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The regulation of ACC synthase protein turnover: a rapid route for modulating plant development and stress responses

Chanung Park, Han Yong Lee and Gyeong Mee Yoon

Abstract

The phytohormone ethylene regulates plant growth, development, and stress responses. The strict fine-tuning of the regulation of ethylene biosynthesis contributes to the diverse roles of ethylene in plants. Pyridoxal 5′-phosphate–dependent 1-aminocyclopropane-1-carboxylic acid synthase, a rate-limiting enzyme in ethylene biosynthesis, is central and often rate-limiting to regulate ethylene concentration in plants. The post-translational regulation of ACS is a major pathway controlling ethylene biosynthesis in response to various stimuli. We conclude that the regulation of ACS turnover may serve as a central hub for the rapid integration of developmental, environmental, and hormonal signals, all of which influence plant growth and stress responses.

Addresses

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Ethylene biosynthesis, ACC synthases, Protein turnover regulation, Plant development and stress responses.

Introduction

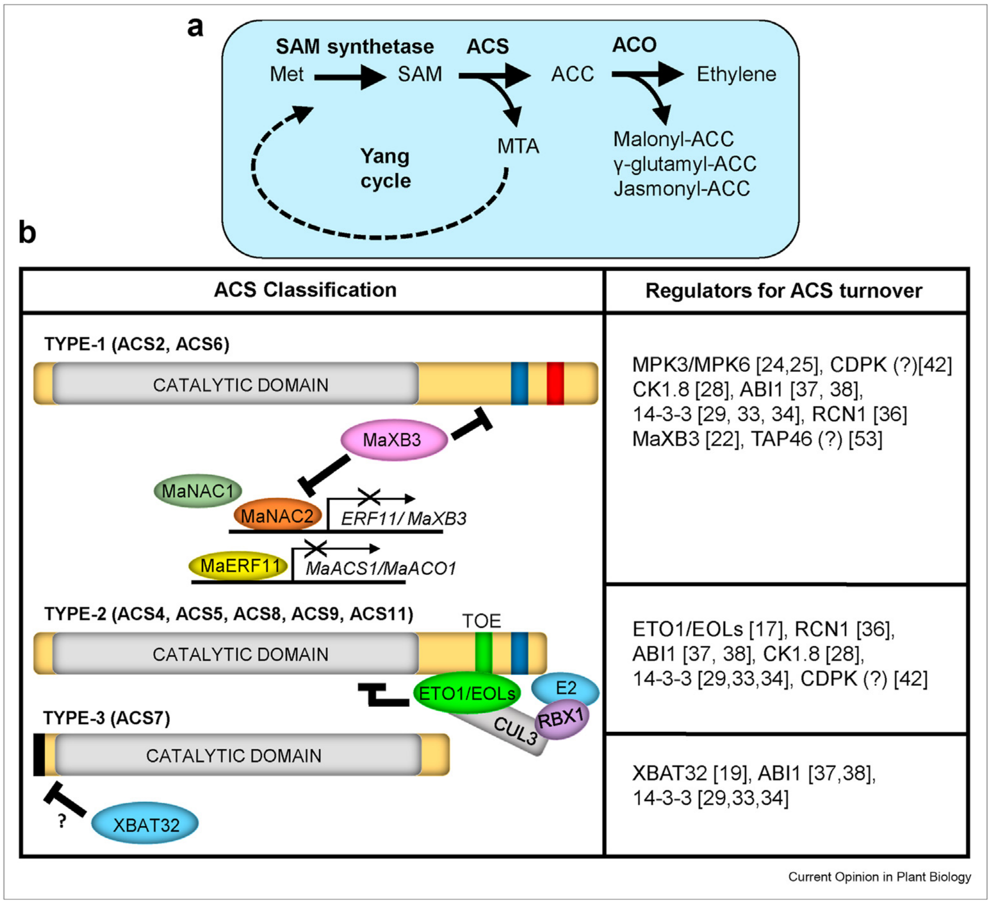
Recent studies have shown that the turnover regulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthases (ACS) in ethylene biosynthesis acts as a hub where internal and external signals governing many developmental and physiological responses merge. Deciphering the mechanisms through which plants regulate ACS protein turnover is a central challenge in understanding the role of ethylene in plant biology. Emerging evidence indicates that the turnover regulation of ACS is important in mediating various ethylene responses in plants, and that ACS turnover is controlled by complicated and multifaceted regulatory mechanisms. In this review, we highlight recent advances in the mechanistic understanding of ACS turnover regulation and the role of this regulation in a subset of plant development and stress responses. Readers are referred to other excellent reviews on recent progress in understanding of the transcriptional regulation of ethylene biosynthesis [1•,2].

