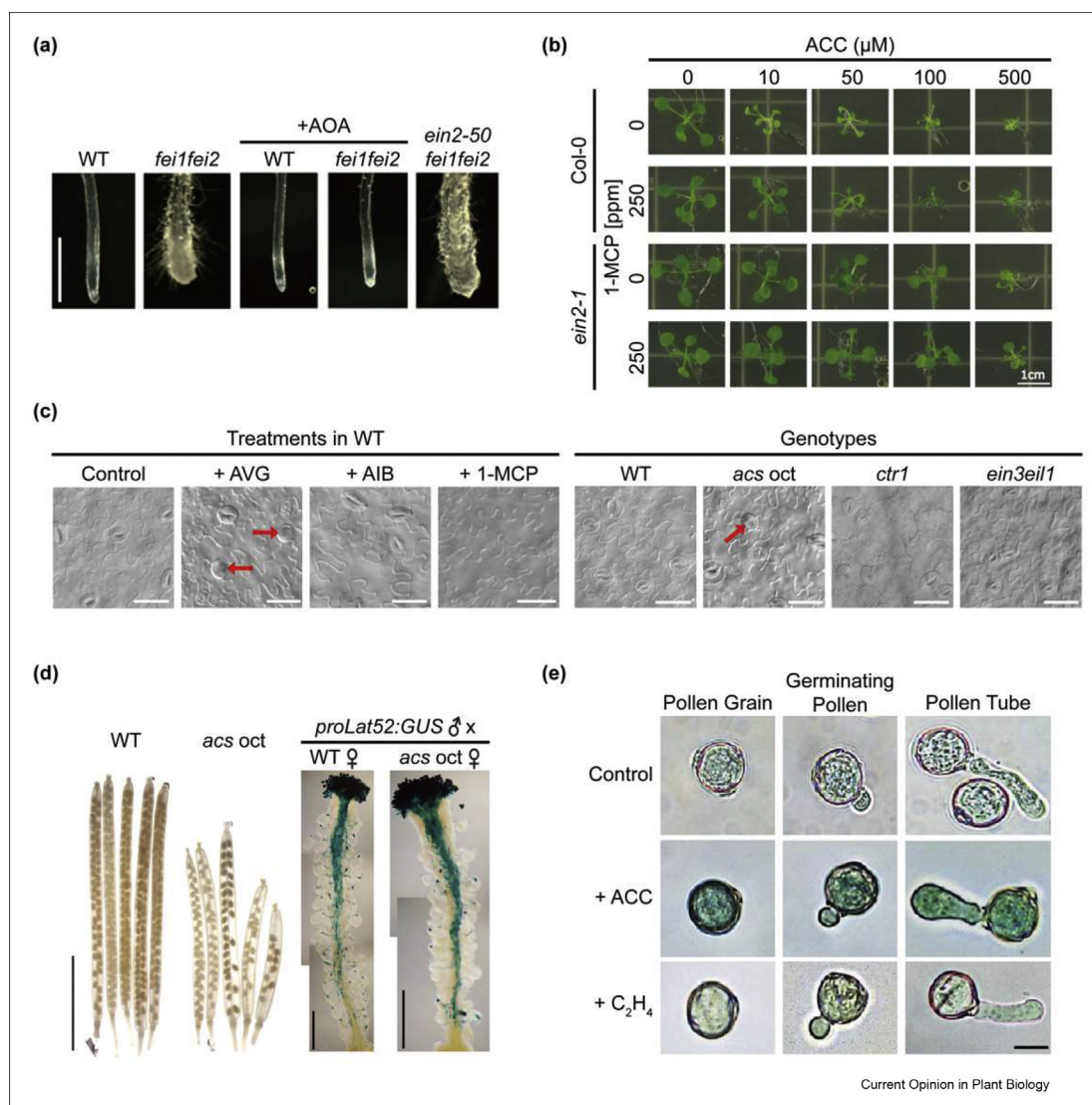
the presence of high concentrations of sucrose or salt resulting from defective cellulose synthesis. The anisotropic expansion defect in the roots was rescued by treatment with either the ACS inhibitor 2-aminooxyacetic acid (AOA) or the ACC structural analog α -aminoisobutyric acid (AIB), which has been typically used as an ACO inhibitor [31]. However, the defect was neither reverted by blocking ethylene perception with 1-methylcyclopropene (1-MCP) or silver ions nor by using mutations that confer ethylene insensitivity (Figure 4a [31]). The authors, therefore, hypothesized that ACC could be a signal that regulates root cell expansion. To explain the rescue of the *fei1fei2* defect by AIB, the authors proposed that AIB acted as a competitive inhibitor of an unknown ACC receptor.

