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The role of protein turnover in ethylene biosynthesis and response

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Abstract

Plant growth and development is controlled by a set of hormones whose responses are tightly regulated in order to direct appropriate responses. In several hormone signaling pathways, protein turnover has emerged as a common regulatory element. Ethylene is a phytohormone that controls a variety of processes, including fruit ripening, senescence, and stress response. This review focuses on the regulation of the ethylene response pathway through protein degradation. Protein turnover has been found to regulate both ethylene biosynthesis and ethylene response. Ethylene production is regulated through the turnover of the biosynthetic enzyme ACS. Recently it was found that ethylene receptors are controlled by protein turnover as well. A third process in the control of ethylene signaling is the targeting of the ethylene response transcription factor ETHYLENE INSENSITIVE3 (EIN3) for degradation by the proteins EIN3-BINDING F-BOX 1 and 2 (EBF1 and EBF2).

Keywords

Ethylene biosynthesis; Ethylene signaling; Protein turnover; Ubiquitin proteasome system

1. Introduction

Plants utilize hormones to control growth and development and to respond quickly to ever-changing environmental conditions. Without precise controls over both the production of and response to these hormones, plants would not be able to survive. In recent years, it has been found that ubiquitin-mediated protein degradation is a key mechanism through which plants are able to quickly modulate their responses to hormones. Protein degradation has been shown to be involved in controlling responses to most hormones, including auxins, gibberellins, jasmonates, abscisic acid, and ethylene [1–9]. The presence of this strategy in hormone response control indicates that it is a vital component of the plant's ability to respond to developmental and environmental changes.

The gaseous hormone ethylene is an important regulator of developmental processes and responses to environmental changes in plants. These processes include fruit ripening, senescence, organ abscission, seed germination, and stress responses [10]. Through a variety of genetic and biochemical approaches, our knowledge of how ethylene is synthesized and perceived by plants has been significantly advanced. Protein degradation is increasingly recognized as an important mechanism governing ethylene biosynthesis and perception. This review focuses on the emerging role that protein degradation plays in these processes. In the first section, we provide an overview of proteasome-mediated protein degradation, and then

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we discuss protein degradation in the ethylene biosynthetic pathway. Next, we cover the recent finding that ethylene receptors are regulated via proteasome-dependent proteolysis. We then discuss what is now considered to be a key step in ethylene signaling: the degradation of the ethylene response transcription factor EIN3. Finally, we consider possible future directions for research in ethylene perception.

2. The ubiquitin proteasome system

The ubiquitin proteasome system (UPS) is comprised of four principle components in targeting a protein for proteasome degradation: the ubiquitin molecule, the ubiquitin activating (E1) enzyme, the ubiquitin conjugation (E2) enzyme, and the ubiquitin ligase (E3) (Fig. 1). (For comprehensive reviews of the UPS pathway in plants, see Refs. [11,12].) The first component, ubiquitin, is a small 76-amino acid protein that becomes attached to a lysine residue on target proteins via a C-terminal glycine residue. Ubiquitin itself contains several available lysine residues, such that polymers of ubiquitin can form on target proteins [13]. The ubiquitin peptide is resistant to degradation via the proteasome due to its strong non-covalent intramolecular bonds, allowing ubiquitin to be reused by the cell [11].

The ubiquitin molecule is activated for attachment to target proteins by ubiquitin activating enzymes, or E1 proteins. E1 enzymes activate the ubiquitin molecule by adenylating the ubiquitin peptide, then forming a covalent linkage with ubiquitin. In the *Arabidopsis thaliana* genome, there are only two E1 proteins present, suggesting that control of substrate specificity is not conferred by the E1 enzymes [14]. After activation, the E1 enzymes transfer the ubiquitin moiety to a ubiquitin conjugating enzyme, or E2 enzyme. It is from the E2 protein that the ubiquitin moiety is transferred to the target protein. It is the function of the ubiquitin ligases, or E3 proteins, to perform the transfer reaction. E3 ligases interact with target proteins to bring the targets to the E2-ubiquitin conjugate and to catalyze the transfer of ubiquitin to the target proteins. The E3 ligases are a very diverse group of proteins, as there are over 1300 genes encoding E3 ligase subunits in *Arabidopsis* [15]. This diversity allows the UPS to target a variety of proteins for degradation.

There are four classes of E3 ligases. One class of ubiquitin E3 ligases is the Homology to E6AP C Terminus (HECT) class of proteins, which is unique due to its ability to covalently bind ubiquitin before transferring the moiety to its target protein [16]. The Really Interesting New Gene (RING)/U-Box family of E3 ligases is a diverse family of proteins in *Arabidopsis thaliana*, with approximately 540 present in the genome [11]. The anaphase promoting complex (APC), which promotes degradation of cell cycle regulators, is a multiple subunit E3 ligase [17]. However, the most diverse set of ubiquitin E3 ligases are the Cullin-RING Ligase (CRL) complexes. The CRL complex consists of a cullin protein that acts as a bridge between a RING protein that recognizes the E2 conjugating enzyme and the target recognition protein [18]. There are several classes of CRL complexes, including the well-known Skp-Cullin-F-box (SCF) complex in which an *Arabidopsis* SKP1-like (ASK) protein bridges CULLIN1 (CUL1) and an F-box protein which acts as the target recognition protein [12]. More than 700 F-box proteins have been annotated in the *Arabidopsis thaliana* genome [11]. Coupled with the other types of CRL complexes, including the CUL3-Broad-Complex, Tamtrack, and Bric-a-Brac (BTB) type of CRL E3 ligases, there are thousands of possible modules to target proteins to the proteasome.