Ethylene biosynthesis pathway

Ethylene, a simple hydrocarbon molecule, is derived from the amino acid methionine [3–5]. After catalysis by S-adenosylmethionine (SAM) synthetase (SAM synthetase), methionine is converted to SAM, which is then catalyzed to ACC and methylthioadenosine (MTA) by ACS (Figure 1) [3–5]. ACC is subsequently oxidized to ethylene by ACC oxidases (ACO), and MTA is recycled back through the Yang cycle, thus salvaging the methylthio group of MTA (Figure 1A) [6,7]. The ACS-dependent conversion of SAM to ACC is the first committed and rate-limiting step in ethylene biosynthesis, although ACO also serves as a rate-limiting step in certain developmental and stress contexts, including flowering, fruit ripening, cotton fiber cell elongation, and flooding [8–11]. The conjugation of ACC into ACC derivatives such as malonyl-ACC also affects ethylene biosynthesis by modulating free ACC pools at the metabolic level (Figure 1A) [12].

ACS is a pyridoxal 5′-phosphate–dependent enzyme in the aminotransferase superfamily [13]. In higher plants, it is generally encoded by a multigene family [13]. In *Arabidopsis*, ACS proteins are encoded by a family of 12 *ACS* genes, only eight of which are functionally active (ACS2, ACS4-9, and ACS11); the remaining ACS enzymes either are inactive or lack ACS activity [14,15]. The classification of eight functional ACS enzymes into three types has been clearly established only in Arabidopsis, on the basis of the presence or absence of phosphorylation sites in their C-termini (Figure 1B) [16]. Type-1 ACS enzymes (ACS2 and ACS6) have calcium-dependent protein kinase (CDPK) and mitogen-activated protein kinase (MPK) phosphorylation sites, whereas type-2 ACS enzymes (ACS4, ACS5, ACS8, ACS9, and ACS11) have only a phosphorylation

Figure 1



Ethylene biosynthesis pathway and the molecular regulators of ACS turnover. a. The ethylene biosynthetic pathway. The core ethylene biosynthetic pathway and the enzymes involved in the pathway are depicted. MTA, a byproduct of the pathway, is recycled through the Yang cycle, and ACC is conjugated to malonyl-ACC, γ-glutamyl ACC, and jasmonyl-ACC, in addition to being oxidized to ethylene. Met, methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; MTA, 5-methylthioadenosine; SAM, S-adenosyl methionine; ACS, ACC synthases; ACO, ACC oxidases. **b**. Model summarizing the three types of ACS isoforms classified in *Arabidopsis* and known molecular regulators of ACS turnover regulation. The regulatory domains, including phosphorylation sites, are located in the N- and C-termini of the ACS isoforms: the blue and red rectangles indicate the phosphorylation sites for CDPK and MPK, respectively. Phosphorylated forms of type-1 and type-2 are generally stable, yet CK1.8- and RCN1-mediated phosphorylation and dephosphorylation of ACS5, respectively, result in destabilization of ACS5. In addition to MaACS1 (type-1 ACS), MaXB3 E3 ligases destabilize MaNAC2. Together with MaNAC1, MaNAC2 inhibits the expression of *ERF11*, thus promoting the expression of *MaACS1* and *MaACO1*. MaNAC2 also suppresses *MaXB3* gene expression, thus providing a negative feedback mechanism for maintaining the protein homeostasis of MaNAC2, MaACS1, and MaACO1. The green rectangle at the C-terminus of type-2 ACS indicates the TOE motif for ETO1 binding. The black box at the N-terminus of type-3 ACS indicates the degradation sequences for the ACS. Whether XBAT32 regulates the degradation of type-3 ACS via the N-terminal degradation sequences remains unknown. Molecular regulators that influence the turnover of ACS are listed to the right of each ACS type. Question marks indicate indirect evidence of ACS turnover regulation and the numbers in brackets indicate reference number.