Additional evidence for ACC having a role in cell expansion involved the cellulose synthase inhibitor isoxaben, which causes root swelling and shortening. Root elongation was restored by the ACS inhibitors AOA, aminoethoxyvinylglycine (AVG), and 2-anilino-7-(4-methoxyphenyl)-7,8-dihydro-5(6H)-quinazolinone (7303) or the ACO inhibitor AIB but not by blocking ethylene perception (with silver ions or norbornadiene) [32]. Similar to isoxaben, ACC treatment induced a short-term inhibition of root elongation of the ethylene-insensitive mutant *ein3eil1*, indicating that ACC, not ethylene, is involved in the root response to cell wall damage stress [32]. Both auxin signaling and superoxide production function downstream of ACC to inhibit root elongation [32].

In a major advance in 2009, genetic evidence for ACC having ethylene-independent signaling roles was obtained through the creation of an *A. thaliana* *acs* octuple mutant in which all eight functional *ACS* genes were disrupted [33]. Because there is incomplete silencing of two of the *ACS* genes in this *acs* octuple mutant, a true knockout mutant of ACC synthesis has yet to be obtained. Nevertheless, as expected, the *acs* octuple mutant displayed phenotypes shared by ethylene-insensitive mutants (e.g., an initial growth delay and delayed senescence) but also had phenotypes that appeared unrelated to having reduced ethylene levels (e.g., smaller cotyledons, a shorter primary root with a proliferation of root hairs, downward curling leaf tips, reduced branching, early flowering, shorter siliques, and fewer seeds) [33].

More recently, some of these potential ACC-specific phenotypes have been investigated in more detail. In the light, ACC treatment inhibited growth of the rosette (50–100 μ M) and primary root (10–50 μ M), as observed when ethylene signaling was blocked by 1-MCP treatment of the wild type or by the *ein2-1* ethylene-insensitive mutation (Figure 4b [34•]). In the dark, ACC treatment (1 μ M) reduced hypocotyl

Figure 4



Ethylene-independent responses of ACC in angiosperms. **(a)** ACC regulates anisotropic expansion of *A. thaliana* root cells via the FEI pathway. The swollen root tip of the *fei1fei2* mutant is rescued by inhibiting ACC biosynthesis (using AOA treatment; 0.375 mM) but is not reverted in an ethylene-insensitive mutant (*ein2-50*). Scale bar = 1 mm. Reproduced from Xu et al. [31] under the Creative Commons CC BY license. **(b)** ACC inhibits the rosette growth of light-grown *A. thaliana* seedlings when ethylene signaling is either chemically blocked by 1-MCP (250 ppm) treatment or genetically blocked by the *ein2-1* mutation. Reproduced from Van Der Straeten et al. [34] under the Creative Commons CC BY license. **(c)** ACC signaling is essential for the symmetric division of *A. thaliana* guard mother cells to develop normal stomata. Reduced ACC synthesis by AVG treatment (25 μ M) leads to the formation of single guard cells (SGC, indicated by red arrows), which are not observed when treated with either AIB (25 μ M) or 1-MCP. Similar SGCs are observed in the *acs* octuple (*oct*) mutant but not in the constitutive ethylene-response mutant *ctr1* nor in the ethylene-insensitive mutant *ein3eil1*. Scale bar = 20 μ m. Modified from Yin et al. [35] under the Creative Commons CC BY license. **(d)** ACC plays a role in *A. thaliana* ovular pollen tube attraction. Left side: The *acs octuple* (*oct*) mutant has a reduced seed set compared with the wild type. Scale bar = 5 mm. Right side: The basis for the reduced seed number is that fewer ovules in the *acs oct* pistil are capable of attracting pollen tubes than that of the wild-type ovules. Pistils were hand pollinated with wild-type pollen expressing β -glucuronidase (GUS) from a pollen-specific promoter (*proLAT52::GUS*) then stained for GUS activity. Each blue dot of GUS staining within the ovule indicates fertilization by the pollen. Scale bar = 0.5 mm. Adapted from Mou et al. under the Creative Commons CC BY license. **(e)** ACC treatment stimulates expression of an ethylene-responsive EBS:GUS reporter in pollen grains, germinating pollen, and pollen tubes of the *Never-ripe* (ethylene-insensitive) mutant of tomato. The control consisted of no treatment. Scale bar = 30 μ m. Reproduced from Althiab-Almasaud et al. [37] with permission. ACC, 1-amino-cyclopropane-1-carboxylic acid; AIB, α -aminoisobutyric acid; AOA, 2-aminooxycetic acid; AVG, aminoethoxyvinylglycine; EBS, EIN3 binding site; 1-MCP, 1-methylcyclopropene.