There are many examples of the UPS regulating hormone signaling. (For a complete review, see [18]). A striking example of protein turnover in hormone signaling is in auxin signaling, where the TIR1 F-box protein is the auxin receptor [1,2]. In gibberellin signaling, the DELLA proteins are targeted for degradation by the F-box proteins SLY1 in *Arabidopsis thaliana* and GID2 in rice *Oryza sativa* [3,4]. Other examples in hormone signaling include

the RING E3 ligases KEG and AIP2 in abscisic acid (ABA) response [5,6] and the F-box protein COI1 in jasmonate signaling [7–9]. The role of protein turnover in ethylene signaling is especially prevalent, as it affects the ethylene response pathway at three separate points: ethylene biosynthesis, ethylene perception, and transcription (Fig. 2).

3. Ethylene biosynthesis

Ethylene is synthesized via a two-step process from the metabolic intermediate S-adenosylmethionine (SAM). SAM is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) as the rate-limiting step in ethylene biosynthesis. ACC oxidase (ACO) then converts ACC into ethylene [19]. Both ACS and ACO enzymes are encoded by multigene families, which are differentially regulated in all plant species studied [20]. In *Arabidopsis thaliana*, there are nine ACS gene products (ACS1–2, ACS4–9, ACS11), one of which produces a non-functional enzyme (ACS1) [21]. ACS gene transcription has been shown to be upregulated by stimuli that induce ethylene synthesis [22]. Gene transcription is a relatively slow process and a rapid change in environment, such as herbivore attack, necessitates immediate responses. Therefore, ethylene synthesis must be induced rapidly to counteract environmental challenges.

A mechanism by which ethylene synthesis is upregulated in a rapid manner has been revealed through the *ethylene overproducer* (*eto*) mutants of *Arabidopsis*. *eto1*, *eto2*, and *eto3* have a constitutive ethylene response phenotype due to the overproduction of ethylene [23,24]. The *eto2* and *eto3* mutations are dominant and were found to be caused by mutations in the C-termini of ACS5 and ACS9, respectively [25,26]. These dominant mutations caused increased stability of their corresponding ACS proteins, suggesting that ACS enzymes are the target of protein degradation [26]. Studies in tomato (*Solanum lycopersicum*) also indicated that protein degradation is responsible for ACS regulation, and that phosphorylation of ACS may play a role in its stabilization. Tomato cell cultures treated with a phosphatase inhibitor had increased ACS activity, but this increase was dependent upon protein synthesis [27]. It was later shown that tomato ACS2 and *Arabidopsis* ACS2 and ACS6 are phosphorylated, leading to increased ethylene production [28,29].

ETO1 encodes a CRL E3 ligase subunit with a BTB motif and six tetratricopeptide repeats [30]. *Arabidopsis* also contains two *ETO1* paralogs, *ETO1-LIKE1* and *ETO1-LIKE2* (*EOL1* and *EOL2*). ETO1, EOL1, and EOL2 interact with ACS5 to decrease its activity [30]. ETO1 also interacts with the CRL complex subunit CUL3, and a proteasome inhibitor increases ACS5 levels, demonstrating that ETO1 and CUL3 form a CUL3-BTB type of E3 ligase [30]. Together, these data support the idea that the UPS regulates ACS5 protein levels and thus, ethylene synthesis. *Arabidopsis* ETO1 interacts specifically with ACS enzymes that have WVF, RLSF, and R/D/E-rich amino acid motifs in their C-termini [31]. These motifs define type 2 ACC synthases and are collectively called the TOE (target of ETO1) sequence [31]. Type 1 ACC synthases lack the WVF motif, while type 3 ACC synthases lack both the WVF and RLSF motifs [31]. The TOE sequence acts as a degradation signal, since the placement of the TOE sequence as a signal peptide on other proteins leads to greater turnover of the target protein [32].

These studies on the ACS proteins and the *eto* mutants have revealed that ethylene biosynthesis is controlled, at least in part, by the UPS. Interestingly, only a subset of ACS proteins is targeted by the ubiquitin E3 ligase subunit ETO1. This raises the question of whether the other types of ACS proteins are regulated by protein turnover as well. It is interesting to note that the ACS enzymes shown to be phosphorylated are all type 1 ACC synthases (AtACS2, AtACS6, LeACS2) [31]. These enzymes might also be targeted for protein turnover, but the mechanism by which this occurs remains unknown. It also remains

unknown whether the type 2 ACS enzymes require phosphorylation to become stable, or vice-versa. The apparent diversity of ACS types may allow plants greater control over the regulation of ethylene production; having many different systems of ACS regulation allows for a finely controlled level of ethylene production.