site for CDPKs and an E3 ligase-binding motif called target of ethylene-overproducing 1 (TOE) [13,16]. Ethylene-overproducing 1 (ETO1) and its paralogs ETO1-like 1 (EOL1) and EOL2 are substrate specificity subunits of the Skp, Cdc53p/Cul1, and F-box protein ubiquitin E3 ligase complex, which regulate the stability of type-2 ACS via the proteasome pathway [17,18]. Type-3 ACS (ACS7) enzyme has no known regulatory motifs (Figure 1). Current knowledge regarding the regulation of ACO in ethylene biosynthesis has been comprehensively discussed in a recent review [1•].

Mechanisms underlying ACS turnover regulation

Ubiquitination-mediated ACS turnover regulation

Analysis of the ethylene overproducer mutants *eto1*, *eto2*, and *eto3* led to the discovery of the protein turnover regulation of ACS [16,18]. The *eto2* and *eto3* mutations affect the C-termini of ACS5 and ACS9, respectively, thus decreasing the ACS turnover rate and consequently resulting in overproduction of ethylene [18]. Analysis of the *eto1* mutant has demonstrated that ACS5 turnover is regulated by the proteasome-dependent pathway, in which ETO1 acts as a substrate-specific adaptor protein for type-2 ACS degradation [17,18].

Whereas ETO1/EOL E3 ligases specifically regulate type-2 ACS turnover, XB3 ortholog 2 in *Arabidopsis* (XBAT32), a RING domain–containing E3 ligase, modulates the protein stability of type-3 ACS7 and type-2 ACS4 [19]. The *xbat32* mutation suppresses the ethylene overproducer phenotypes induced by the overexpression of ACS7 and increases ACS4 protein stability. ACS7 turnover is also negatively regulated by the first 14 amino acids in the N-terminus via the ubiquitin-proteasome pathway [20,21•]. Intriguingly, the N-terminus confers ACS7 protein instability only in light-grown seedlings, and ethylene treatment and salt stress suppress the N-terminus–mediated degradation of ACS7 protein. These results suggest that the degradation of ACS7 is regulated by developmental and environmental signals [20,21•]. In addition to XBAT32, a recent study has identified and characterized the role of *Musa acuminata* XA21 binding protein 3 (MaXB3), a banana ortholog of XBAT32, in ethylene biosynthesis regulation. MaXB3 regulates the stability of MaACS1 and MaACO1, and the overexpression of MaXB3 in a banana and tomato delays fruit ripening [22••]. MaXB3 also promotes the ubiquitination of banana NAC domain–containing protein 2 (MaNAC2), which positively controls the gene expression of *MaACS1* and *MaACO1*. Furthermore, MaNAC1/MaNAC2 represses *MaXB3* gene expression, thus providing a feedback mechanism that balances MaNAC2, MaACS1, and MaACO1 levels (Figure 1B) [22••]. These studies have revealed that multilayered cascades suppress ethylene biosynthesis at both the transcriptional and post-translational levels.