elongation and primary root growth in wild-type and *ein2-1* seedlings in the presence of AIB, which presumably inhibited ethylene biosynthesis as an antagonist of

ACO; however, AIB acting as an agonist or antagonist of a hypothetical ACC receptor has not been ruled out [34].

ACC also modulates the symmetric division of guard mother cells (GMCs) into two guard cells (GCs) during stomatal development. Treatment of epidermal cells with AVG led to the formation of single guard cells, whereas no response was observed when ACO activity was blocked (using AIB or Co^{2+}), neither in the wild type treated with 1-MCP nor in ethylene-insensitive mutants (Figure 4c [35•]). A similar single guard cell defect was observed in the *acs* octuple mutant, and treatment with ACC (10 μM) partially rescued the defect, whereas ethylene had no effect [35•]. ACC was found to regulate GMC division via a cell cycle-dependent pathway [35•].

Whereas Tsuchisaka et al. [33] proposed that embryonic lethality was an underlying cause of the lower number of seeds in the *acs* octuple mutant, a recent study determined that the reduced seed set was the result of reduced pollen tube attraction by the octuple mutant ovules (Figure 4d [36••]). Mou et al. [36••] found that ACC in the sporophytic tissue of the ovules plays an ethylene-independent role in pollen tube attraction. Compared with wild-type ovules, a higher proportion of *acs* octuple mutant ovules showed greater retention of the LURE1.2 peptide in the synergid cells instead of trafficking to the filiform apparatus or micropyle where LURE1.2 (along with other LURE1 peptides) is known to serve as a pollen tube attractant [36••]. ACC treatment (1 μM) of *acs* octuple ovules promoted LURE1.2-eGFP secretion and restored pollen tube attraction, whereas ethylene did not [36••].

ACC might play a role in pollen tube growth as well. In *Solanum lycopersicum*, *in vitro* pollen tube growth was promoted by applying low concentrations of ACC (0.1–100 μM) when ethylene receptor signaling was inhibited [37•]. Unlike the ACC-specific responses discussed previously, promotion of tomato pollen tube growth can be induced by both ethylene and ACC. Notably, treating pollen grains and pollen tubes with ACC stimulated the expression of an ethylene-responsive reporter fusion (EIN3-binding site: β -glucuronidase) either in the ethylene-insensitive *Never-ripe* mutant (e.g. Figure 4e) or in presence of 1-MCP, suggesting that ACC might activate ethylene-response signaling at a point downstream of the ethylene receptor [37•].

Speculation on 1-amino-cyclopropane-1-carboxylic acid signaling mechanisms

A candidate 1-amino-cyclopropane-1-carboxylic acid receptor

The ethylene-independent functions of ACC above suggest that plants have ACC-specific signaling pathways. Mou et al. [36••] raised the possibility that ACC could signal via the gating of glutamate receptor-like (GLR) ionotropic channels. GLRs are homologs of mammalian ionotropic glutamate receptors, which are

ligand-gated ion channels; when activated by glutamate and other amino acids, they allow cations to flow across the cell membrane [38]. ACC, a nonproteinogenic amino acid (not naturally present in animal tissues), was found to be a partial agonist of mammalian ionotropic glutamate receptors (e.g. studies reported by Wudick et al., Inanobe et al., and Kristensen et al. [38–40]). In plants, GLRs can be stimulated by various amino acids [41], but the physiological ligands remain unknown, and the roles of GLRs are only starting to come to light. Mou et al. [36••] demonstrated that ACC elicits GLR-dependent Ca^{2+} -containing ion currents in *A. thaliana* root protoplasts (using 250–500 μM ACC) and triggers transient cytosolic Ca^{2+} elevation in ovules (using 500 μM ACC). Furthermore, in a mammalian cell expression system, ACC treatment (500 μM) stimulated *P. patens* PpGLR1-dependent elevation of cytosolic Ca^{2+} fluxes to a greater extent than any of the 20 proteinogenic amino acids. (The high concentrations of ACC used as a ligand in these studies are typical of electrophysiological studies and imaging in heterologous systems.) The LURE1.2 secretion defect in *acs* octuple ovules was rescued by increasing cellular Ca^{2+} levels with a Ca^{2+} ionophore, thus leading Mou et al. [36••] to propose that ACC might serve as a GLR-gating ligand that induces Ca^{2+} elevation in the ovule, which then promotes LURE1.2 secretion [36••]. Whether ACC signaling operates through GLRs and Ca^{2+} spikes that evoke downstream responses requires further investigation. As implicated by other studies, downstream signaling components of ACC signaling appear to involve auxin signaling [31], superoxide production [31], and/or the cell cycle [35•].