4. Ethylene receptor degradation

In *Arabidopsis thaliana*, ethylene is perceived by a family of five receptors (ETR1, ERS1, ETR2, EIN4, ERS2), which negatively regulate response [33]. In the absence of ethylene, these receptors activate the Raf-like kinase CTR1, which also negatively regulates response [24]. The ethylene receptors are present as disulfide-linked dimers, which require a copper cofactor to bind ethylene [34,35]. The ethylene receptors have similarity to bacterial two-component regulators, containing an N-terminal transmembrane domain responsible for ethylene binding [36], a cGMP phosphodiesterase/adenylate cyclase/FhlA (GAF) domain that to date has no known function in the ethylene receptors, and a histidine kinase-like domain. Some receptors (ETR1, ETR2, and EIN4) also have a C-terminal domain that shows similarity to bacterial two-component receiver domains. Ethylene receptors can be divided into two subfamilies based on sequence similarity. Subfamily I members (ETR1 and ERS1) contain all of the necessary residues for histidine kinase activity, while subfamily II members (ETR2, EIN4, and ERS2) do not [37]. It has been shown that subfamily I members have histidine kinase activity *in vitro* [38,39]. However, this activity does not appear to be required for wild-type receptor function in ETR1 [40]. The ethylene receptor ETR1 has been localized to the endoplasmic reticulum (ER) membrane and the Golgi apparatus [41,42], and has been shown to interact with CTR1 [43,44]. For a complete review of ethylene receptor action in plants, see Binder, this issue.

The mechanisms by which the ethylene receptors signal to downstream components remain unclear. However, a mechanism that regulates the level of receptors has recently emerged. A new study by Chen et al. [45] has shown that the *Arabidopsis* subfamily II ethylene receptor ETR2 is degraded when high amounts (10 ppm) of ethylene are present. This effect is independent of transcriptional control, as mRNA levels of *ETR2* increase when ethylene is present [37], and can still take place when transcription of ethylene response genes are constitutively active [45]. The targeting of ETR2 for degradation is dependent on receptor signaling, since the ethylene-insensitive receptor mutants *etr1-1* and *etr2-1* are defective in ETR2 protein degradation [45]. Perhaps most surprising about this study is the finding that the degradation of ETR2 is dependent on the proteasome [45]. The typical model for receptor-ligand induced protein degradation is endocytosis of the receptor-ligand complex, followed by degradation in the lysosome [46]. Contrary to this notion, ETR2 degradation is prevented by inhibitors of the proteasome, implicating that ETR2 degradation takes place through the UPS [45]. Furthermore, degradation of ETR2 does not require ETR2 transport from the ER membrane to the lysosome, as an inhibitor of secretory pathway transport does not affect the degradation process [45].

Similar work by Kevany et al. [47] shows that multiple ethylene receptors in tomato are degraded in response to ethylene. Unlike *Arabidopsis thaliana*, which has five ethylene receptors, tomato has six receptors (LeETR1, LeETR2, NR, LeETR4, LeETR5, LeETR6) [48–50]. The study by Kevany et al. shows that the protein levels of three ethylene receptors, NR, LeETR4, and LeETR6, are all reduced during fruit ripening. This is in contrast to ethylene receptor gene mRNA levels, which increase during fruit ripening in tomato [47,48,51]. The authors also show that these same ethylene receptors are degraded in response to ethylene treatment in immature fruits and vegetative tissues, again despite increases in receptor gene transcript levels. Similar to the *Arabidopsis* ETR2 receptor, a proteasome inhibitor abrogated the degradation of tomato ethylene receptors, demonstrating

that this process is dependent on the UPS [47]. Contrary to the effect seen in *Arabidopsis*, the ethylene-insensitive receptor mutant *Nr* is still able to degrade the ethylene receptors LeETR4 and LeETR6 when treated with ethylene. However, this effect could be due to the semi-dominant nature of *Nr*, which suggests a weaker ethylene-insensitivity than the effect of the fully dominant *etr2-1* in *Arabidopsis* [52,53]. Interestingly, both the *Nr* and *etr2-1* mutations result in the same proline to leucine substitution in their respective receptors. The reason for the differences between the *Arabidopsis* and tomato ethylene receptor mutation phenotypes remains unknown.

The study by Kevany et al. suggests that ethylene responses during fruit ripening in tomato are controlled by ethylene receptor levels. The apparent duality of increased transcript production coupled with decreased protein levels seems counterintuitive. However, this may allow the plant greater control over ethylene responses during fruit ripening. The authors also found that mutations in the *Nr* protein that cause ethylene-insensitivity will partially abrogate *Nr* degradation, which indicates that ethylene-receptor binding is required for turnover. This is supported by Chen et al., who similarly found that ethylene-insensitive mutations in the *Arabidopsis* receptors ETR1 and ETR2 will abrogate ethylene receptor turnover, even in constitutive ethylene response mutants, suggesting that it is ligand-receptor binding that induces turnover, and not downstream ethylene responses.