Phosphorylation- and dephosphorylation-mediated ACS turnover regulation

The phosphorylation of ACS was first implicated in tomato suspension cells treated with a kinase or phosphatase inhibitor [23]. Subsequently, molecular genetics and biochemical studies identified several molecular components and the underlying mechanisms governing phosphorylation-mediated ACS turnover regulation. For example, necrotrophic fungi *Botrytis cinerea*–activated MPK3/MPK6 increases ethylene biosynthesis by decreasing the turnover of ACS2/ACS6 via C-terminal phosphorylation [24,25]. Casein kinase 1.8 (CK1.8), a Ser/Thr kinase involved in blue light signaling [26], flowering [26], and brassinosteroid signaling [27], regulates the protein turnover of ACS5 by promoting interaction with ETO1 [28]. Additional evidence of phosphorylation-mediated ACS turnover regulation has come from the observation that ACS directly interacts with 14-3-3 proteins, a family of evolutionarily well-conserved regulatory proteins in eukaryotes [29,30]. The 14-3-3 protein modulates the activity, stability, and localization of the interacting proteins that are phosphorylated by upstream kinases [31,32]. The interaction of ACS with 14-3-3 has been observed in several plant species, including *Arabidopsis*, barley, and rice [29,33,34]. In *Arabidopsis*, 14-3-3ω, one of the 13 isoforms of 14-3-3, increases the stability of ACS5 by destabilizing ETO1/EOL E3 ligases [29]. Moreover, 14-3-3ψ, also known as rare cold inducible 1A (RCI1A), negatively regulates freezing tolerance by decreasing ACS stability via direct interaction [35]. The 14-3-3-mediated regulation of ACS turnover suggests the involvement of protein kinases that have yet to be identified.

Protein phosphatases such as roots curl in 1-N-naphthylphthalamic acid (RCN1) and ABA-insensitive 1 (ABI1) have been found to control ethylene biosynthesis. RCN1 interacts with and destabilizes type-1 ACS6; concordantly, ACS6 in the *rcn1* mutant shows a delayed turnover rate. By contrast, the *rcn1* mutation decreases the stability of type-2 ACS5, suggesting type-specific regulation of ACS stability by RCN1 and phosphorylation/dephosphorylation [36]. Similarly, ABI1 dephosphorylates the C-terminus of type-1 ACS6, thus destabilizing ACS6. ABI1 and ABI1-like protein phosphatase 2C are also involved in regulating ethylene biosynthesis via direct interaction with ACS7 [37,38].

Homodimerization- and heterodimerization-mediated ACS turnover regulation

ACS functions as homodimers or heterodimers by forming active sites via the interaction of residues in the monomeric subunit [39]. Subsequent studies have suggested that the eight functional ACS proteins may form as many as 45 different combinations of homodimers or heterodimers, only 25 of which are functional, owing to structural restraints that prevent the formation of functional active sites [15,39,40]. Although the roles of ACS homodimerization and heterodimerization in regulating ACS activity are recognized, their roles in ACS stability were unknown until very recently. Lee et al. have demonstrated that the homodimerization and heterodimerization of ACS modulate the stability of ACS enzymes [41••]. For example, the half-lives of type-1 ACS2 and ACS6, the most labile ACS isoforms, are significantly increased (∼5-fold) when these isoforms form heterodimers with ACS7 rather than cognate homodimers [41••]. Interestingly, the stability of ACS7 is not influenced by heteromerization with other ACSs [41••], suggesting that the expression patterns of *ACS7* contribute to the homodimerization- or heterodimerization-mediated regulation of ACS turnover.