1-amino-cyclopropane-1-carboxylic acid as an internal or external sensor?

We speculate that ACC, which is typically present at low concentrations, may conceivably reflect cellular metabolic homeostasis, similar to how the nonproteinogenic amino acid GABA is considered to be a proxy for the plant cell nitrogen or amino acid content [42] or how trehalose-6-phosphate, a low-abundant sugar phosphate, mirrors sucrose levels in plants and controls the energy status [43]. Although this has not been investigated, ACC might have a nitrogen or metabolic sensing function, perhaps, linked with changes in Ca^{2+} fluxes caused by GLR channels. *A. thaliana* has 20 GLRs with distinct subcellular membrane localizations, raising the question of whether GLR-mediated Ca^{2+} fluxes could be linked to ACC-specific responses in different organs.

ACC, or one of its derivatives [4], could potentially serve in a role outside of the cell. This idea is corroborated by the identification of γ -glutamyl-ACC, a low abundance ACC conjugate [44] that is made in the apoplast by γ -glutamyl transpeptidase in *A. thaliana* [45]. Secreted ACC could serve as a cellular messenger to alter the extracellular environment to control, for

example, cell wall integrity and/or flexibility, perhaps involving plasma membrane-localized GLRs. Other extracellular Ca^{2+} channels in plants operate as environmental sensors, such as the Na^+ sensor monocation-induced $[\text{Ca}^{2+}]_i$ increases1 (MOCA1) [46] and the osmotic sensor reduced hyperosmolality-induced $[\text{Ca}^{2+}]_i$ increase1 (OSCA1) [47]. Apoplastic ACC sensing might play a role in plant–microbe interactions, as certain beneficial bacteria are known to consume ACC as a nitrogen source using ACC deaminase [48]; so, perhaps, apoplastic ACC can modulate the symbiotic interaction with plant growth-promoting bacteria.

Dual 1-amino-cyclopropane-1-carboxylic acid functions

How might seed plants use ACC for both ethylene production and independent signaling? The function of ACC could be dependent on ACO expression/activity for ethylene production and the availability of ACC signaling components. A switch between ACC signaling and ethylene production could conceivably involve feedback mechanisms based on ACC and/or ethylene levels. ACC levels are controlled through ACC homeostasis, which is mainly regulated through synthesis (by ACS), conversion to ethylene, conjugation to malonyl, γ -glutamyl, or jasmonyl ACC [4], transport (e.g. via lysine histidine transporter 1 (LHT1) and LHT2; [49,50]) and storage (e.g. in the vacuole [51]). There might also be feedback mechanisms involving an interplay of ACC and ethylene based on their downstream responses.

Conclusions and future directions

It is increasingly clear that ACC can function as a signaling molecule independent of its role as the ethylene precursor. ACC is active at low concentrations (e.g. 1 μM), can induce local or distant responses (e.g. Figure 4b), and can be transported via LHT1 and LHT2. Current data suggest that ACC may have been a signaling molecule before acquiring its predominant role in ethylene biosynthesis. This scenario has some similarities with the biosynthesis of the plant hormone jasmonate (JA) in that bryophytes lack the enzymes that convert the precursor dinor-12-oxo-phytodienoic acid (dn-OPDA) to JA-Ile, and dn-OPDA itself serves as a signaling molecule. However, unlike dn-OPDA, which is the ligand of a coevolved form of the JA receptor COI1 [52], there is no evidence that ACC signals through the ethylene receptors, given that ACC and ethylene responses are generally distinct, and ethylene-insensitive mutants still respond to ACC.