The two new studies by Chen et al. and Kevany et al. combine to demonstrate that ethylene receptors in plants can be regulated by the UPS. The ethylene receptor ETR2 in *Arabidopsis* is localized to the ER membrane [45], meaning that ETR2 would have to be extracted from the membrane for degradation to occur. ER-associated protein degradation (ERAD) is mostly known to act upon misfolded proteins [54], although it has been shown in yeast that native integral ER-membrane proteins can be ubiquitinated and processed by the proteasome [55,56]. This suggests that the ETR2 ethylene receptor could be processed by the same mechanism. Chen et al. state that ETR1 protein levels changed little when *Arabidopsis* plants were exposed to ethylene. It thus remains to be seen whether other ethylene receptors in *Arabidopsis* are regulated via protein turnover.

5. Regulation of the transcription factor EIN3

Once ethylene binds to the receptors, receptor signaling is deactivated, leading to deactivation of CTR1. Since CTR1 is homologous to the Raf-like MAPKKK family of proteins, it has been hypothesized that the ethylene signaling pathway contains a MAP kinase module. However, no conclusive evidence has been reported to confirm this hypothesis [57]. The next downstream component known to be a part of the ethylene signaling pathway is *EIN2*. The *EIN2* protein shows homology to the N-ramp family of metal ion transporters, although no molecular function for this protein in the ethylene signaling pathway is known [58]. Downstream of *EIN2* in the ethylene signaling pathway is *EIN3*, which encodes a transcriptional activator of ethylene responses [59,60]. There are also five *EIN3-LIKE* (*EIL*) genes in *Arabidopsis*, although only two of the proteins (*EIL1*, *EIL2*) have been shown to be involved in ethylene responses [19,59]. *EIN3* and the *EIL* proteins regulate ethylene response through the transcriptional activation of another transcription factor, *ERF1* [60]. *ERF1* then transcriptionally activates ethylene response genes through binding of a common element located within the promoters of response genes, the ethylene response element (ERE) [60].

Although the downstream events in the activation of ethylene response genes had been characterized, the question of how the *EIN3* protein is activated remained unclear. A trio of papers resolved this question, when it was revealed that the F-box proteins *EBF1* and *EBF2* are members of the ethylene signaling pathway [61–63]. *EIN3* levels accumulate when

Arabidopsis is treated with ethylene, and this effect is dependent upon the proteasome [61–63]. The F-box proteins EBF1 and EBF2 were found to interact with EIN3, EIL1, and the *Arabidopsis* SCF components ASK1, ASK2, and ASK11 [61–63]. In *Arabidopsis*, single *ebf1* and *ebf2* loss-of-function mutants are hypersensitive to ethylene [61–63], while *ebf1 ebf2* double mutants display constitutive ethylene phenotypes, severe growth defects, and are non-viable [61–63]. These phenotypes are rescued by *ein3* or *eil1* loss-of-function alleles [62,64]. Furthermore, EIN3 protein accumulates in *ebf* loss-of-function mutants [61–63], while over-expression of EBF1 or EBF2 leads to decreased accumulation of EIN3 [61,62]. These data combine to support the idea that the EIN3 transcription factor is targeted to the UPS by the F-box proteins EBF1 and EBF2.

The precise mechanisms by which EBF1 and EBF2 target EIN3 for degradation remain unknown. However, additional insight has come from studies into the genetic and kinetic behavior of various *ein3* and *ebf* mutants. Kinetic analysis of etiolated *Arabidopsis* seedlings treated with ethylene has shown a biphasic response to the hormone [65]. After a short delay, *Arabidopsis* seedlings first decrease their growth rate rapidly to a new, slower rate. After equilibrating at the lower rate for a short period, growth rate is decreased even more, although the change is slower. This new, even lower growth rate is maintained until ethylene is removed [65]. Surprisingly, EIN3 and EIL1 are only involved in the second, slower response to ethylene, since *ein3 eil1* double mutants are not inhibited in the first, rapid response [66]. This is in contrast to *ein2* mutants, which are inhibited in both phases of ethylene response [66]. *ebf1* mutants fail to equilibrate after the first response to ethylene and enter directly into the second stage of growth inhibition. Meanwhile, *ebf2* mutants are defective in growth rate recovery after the removal of ethylene [64]. Overexpression lines of both *EBF1* and *EBF2* are defective only in the second phase of growth inhibition, supporting the idea that the first phase of growth inhibition in response to ethylene is EIN3/EIL1 independent [64]. The differences in kinetic responses to ethylene between *ebf1* and *ebf2* mutants suggest that they have differing roles in response. This is supported by genetic evidence, where *ctr1 ebf1* double mutants have a similar phenotype to *ctr1* mutants, but *ctr1 ebf2* double mutants have a more severe constitutive ethylene phenotype [64]. Binder et al. propose that this is due to a difference in the regulation of EBF1 and EBF2, where EBF1 is mostly responsible for the regulation of initiation of EIN3-dependent ethylene responses, while EBF2 is responsible for feedback regulation under high doses of ethylene and recovery after ethylene is no longer present.