The role of ACS turnover regulation in growth and development

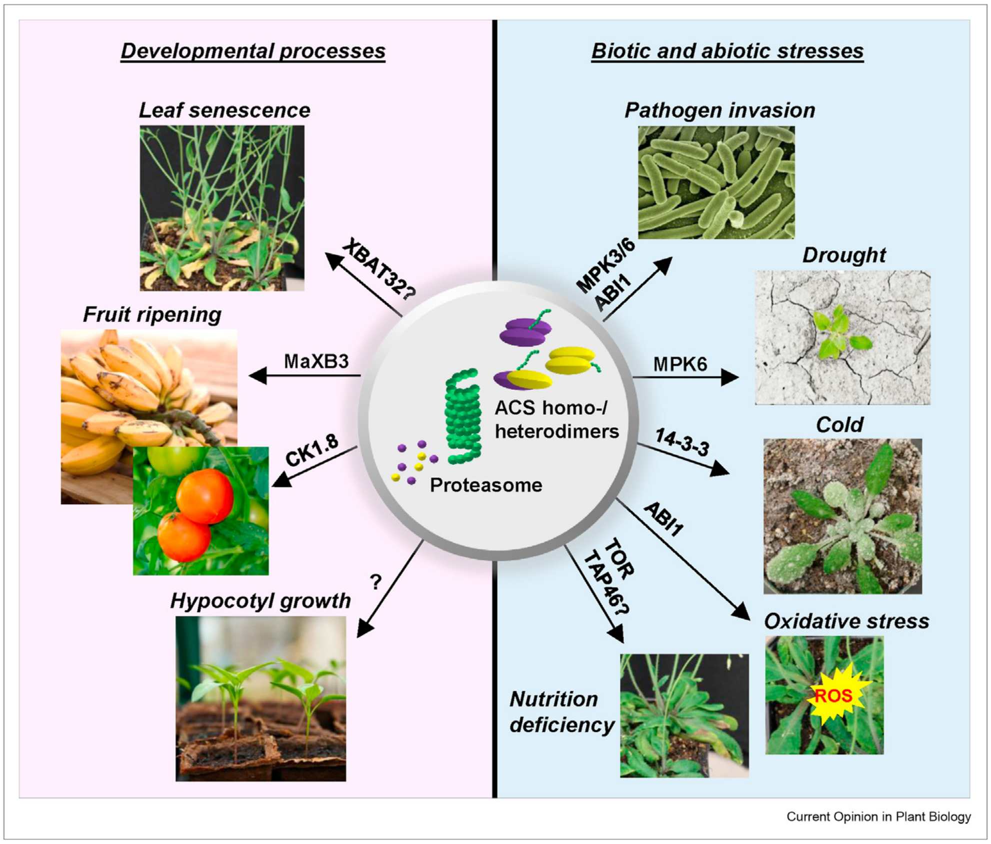
ACS turnover-regulated ripening in climacteric fruits

Studies in wounded ripening tomato discs (*Solanum lycopersicum*) first demonstrated the rapid turnover of ACS [23]. A radio-labeled ACS in a wounded tomato disc has been found to rapidly decay *in vivo*, with a half-life of approximately 58 min; this decay has been suggested to be mediated by an ATP-dependent process, such as the ubiquitin-dependent pathway [23]. A decade later, Kamiyushidara et al. showed that the phosphorylation of LeACS2 stabilizes LeACS2, thereby altering cellular ACS activity, ACC content, and *in vivo* ethylene levels [42]. Although the direct involvement of ACS turnover has not been demonstrated, overexpression of CK1.8 in tomatoes significantly delays tomato fruit ripening (Figure 2) [28], thus indirectly demonstrating the role of ACS turnover regulation in fruit ripening. Furthermore, an analysis of immunoprecipitated ^{32}P-labeled phosphorylated protein extracts using anti-MaACS1 antibody has shown relatively higher abundance of phosphorylated MaACS1 in ripe banana than in unripe banana [43]. A recent study also shows that overexpression of MaXB3 RING E3 ligase, the cognate E3 ligase for MaACS1 and MaACO1 in a banana and tomato, delays the ripening of both fruits (Figure 2) [22••].

ACS turnover–regulated hypocotyl elongation

Ethylene inhibits hypocotyl growth in the dark but stimulates it in the light [44–47]. The role of ACS turnover regulation in hypocotyl growth has been demonstrated in ethylene overproducer mutants (*eto1*, *eto2*, and *eto3*), which exhibit pronounced inhibition of

Figure 2



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ACS turnover regulation controls development and stress responses. The stability of ACS proteins is regulated via the proteasome pathway. Yellow and purple oval dimers indicate ACS homodimers or heterodimers. The green tails attached to ACS dimers indicate polyubiquitin chains and small purple and yellow dots represent digested ACS polypeptides. The listed developmental and stress responses involve ACS turnover regulation and associated regulatory components, shown above each arrow. The question marks indicate regulatory components with indirect or unknown evidence. XBAT32, XB3 ortholog 2 in *Arabidopsis*; MaXB3, *Musa acuminata* XA21 binding protein 3; MaNAC1 and MaNAC2, *Musa acuminata* NAC domain–containing protein 1 and 2; ERF11, ethylene response factor 11; MaACS1, *Musa acuminata ACC synthase*; *MaACO1, Musa acuminata ACC oxidase*; CK1.8, casein kinase 1.8; MPK3/6, mitogen-activated protein kinase 3 and 6; ABI1, ABA-insensitive 1; TOR, target of rapamycin; TAP46, type 2A phosphatase–associated protein of 46 kDa; ROS, reactive oxygen species. Free images from Pixabay were used to generate the figure.

hypocotyl elongation in the dark [18,48]. Etiolated seedlings treated with cytokinin also show inhibited hypocotyl growth due to the stabilization of ACS proteins [49]. In contrast to the role of ethylene in the dark, treatment with exogenous ethylene or ACC stimulates hypocotyl elongation in the light [46,47]; however, the role of ACS turnover regulation in this process remains unclear.