Given the role of ACC as a signal, caution is required when using ACC as a substitute for ethylene treatment. In maize, for example, ACC-induced resistance to the fungal pathogen *Fusarium graminearum* was attributed to ethylene but could have been caused by ACC [53]. In the red alga *Pyropia yezoensis*, ACC-induced (50 μM)

sexual reproduction was attributed to ethylene [14], whereas subsequent findings suggest that the response was likely caused by ACC [14,54]. This highlights the importance of verifying the effects of exogenous ACC using ethylene gas. Some other limitations of treating plants with ACC, particularly when investigating ACC signaling responses, include not knowing the fate of the ACC taken up by the plant and distinguishing between ACC signaling and a stress/toxicity response at particular doses of ACC.

There are a number of future directions for the emerging topic of ACC signaling. The likely identification of additional ACC responses in the plant lineage will provide further impetus for a new focal point on the elucidation of ACC signaling pathways. This will include how ACC signaling is regulated, particularly in seed plants that rely on ACC for ethylene production, and whether there is interplay with ethylene signaling and other plant hormones. In nonseed plants, there are basic questions concerning whether endogenous ACC is synthesized and transported, how ACC is converted to ethylene, and what the primary mechanisms of non-ACC-based ethylene production are. An interesting broader question involves the evolutionary relationships between ACC biosynthesis, ethylene biosynthesis, ACC signaling, and ethylene signaling.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Abeles FB, Morgan PW, Saltveit Jr ME: *Ethylene in plant biology*. 2nd ed. Academic Press; 2012.
2. Ju C, Van de Poel B, Cooper ED, Thierier JH, Gibbons TR, Delwiche CF, Chang C: **Conservation of ethylene as a plant hormone over 450 million years of evolution**. *Native Plants* 2015, **1**:14004.
3. Mount SM, Chang C: **Evidence for a plastid origin of plant ethylene receptor genes**. *Plant Physiol* 2002, **130**:10–14.
4. Pattyn J, Vaughan-Hirsch J, Van de Poel B: **The regulation of ethylene biosynthesis: a complex multilevel control circuitry**. *New Phytol* 2021, **229**:770–782.
5. Park C, Lee HY, Yoon GM: **The regulation of ACC synthase protein turnover: a rapid route for modulating plant**