A key question that remains is how the EBF F-box proteins are regulated. Recent findings indicate that the exoribonuclease XRN4 regulates *EBF1* and *EBF2* transcript levels [67,68]. The *EIN5* locus, which was first identified in a screen for ethylene-insensitive *Arabidopsis* mutants, was found to encode XRN4, and epistasis analysis showed that *EIN5/XRN4* lies downstream of *CTR1* but upstream of *EBF1* and *EBF2* [67,68]. *EBF1* and *EBF2* transcripts are upregulated in *ein5/xrn4* mutants [67,68]. However, *EBF* transcript turnover rate is the same between wild type and *ein5/xrn4* mutants, indicating that XRN4 fails to directly degrade *EBF* transcripts [68]. Furthermore, *ein5/xrn4* and *ein3* mutants display a similar kinetic response to ethylene, where both are defective in the second phase of growth inhibition [68]. While the mechanism of how XRN4 regulates *EBF1/2* transcript levels is unknown, these data show that XRN4 regulates the ethylene signaling pathway through negative regulation of EBF1/2. This finding reveals a new method of regulation within the ethylene signaling pathway. The involvement of the F-box E3 ligase subunits EBF1 and EBF2, with differing roles in EIN3 regulation, as well as the finding that the exoribonuclease XRN4 regulates EBF1 and EBF2, indicates the need for a fine level of control over transcription of ethylene response genes.

6. Future directions

There are currently many examples of protein turnover in the regulation of hormone signaling. The ethylene biosynthesis and signaling pathways are targets of the UPS at three different levels, demonstrating the importance of the UPS in controlling ethylene responses in plants. One level of control is at the level of ethylene biosynthesis, where the *Arabidopsis* CRL E3 ligase subunit ETO1 targets a subclass of ACC synthases for protein degradation [30]. However, there are still unanswered questions about the mechanism of regulation of the ACC synthases. The mode of activation that releases the type 2 ACC synthases from targeting by ETO1 remains unclear. Although it has been shown that some ACS proteins are phosphorylated, which stabilizes the protein, all examples so far have been for the type 1 ACC synthases [28,29,31]. Conversely, while it is known that phosphorylation is involved in the regulation of turnover of type 1 ACS enzymes, it is unknown which protein(s) target them for degradation.

An additional level of regulation of ethylene response occurs by the UPS-mediated degradation of ethylene receptors in plants [45,47]. The *Arabidopsis* ethylene receptor ETR2 is targeted for degradation via the UPS [45], and the tomato ethylene receptors NR, LeETR4 and LeETR6 are also regulated by the UPS [47]. However, the proteins that target the ethylene receptors for degradation remain unknown. The characterization of the method by which the ethylene receptors are degraded will be an important step in the understanding of the regulation of the ethylene response pathway. Another point that remains unresolved is the difference between the *Arabidopsis* and tomato ethylene receptor systems. In *Arabidopsis*, ethylene response inhibitors and ethylene-insensitive mutations prevented ETR2 turnover [45], while in tomato, the *Nr* mutation did not prevent turnover of ETR4 and ETR6 [47]. While it was noted that the *Nr* mutation is semi-dominant and may not fully inactivate ethylene response, NR protein turnover was prevented by this mutation [47,52]. It also remains unknown whether other ethylene receptors in *Arabidopsis* and tomato are targets of protein degradation. While it was found that the other tomato ethylene receptors' turnover rate was not affected by ethylene treatment in fruit tissue, it is possible other conditions or tissues may induce turnover of these receptors [47]. In *Arabidopsis*, ETR2 has been found to be degraded in response to ethylene binding [45]. Whether any of the other *Arabidopsis* receptors are degraded in response to ethylene remains unknown.

A third level of control of ethylene response is the targeting of the EIN3 transcription factor for degradation by the F-box proteins EBF1 and EBF2 [61–63]. The EBF1 and EBF2 proteins seem to have differing roles in ethylene response [64], and the exoribonuclease XRN4 is responsible for regulation of the *EBF1* and *EBF2* transcripts, but its mode of action is unknown [67,68]. The *ein5/xrn4* mutant is not defective in *EBF1* or *EBF2* mRNA turnover [68], although increased transcript levels are seen in *ein5/xrn4* mutants [67,68]. A possible explanation would be that XRN4 is indirectly involved in EBF1/2 mRNA regulation. XRN4 could regulate the levels of a transcript whose protein regulates EBF1/2 mRNA levels, since XRN4 has been demonstrated to be a functional exoribonuclease, specifically in the degradation of cleavage products from miRNA-mediated mRNA turnover [69]. However, the control of EBF1/2 is unlikely to involve miRNAs, since miRNA pathway mutants had no effect on *EBF1/2* transcript levels [67]. Therefore, the question remains of how XRN4 regulates the levels of *EBF1* and *EBF2* transcripts.

Clearly, the UPS plays a significant role in ethylene biosynthesis and response. Although the UPS is involved in the synthesis and signaling responses of other hormones as well, the full extent to which this occurs remains to be seen. As research progresses, it may be revealed that protein turnover is the primary mode of regulation in controlling hormone responses in plant systems.