Seo and Yoon (2019) have shown that ACS stabilization stimulates the hypocotyl elongation of *Arabidopsis* seedlings during the dark-to-light transition [50•]. During this transition, light stabilizes ACS5, thus resulting in a transient increase in hypocotyl growth. Analyses of various single- and higher-order *acs* mutants have revealed that type-2 ACS4 and ACS9, but not type-1 or type-3 ACS, also play a role in the process [50•]. These results suggest an ACS isoform—specific role in light-induced hypocotyl growth. However, the underlying mechanism is unknown.

ACS turnover–regulated leaf senescence

The studies from Tsuchisaka et al. have provided a comprehensive view of the role of ACS isoforms and unmasked unique and overlapping function of various ACS isoforms in different developmental processes [15]. For example, the *Arabidopsis* octuple *acs* mutant exhibits significantly delayed leaf senescence compared with that in wild-type plants [15]. Although many studies have shown that the altered transcript levels of *ACS* affect plant senescence, which is induced by stress or aging, less is known about the role of ACS turnover regulation in the process. However, a recent study has shown that the rapid accumulation of ACS7 accelerates leaf senescence in *Arabidopsis*, in agreement with the delayed leaf senescence observed in the *acs7-1* mutant [21•]. Intriguingly, the accumulation of ACS7 protein depends on the age of the plant, which is regulated by the N-terminal degradation motif [20]. Seedlings overexpressing truncated ACS7 without the first 14 amino acids in the N-terminus show significantly earlier leaf senescence than seedlings expressing wild-type ACS7 [21•]. These results suggest that senescence signals negatively regulate the N-terminus—mediated degradation of ACS7, thereby increasing ethylene biosynthesis.

The role of ACS turnover regulation in plant stress responses

The MPK signaling pathway appears to regulate several stress responses that involve ACS turnover regulation (Figure 2). As described in the previous section, in *Arabidopsis Botrytis cinerea*-activated MPK3/6 stabilizes ACS2/ACS6 via the phosphorylation of the noncatalytic C-termini of ACSs, thus increasing ethylene production during pathogen infection [24,25]. The MPK pathway also controls drought response by modulating the stability of ACS in rose flowers [51]. Rehydration of rose flowers after 24 h of dehydration leads to a burst of ethylene production resulting from the accumulation of RhACS1/RhACS2 and enhanced RhMPK6 activity during the rehydration [51]. The protein phosphatase ABI1 negatively regulates the stability of ACS6 by dephosphorylating the C-terminus of ACS6, which is a target of MPK6 during oxidative stress (Figure 2) [37]. In addition, ABI1 controls the activity of MPK6, thus modulating the levels of phosphorylated ACS6 protein in plants [37]. Furthermore, under Fe-deficient conditions, the expression and activity of MPK3/MPK6 are enhanced, and *ACS2* and *ACS6* transcript levels are increased [52]. In line with the results, the *mpk3*, *mpk6*, and *acs2* mutants show a lower induction of ethylene biosynthesis under Fe deficiency [52]. Ye et al. have not examined the changes in the protein abundance of ACS2 and ACS6 [52]; however, in accordance with prior studies demonstrating the role of MPK3/MPK6 in ACS2/ACS6 protein stability, Fe deficiency may modulate the stability of ACS2 and ACS6. The role of ACS stabilization in sensing nutrient deficiency has also been implicated in a recent study demonstrating that the protein abundance of ACS2 and ACS6 is decreased in *Arabidopsis* seedlings under suppression of the target of rapamycin (TOR) signaling pathway, a major route controlling nutrition and energy levels in plants [53]. Zhuo et al. have shown that the suppression of TOR signaling inhibits hypocotyl growth in etiolated *Arabidopsis* seedlings, a response that may be associated with an increased protein abundance of ACS2 and ACS6 [53]. Intriguingly, ACS2/ACS6 interacts with type 2A phosphatase-associated protein of 46 kDa (TAP46), a downstream component of the TOR signaling pathway (Figure 2) [53]. Notably, RCN1, a regulatory A subunit of the type-2A phosphatase complex, negatively regulates the stability of ACS6. Although the functional connection between RCN1 and TAP46 is unknown and the TAP46-mediated destabilization of ACS2/ACS6 remains to be determined, these results suggest that the TOR signaling pathway may control ethylene biosynthesis, presumably in part via ACS turnover regulation.