- development and stress responses. *Curr Opin Plant Biol* 2021, **63**:102046.
6. Li FW, Brouwer P, Carretero-Paulet L, Cheng S, de Vries J, Delaux PM, Eily A, Koppers N, Kuo LY, Li Z, *et al.*: Fern genomes elucidate land plant evolution and cyanobacterial symbioses. *Native Plants* 2018, **4**:460–472.
 7. Chernys J, Kende H: Ethylene biosynthesis in *Regnellidium diphyllum* and *Marsilea quadrifolia*. *Planta* 1996, **200**:113–118.
 8. Osborne DJ, Walters J, Milborrow BV, Norville A, Stange LMC: Evidence for a non-ACC ethylene biosynthesis pathway in lower plants. *Phytochemistry* 1996, **42**:51–60.
 9. Rohwer F, Bopp M: Ethylene synthesis in moss protonema. *J Plant Physiol* 1985, **117**:331–338.
 10. Li DD, Flores-Sandoval E, Ahtesham U, Coleman A, Clay JM, Bowman JL, Chang C: Ethylene-independent functions of the ethylene precursor ACC in *Marchantia polymorpha*. *Nature Plants* 2020, **6**:1335–1344.
- In this study, ethylene and ACC responses in the liverwort *Marchantia polymorpha* are described and found to be distinct, based on ethylene and ACC treatments, as well as on analyses of CRISPR/Cas9-generated mutants in predicted ethylene signaling and ACS genes. Genetic analyses of *Mpacs1Mpacs2* double mutants confirmed that ACC is not primarily used for ethylene biosynthesis, but has its own ethylene-independent roles in aspects of growth and development, such as the size and number of gemmae cups and the development of apical notches (meristems).
11. Katayose A, Kanda A, Kubo Y, Takahashi T, Motose H: Distinct functions of ethylene and ACC in the basal land plant *Marchantia polymorpha*. *Plant Cell Physiol* 2021, **62**:858–871.
- Distinct functions of ethylene and ACC are demonstrated in the liverwort *Marchantia polymorpha*, based on ethylene and ACC treatments. Additionally, ACC treatment is found to activate the expression of genes not induced by ethylene. *Mpacs1Mpacs2* double mutants do not show a decrease in ACC levels (nor in ethylene levels) nor do they display any obvious phenotypes during vegetative thallus growth, leading to the conclusions that these ACS homologs are unessential for ACC and ethylene production, and do not play substantial roles in thallus growth.
12. Sun L, Dong H, Nasrullah, Mei Y, Wang NN: Functional investigation of two 1-aminocyclopropane-1-carboxylate (ACC) synthase-like genes in the moss *Physcomitrella patens*. *Plant Cell Rep* 2016, **35**:817–830.
 13. Kwa SH, Wee YC, Kumar PP: Role of ethylene in the production of sporophytes from *Platyserium coronarium* (Koenig) Desv. frond and rhizome pieces cultured in vitro. *J Plant Growth Regul* 1995, **14**:183–189.
 14. Uji T, Matsuda R, Takechi K, Takano H, Mizuta H, Takio S: Ethylene regulation of sexual reproduction in the marine red alga *Pyropia yezoensis* (Rhodophyta). *J Appl Phycol* 2016, **28**:3501–3509.
 15. Vanden Driessche T, Kevers C, Collet M, Gaspar T: *Acetabularia mediterranea* and ethylene production in relation with development, circadian rhythm in emission and response to external application. *J Plant Physiol* 1988, **133**:635–639.
 16. Maillard P, Thepenier C, Gudin C: Determination of an ethylene biosynthesis pathway in the unicellular green-alga, *Haematococcus-Pluvialis* - relationship between growth and ethylene production. *J Appl Phycol* 1993, **5**:93–98.
 17. Plettner I, Steinke M, Malin G: Ethene (ethylene) production in the marine macroalga *Ulva* (Enteromorpha) *intestinalis* L. (Chlorophyta, Ulvophyceae): effect of light-stress and co-production with dimethyl sulphide. *Plant Cell Environ* 2005, **28**:1136–1145.
 18. Tittle FL: Auxin-stimulated ethylene production in fern gametophytes and sporophytes. *Physiol Plantarum* 1987, **70**:499–502.
 19. Nagahama K, Ogawa T, Fujii T, Fukuda H: Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene. *J Ferment Bioeng* 1992, **73**:1–5.
 20. Shipston N, Bunch AW: The physiology of L-methionine catabolism to the secondary metabolite ethylene by *Escherichia coli*. *J Gen Microbiol* 1989, **135**:1489–1497.
 21. Lee CC, Hu YL, Ribbe MW: Vanadium nitrogenase reduces CO. *Science* 2010, **329**:642–642.
 22. Hu YL, Lee CC, Ribbe MW: Extending the carbon chain: hydrocarbon formation catalyzed by vanadium/molybdenum nitrogenases. *Science* 2011, **333**:753–755.
 23. North JA, Narrowe AB, Xiong WL, Byerly KM, Zhao GQ, Young SJ, Murali S, Wildenthal JA, Cannon WR, Wrighton KC, *et al.*: A nitrogenase-like enzyme system catalyzes methionine, ethylene, and methane biogenesis. *Science* 2020, **369**:1094–1098.
 24. Summers JE, Voeselek L, Blom C, Lewis MJ, Jackson MB: *Potamogeton pectinatus* is constitutively incapable of synthesizing ethylene and lacks 1-aminocyclopropane-1-carboxylic acid oxidase. *Plant Physiol* 1996, **111**:901–908.
 25. Golicz AA, Schliep M, Lee HT, Larkum AW, Dolferus R, Batley J, Chan CK, Sablok G, Ralph PJ, Edwards D: Genome-wide survey of the seagrass *Zostera muelleri* suggests modification of the ethylene signalling network. *J Exp Bot* 2015, **66**:1489–1498.
 26. Olsen JL, Rouze P, Verhelst B, Lin YC, Bayer T, Collen J, Dattolo E, De Paoli E, Dittami S, Maumus F, *et al.*: The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature* 2016, **530**:331–335.
 27. Wang W, Haberer G, Gundlach H, Glasser C, Nussbaumer T, Luo MC, Lomsadze A, Borodovsky M, Kerstetter RA, Shanklin J, *et al.*: The *Spirodela polyrrhiza* genome reveals insights into its neotenus reduction fast growth and aquatic lifestyle. *Nat Commun* 2014, **5**:3311.
 28. Stange L, Osborne DJ: Cell specificity in auxin- and ethylene-induced 'supergrowth' in *Riella helicophylla*. *Planta* 1988, **175**:341–347.
 29. Stange LMC, Osborne DJ: Contrary effects of ethylene and ACC on cell growth in the liverwort *Riella helicophylla*. In *Biochemical and physiological aspects of ethylene production in lower and higher plants*. Edited by Clijsters H, de Proft M, Marcelle R, van Poucke M, Springer; 1989:341–348.
 30. Depaepe T, Van Der Straeten D: Tools of the ethylene trade: a chemical kit to influence ethylene responses in plants and its use in agriculture. *Small Methods* 2020, **4**:1900267.
 31. Xu SL, Rahman A, Baskin TI, Kieber JJ: Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in *Arabidopsis*. *Plant Cell* 2008, **20**:3065–3079.
 32. Tsang DL, Edmond C, Harrington JL, Nuhse TS: Cell wall integrity controls root elongation via a general 1-aminocyclopropane-1-carboxylic acid-dependent, ethylene-independent pathway. *Plant Physiol* 2011, **156**:596–604.
 33. Tsuchisaka A, Yu G, Jin H, Alonso JM, Ecker JR, Zhang X, Gao S, Theologis A: A combinatorial interplay among the 1-aminocyclopropane-1-carboxylate isoforms regulates ethylene biosynthesis in *Arabidopsis thaliana*. *Genetics* 2009, **183**:979–1003.
 34. Vanderstraeten L, Depaepe T, Bertrand S, Van Der Straeten D: The ethylene precursor ACC affects early vegetative development independently of ethylene signaling. *Front Plant Sci* 2019, **10**:1591.
- In this study, genetic and chemical analyses reveal several distinct responses between ACC and ethylene in both light- and dark-grown seedlings of *Arabidopsis thaliana*. Independently of ethylene, ACC influences several vegetative developmental processes, such as inhibiting rosette growth and hypocotyl elongation.
35. Yin J, Zhang X, Zhang G, Wen Y, Liang G, Chen X: Amino-cyclopropane-1-carboxylic acid is a key regulator of guard mother cell terminal division in *Arabidopsis thaliana*. *J Exp Bot* 2019, **70**:897–908.
- This study uncovers an ethylene-independent role of ACC in *Arabidopsis thaliana* guard cell development. Using both chemical and genetic analyses, the authors find that ACC is required for the symmetrical division of the single guard mother cell into a fully functional two-celled guard cell.