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References

1. Dharmasiri N, Dharmasiri S, Estelle M. The F-box protein TIR1 is an auxin receptor. *Nature*. 2005; 435:441–445. [PubMed: 15917797]
2. Kepinski S, Leyser O. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature*. 2005; 435:446–451. [PubMed: 15917798]
3. Dill A, Thomas SG, Hu J, Steber CM, Sun TP. The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell*. 2004; 16:1392–1405. [PubMed: 15155881]
4. Gomi K, Sasaki A, Itoh H, Ueguchi-Tanaka M, Ashikari M, Kitano H, Matsuoka M. GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J*. 2004; 37:626–634. [PubMed: 14756772]
5. Stone SL, Williams LA, Farmer LM, Vierstra RD, Callis J. KEEP ON GOING, a RING E3 ligase essential for *Arabidopsis* growth and development, is involved in abscisic acid signaling. *Plant Cell*. 2006; 18:3415–3428. [PubMed: 17194765]
6. Zhang X, Garretton V, Chua NH. The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. *Genes Dev*. 2005; 19:1532–1543. [PubMed: 15998807]
7. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonates signalling. *Nature*. 2007; 448:661–665. [PubMed: 17637677]
8. Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R. The JAZ family of repressors is the missing link in jasmonate signaling. *Nature*. 2007; 448:666–671. [PubMed: 17637675]
9. Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D. The SCF(COI1) ubiquitin-ligase complexes are required for jasmonates responses in *Arabidopsis*. *Plant Cell*. 2002; 14:1919–1935. [PubMed: 12172031]
10. Abeles, FB.; Morgan, PW.; Saltveit, ME, Jr. Ethylene in Plant Biology. 2nd. Academic Press, Inc.; San Diego: 1992.
11. Smalle J, Vierstra RD. The ubiquitin 26S proteasome proteolytic pathway. *Annu Rev Plant Biol*. 2004; 55:555–590. [PubMed: 15377232]
12. Dreher K, Callis J. Ubiquitin, hormones, and biotic stress in plants. *Ann Bot (Lond)*. 2007; 99:787–822. [PubMed: 17220175]
13. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem*. 2001; 70:503–533. [PubMed: 11395416]
14. Hatfield PM, Gosink MM, Carpenter TB, Vierstra RD. The ubiquitin-activating enzyme (E1) gene family in *Arabidopsis thaliana*. *Plant J*. 1997; 11:213–226. [PubMed: 9076989]
15. Vierstra RD. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends Plant Sci*. 2003; 8:135–142. [PubMed: 12663224]
16. Downes BP, Stupar RM, Gingerich DJ, Vierstra RD. The HECT ubiquitin-protein ligase (UPL) family in *Arabidopsis*: UPL3 has a specific role in trichome development. *Plant J*. 2003; 35:729–742. [PubMed: 12969426]
17. Capron A, Okresz L, Genschik P. First glance at the plant APC/C, a highly conserved ubiquitin-protein ligase. *Trends Plant Sci*. 2003; 8:83–89. [PubMed: 12597875]
18. Stone SL, Callis J. Ubiquitin ligases mediate growth and development by promoting protein death. *Curr Opin Plant Biol*. 2007; 10:624–632. [PubMed: 17851112]