Phytohormone-mediated ACS turnover regulation

The biosynthesis of ethylene is largely influenced by its interaction with other phytohormones [41,49]. Analysis of the cytokinin-insensitive 5 (*cin5*) mutant has revealed that cytokinin stimulates ethylene biosynthesis via ACS5 in etiolated seedlings [54]. Subsequent biochemical studies of seedlings expressing ACS5 protein have shown that not only cytokinin but also brassinosteroids increase ethylene production by stabilizing ACS5 [18,49]. A recent study has further demonstrated that other phytohormones, including auxin, abscisic acid

(ABA), gibberellic acid, methyl jasmonate (MJ), and salicylic acid, also modulate ACS protein turnover with distinct effects on different ACS isoforms [41••]. For instance, ABA and MJ decrease the turnover rate of type-2 ACS; however, neither influences the stability of type-1 ACS. None of these phytohormones affect type-3 ACS stability [41••]. Phytohormones are major signaling molecules controlling numerous cellular processes, and consequently plant development and stress responses. Given that ACS protein turnover is regulated by most plant hormones, phytohormone-mediated ACS turnover regulation may be a central mechanism underlying the rapid transduction of internal and external stimuli into cells without transcriptional regulation.

Conclusion and future prospects

Post-translational regulatory mechanisms are typically exerted quickly and often independently of gene expression. Therefore, plants use post-translational regulatory mechanisms to rapidly respond to internal and external stimuli, thereby promptly modulating plant growth and responses to stresses. Recent studies have made great progress in exploring the molecular mechanisms and novel regulators of ACS turnover regulation, providing far-reaching implications regarding the role of ACS turnover in plant development and stress responses, including fruit ripening, hypocotyl elongation, leaf development, and a subset of abiotic and biotic stresses. However, additional studies are needed to elucidate the role of ACS turnover regulation and the underlying mechanisms in other developmental and stress responses as well as hormonal crosstalk. For example, most plant hormones regulate the stability of ACS proteins and have distinct effects on the stability of different ACS isoforms, yet the components and mechanisms involved are largely unknown. Additional post-translational modifications other than phosphorylation and ubiquitination also need to be elucidated for further mechanistic understanding of ACS turnover regulation. A recent bioinformatic analysis of ethylene biosynthetic proteins has shown that multiple ACS isoforms in *Arabidopsis* and rice contain sites for glycosylation [55], a post-translational modification known to control protein stability and activity, implying a novel modification of ACS at the post-translational level [56]. Furthermore, emerging studies indicate that ACC acts as a signaling molecule independently of ethylene signaling in multiple processes [12,57•], including cell wall function [58], cell expansion [59], guard cell differentiation [60], pollen tube attraction [61••], and pathogen interactions [62]. The contrasting functions of ACC and ethylene in thallus and gemma development in *Marchantia polymorpha* also support a function of ACC itself as a signaling molecule [63•]. Given that ACS modulates cellular ACC levels, investigating whether ACS turnover regulation plays a role in ACC-mediated cellular processes is of great interest.

Author's contribution

Chanung Park: Writing - review & editing; **Han Yong Lee:** Writing - review & editing, Visualization; **Gyeong Mee Yoon:** Conceptualization, Writing - original draft, review & editing, Visualization.

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 article.

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