36. Mou W, Kao YT, Michard E, Simon AA, Li D, Wudick MM, Lizzio MA, Feijo JA, Chang C: **Ethylene-independent signaling by the ethylene precursor ACC in Arabidopsis ovular pollen tube attraction.** *Nat Commun* 2020, **11**:4082.

This paper investigates the underlying basis for why the Arabidopsis *acs* octuple mutant has a reduced seed set, revealing that ACC in the sporophytic tissue of the ovules plays a role in pollen tube attraction by promoting the secretion of the peptide LURE1.2, a pollen tube chemoattractant, needed for successful fertilization. Furthermore, the paper showed that ACC can activate glutamate receptor-like (GLR) channels, triggering elevation of cytosolic Ca^{2+} levels that are involved in secretion of LURE2.1 peptide. ACC, a nonproteinogenic amino acid, induces the highest PpGLR1-dependent cytosolic Ca^{2+} elevation levels (in a heterologous expression system) compared to all twenty amino acids, raising the possibility that ACC is a physiological ligand for GLRs.

37. Althiab-Almasaud R, Sallanon H, Chang C, Chervin C: **1-aminocyclopropane-1-carboxylic acid (ACC) stimulates tomato pollen tube growth independently of ethylene receptors.** *Physiol Plantarum* 2021, <https://doi.org/10.1111/ppl.13579>.
This study reveals that treatment with either ethylene or ACC stimulates tomato pollen tube growth, yet ACC does so even when ethylene receptor signaling is inhibited. Moreover, ACC treatment stimulates expression of an ethylene-responsive *EBS:GUS* reporter in pollen grains and pollen tubes when the ethylene receptors are inhibited by 1-MCP or by the ethylene-insensitive Never-ripe mutation, indicating the possible activation of ethylene signaling by ACC downstream of the ethylene receptors.
38. Wudick MM, Michard E, Oliveira Nunes C, Feijó JA: **Comparing plant and animal glutamate receptors: common traits but different fates?** *J Exp Bot* 2018, **69**:4151–4163.
39. Inanobe A, Furukawa H, Gouaux E: **Mechanism of partial agonist action at the NR1 subunit of NMDA receptors.** *Neuron* 2005, **47**:71–84.
40. Kristensen AS, Hansen KB, Naur P, Olsen L, Kurtkaya NL, Dravid SM, Kvist T, Yi F, Pohlsgaard J, Clausen RP, et al.: **Pharmacology and structural analysis of ligand binding to the orthosteric site of glutamate-like GluD2 receptors.** *Mol Pharmacol* 2016, **89**:253–262.
41. Tapken D, Anshütz U, Liu L-H, Huelsken T, Seebohm G, Becker D, Hollmann M: **A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids.** *Sci Signal* 2013, **6**:ra47.
42. Barbosa JM, Singh NK, Cherry JH, Locy RD: **Nitrate uptake and utilization is modulated by exogenous gamma-aminobutyric acid in Arabidopsis thaliana seedlings.** *Plant Physiol Biochem* 2010, **48**:443–450.
43. Baena-Gonzalez E, Lunn JE: **SnRK1 and trehalose 6-phosphate - two ancient pathways converge to regulate plant metabolism and growth.** *Curr Opin Plant Biol* 2020, **55**:52–59.
44. Martin MN, Cohen JD, Saftner RA: **A new 1-aminocyclopropane-1-carboxylic acid-conjugating activity in tomato fruit.** *Plant Physiol* 1995, **109**:917–926.