19. Wang KL, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. *Plant Cell*. 2002; 14:S131–S151. [PubMed: 12045274]
20. Bleecker AB, Kende H. Ethylene: a gaseous signal molecule in plants. *Annu Rev Cell Dev Biol*. 2000; 16:1–18. [PubMed: 11031228]
21. Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, Theologis A. Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. *J Biol Chem*. 2003; 278:49102–49112. [PubMed: 12968022]
22. Liang X, Abel S, Keller JA, Shen NF, Theologis A. The 1-amino-cyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. *Proc Natl Acad Sci USA*. 1992; 89:11046–11050. [PubMed: 1438312]
23. Guzman P, Ecker JR. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *Plant Cell*. 1990; 2:513–523. [PubMed: 2152173]
24. Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell*. 1993; 72:427–441. [PubMed: 8431946]
25. Vogel JP, Woeste KE, Theologis A, Kieber JJ. Recessive and dominant mutations in the ethylene biosynthetic gene *ACS5* of *Arabidopsis* confer cytokinin insensitivity and ethylene overproduction, respectively. *Proc Natl Acad Sci USA*. 1998; 95:4766–4771. [PubMed: 9539813]
26. Chae HS, Faure F, Kieber JJ. The *eto1*, *eto2*, and *eto3* mutations and cytokinin treatment increase ethylene biosynthesis in *Arabidopsis* by increasing the stability of ACS protein. *Plant Cell*. 2003; 15:545–559. [PubMed: 12566591]
27. Spanu P, Grosskopf DG, Felix G, Boller T. The apparent turnover of 1-aminocyclopropane-1-carboxylate synthase in tomato cells is regulated by protein phosphorylation and dephosphorylation. *Plant Physiol*. 1994; 106:529–535. [PubMed: 12232347]
28. Tatsuki M, Mori H. Phosphorylation of tomato 1-aminocyclopropane-1-carboxylic acid synthase, LE-ACS2, at the C-terminal region. *J Biol Chem*. 2001; 276:28051–28057. [PubMed: 11375393]
29. Liu Y, Zhang S. Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. *Plant Cell*. 2004; 16:3386–3399. [PubMed: 15539472]
30. Wang KL, Yoshida H, Lurin C, Ecker JR. Regulation of ethylene gas biosynthesis by the *Arabidopsis* ETO1 protein. *Nature*. 2004; 428:945–950. [PubMed: 15118728]
31. Yoshida H, Nagata M, Saito K, Wang KL, Ecker JR. *Arabidopsis* ETO1 specifically interacts with and negatively regulates type 2 1-aminocyclopropane-1-carboxylate synthases. *BMC Plant Biol*. 2005; 5:14. [PubMed: 16091151]
32. Yoshida H, Wang KL, Chang CM, Mori K, Uchida E, Ecker JR. The ACC synthase TOE sequence is required for interaction with ETO1 family proteins and destabilization of target proteins. *Plant Mol Biol*. 2006; 62:427–437. [PubMed: 16897471]
33. Hua J, Meyerowitz EM. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis*. *Cell*. 1998; 94:261–271. [PubMed: 9695954]
34. Schaller GE, Ladd AN, Lanham MB, Spanbauer JM, Bleecker AB. The ethylene response mediator ETR1 from *Arabidopsis* forms a disulfide-linked dimer. *J Biol Chem*. 1995; 270:12526–12530. [PubMed: 7759498]
35. Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. *Science*. 1999; 283:996–998. [PubMed: 9974395]
36. Schaller GE, Bleecker AB. Ethylene-binding sites generated in yeast expressing the *Arabidopsis* *ETR1* gene. *Science*. 1995; 270:1809–1811. [PubMed: 8525372]
37. Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM. *EIN4* and *ERS2* are members of the putative ethylene receptor gene family in *Arabidopsis*. *Plant Cell*. 1998; 10:1321–1332. [PubMed: 9707532]
38. Gamble RL, Coonfield ML, Schaller GE. Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. *Proc Natl Acad Sci USA*. 1998; 95:7825–7829. [PubMed: 9636235]
39. Moussatche P, Klee HJ. Autophosphorylation activity of the *Arabidopsis* ethylene receptor multigene family. *J Biol Chem*. 2004; 279:48734–48741. [PubMed: 15358768]

40. Wang W, Hall AE, O'Malley R, Bleecker AB. Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from *Arabidopsis* is not required for signal transmission. *Proc Natl Acad Sci USA*. 2003; 100:352–357. [PubMed: 12509505]
41. Chen YF, Randlett MD, Findell JL, Schaller GE. Localization of the ETR1 ethylene receptor to the endoplasmic reticulum of *Arabidopsis*. *J Biol Chem*. 2002; 277:19861–19866. [PubMed: 11916973]
42. Dong CH, Rivarola M, Resnick JS, Maggin BD, Chang C. Subcellular co-localization of *Arabidopsis* RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 signaling. *Plant J*. 2008; 53:275–286. [PubMed: 17999643]
43. Clark KL, Larsen PB, Wang X, Chang C. Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS1 ethylene receptors. *Proc Natl Acad Sci USA*. 1998; 95:5401–5406. [PubMed: 9560288]
44. Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, Schaller GE. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. *J Biol Chem*. 2003; 278:34725–34732. [PubMed: 12821658]
45. Chen YF, Shakeel SN, Bowers J, Zhao XC, Etheridge N, Schaller GE. Ligand-induced degradation of the ethylene receptor ETR2 through a proteasome-dependent pathway in *Arabidopsis*. *J Biol Chem*. 2007; 282:24752–24758. [PubMed: 17595158]
46. Sorkin A, Von Zastrow M. Signal transduction and endocytosis: close encounters of many kinds. *Nat Rev Mol Cell Biol*. 2002; 3:600–614. [PubMed: 12154371]
47. Kevany BM, Tieman DM, Taylor MG, Cin VD, Klee HJ. Ethylene receptor degradation controls the timing of ripening in tomato fruit. *Plant J*. 2007; 51:458–467. [PubMed: 17655616]
48. Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. An ethylene-inducible component of signal transduction encoded by *never-ripe*. *Science*. 1995; 270:1807–1809. [PubMed: 8525371]
49. Zhou D, Kalaitzis P, Mattoo AK, Tucker ML. The mRNA for an *ETR1* homologue in tomato is constitutively expressed in vegetative and reproductive tissues. *Plant Mol Biol*. 1996; 30:1331–1338. [PubMed: 8704141]
50. Lashbrook CC, Tieman DM, Klee HJ. Differential regulation of the tomato *ETR* gene family throughout plant development. *Plant J*. 1998; 15:243–252. [PubMed: 9721682]
51. Tieman DM, Taylor MG, Ciardi JA, Klee HJ. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proc Natl Acad Sci USA*. 2000; 97:5663–5668. [PubMed: 10792050]
52. Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. The *never ripe* mutation blocks ethylene perception in tomato. *Plant Cell*. 1994; 6:521–530. [PubMed: 8205003]
53. Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM. *ETR2* is an *ETR1*-like gene involved in ethylene signaling in *Arabidopsis*. *Proc Natl Acad Sci USA*. 1998; 95:5812–5817. [PubMed: 9576967]
54. Meusser B, Hirsch C, Jarosch E, Sommer T. ERAD: the long road to destruction. *Nat Cell Biol*. 2005; 7:766–772. [PubMed: 16056268]
55. Hampton RY, Gardner RG, Rine J. Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum protein. *Mol Biol Cell*. 1996; 7:2029–2044. [PubMed: 8970163]
56. Hampton RY, Bhakta H. Ubiquitin-mediated regulation of 3-hydroxy-3-methylglutaryl-CoA reductase. *Proc Natl Acad Sci USA*. 1997; 94:12944–12948. [PubMed: 9371780]
57. Stepanova AN, Alonso JM. Ethylene signaling pathway. *Sci STKE*. 2005; 2005:cm3. [PubMed: 15784879]
58. Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science*. 1999; 284:2148–2152. [PubMed: 10381874]
59. Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell*. 1997; 89:1133–1144. [PubMed: 9215635]

60. Solano R, Stepanova A, Chao Q, Ecker JR. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev.* 1998; 12:3703–3714. [PubMed: 9851977]
61. Guo H, Ecker JR. Plant responses to ethylene gas are mediated by SCF^{EBF1/EBF2}-dependent proteolysis of EIN3 transcription factor. *Cell.* 2003; 115:667–677. [PubMed: 14675532]
62. Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P. EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. *Cell.* 2003; 115:679–689. [PubMed: 14675533]
63. Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo SD, Yanagisawa S, Vierstra RD. *Arabidopsis* EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. *Proc Natl Acad Sci USA.* 2004; 101:6803–6808. [PubMed: 15090654]
64. Binder BM, Walker JM, Gagne JM, Emborg TJ, Hemmann G, Bleecker AB, Vierstra RD. The *Arabidopsis* EIN3 binding F-box proteins EBF1 and EBF2 have distinct but overlapping roles in ethylene signaling. *Plant Cell.* 2007; 19:509–523. [PubMed: 17307926]
65. Binder BM, O'Malley RC, Wang W, Moore JM, Parks BM, Spalding EP, Bleecker AB. *Arabidopsis* seedling growth response and recovery to ethylene. A kinetic analysis. *Plant Physiol.* 2004; 136:2913–2920. [PubMed: 15466220]
66. Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB. Short-term growth responses to ethylene in *Arabidopsis* seedlings are EIN3/EIL1 independent. *Plant Physiol.* 2004; 136:2921–2927. [PubMed: 15466219]
67. Olmedo G, Guo H, Gregory BD, Nourizadeh SD, Aguilar-Henonin L, Li H, An F, Guzman P, Ecker JR. *ETHYLENE-INSENSITIVE5* encodes a 5' → 3' exoribonuclease required for regulation of the EIN3-targeting F-box proteins EBF1/2. *Proc Natl Acad Sci USA.* 2006; 103:13286–13293. [PubMed: 16920797]
68. Potuschak T, Vansiri A, Binder BM, Lechner E, Vierstra RD, Genschik P. The exoribonuclease XRN4 is a component of the ethylene response pathway in *Arabidopsis*. *Plant Cell.* 2006; 18:3047–3057. [PubMed: 17085683]
69. Souret FF, Kastenmayer JP, Green PJ. AtXRN4 degrades mRNA in *Arabidopsis* and its substrates include selected miRNA targets. *Mol Cell.* 2004; 15:173–183. [PubMed: 15260969]

Fig. 1.

The ubiquitin proteasome system. Ubiquitin (Ub) is activated by a ubiquitin-activating enzyme (E1). The ubiquitin moiety is transferred to a ubiquitin-conjugating enzyme (E2). The ubiquitin molecule is then attached to a target protein by a ubiquitin ligase (E3) protein, which can trigger formation of a ubiquitin polymer chain on the target protein. The E3 ligase is thought to dictate target specificity, as there are over a thousand different E3 ligases in the *Arabidopsis thaliana* genome [15]. Ubiquitin is a signal for protein degradation via the 26S proteasome.

Fig. 2. Protein turnover within the ethylene biosynthesis and signaling pathways of *Arabidopsis thaliana*. The BTB E3 ligase subunit ETO1 targets the ethylene biosynthetic enzyme ACS for protein degradation. The ethylene receptor ETR2 is targeted to the 26S proteasome by an unknown E3 ligase. It is unknown whether any of the other *Arabidopsis* ethylene receptors are degraded by the proteasome. The ethylene response transcription factor EIN3 is regulated by the F-box proteins EBF1 and EBF2. EBF1 and EBF2 are regulated by the exoribonuclease XRN4. SAM: S-adenosylmethionine; ACC: 1-aminocyclopropane-1-carboxylic acid; ACO: ACC oxidase; ACS: ACC synthase.