45. Martin MN, Saladores PH, Lambert E, Hudson AO, Leustek T: **Localization of members of the gamma-glutamyl transpeptidase family identifies sites of glutathione and glutathione S-conjugate hydrolysis.** *Plant Physiol* 2007, **144**:1715–1732.
46. Jiang Z, Zhou X, Tao M, Yuan F, Liu L, Wu F, Wu X, Xiang Y, Niu Y, Liu F, et al.: **Plant cell-surface GIPC sphingolipids sense salt to trigger Ca^{2+} influx.** *Nature* 2019, **572**:341–346.
47. Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B, et al.: **OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis.** *Nature* 2014, **514**:367–371.
48. Glick BR: **Bacteria with ACC deaminase can promote plant growth and help to feed the world.** *Microbiol Res* 2014, **169**:30–39.
49. Shin K, Lee S, Song WY, Lee RA, Lee I, Ha K, Koo JC, Park SK, Nam HG, Lee Y, et al.: **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 Physiol* 2015, **56**:572–582.
50. Choi J, Eom S, Shin K, Lee RA, Choi S, Lee JH, Lee S, Soh MS: **Identification of lysine histidine transporter 2 as an 1-aminocyclopropane carboxylic acid transporter in Arabidopsis thaliana by transgenic complementation approach.** *Front Plant Sci* 2019, **10**:1092.
Little is known regarding ACC transport in plants. The authors follow-up on the prior discovery that the lysine histidine transporter, LHT1, can transport ACC, by now showing that overexpression of the LHT1 paralog, LHT2, rescues the *lht1* mutant, restoring uptake of ACC and rescuing an early senescence phenotype. In contrast, overexpression of *LHT3* and *AMINO ACID PERMEASE 5* (AAP5) do not confer rescue. Transport assays in *Xenopus laevis* oocytes confirmed that LHT1 and LHT2 transport ACC, but also transport overlapping sets of other amino acids.
51. Tophof S, Martinoia E, Kaiser G, Hartung W, Amrhein N: **Compartmentation and transport of 1-aminocyclopropane-1-carboxylic acid and N-malonyl-1-aminocyclopropane-1-carboxylic acid in barley and wheat mesophyll cells and protoplasts.** *Physiol Plantarum* 1989, **75**:333–339.
52. Monte I, Franco-Zorrilla JM, Garcia-Casado G, Zamarreno AM, Garcia-Mina JM, Nishihama R, Kohchi T, Solano R: **A single JAZ repressor controls the jasmonate pathway in Marchantia polymorpha.** *Mol Plant* 2019, **12**:185–198.
53. Zhou S, Zhang YK, Kremling KA, Ding Y, Bennett JS, Bae JS, Kim DK, Ackerman HH, Kolomiets MV, Schmelz EA, et al.: **Ethylene signaling regulates natural variation in the abundance of antifungal acetylated diferuloylsucroses and Fusarium graminearum resistance in maize seedling roots.** *New Phytol* 2019, **221**:2096–2111.
54. Endo H, Mizuta H, Uji T: **α -aminoisobutyric acid mimics the effect of 1-aminocyclopropane-1-carboxylic acid to promote sexual reproduction in the marine red alga Pyropia yezoensis (Rhodophyta).** *J Appl Phycol* 2021, **33**:1081–1087.