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REVIEW ESSAY

Subcellular dynamics of ethylene signaling drive plant plasticity to growth and stress

Spatiotemporal control of ethylene signaling in *Arabidopsis*

Yuan-Chi Chien | **Gyeong Mee Yoon**

Department of Botany and Plant Pathology and Center for Plant Biology, Purdue University, West Lafayette, Indiana, USA

Correspondence
Gyeong Mee Yoon, Department of Botany and Plant Pathology and Center for Plant Biology, Purdue University, West Lafayette, IN 47907, USA.
Email: yoong@purdue.edu

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Abstract

Volatile compounds, such as nitric oxide and ethylene gas, play a vital role as signaling molecules in organisms. Ethylene is a plant hormone that regulates a wide range of plant growth, development, and responses to stress and is perceived by a family of ethylene receptors that localize in the endoplasmic reticulum. Constitutive Triple Response 1 (CTR1), a Raf-like protein kinase and a key negative regulator for ethylene responses, tethers to the ethylene receptors, but undergoes nuclear translocation upon activation of ethylene signaling. This ER-to-nucleus trafficking transforms CTR1 into a positive regulator for ethylene responses, significantly enhancing stress resilience to drought and salinity. The nuclear trafficking of CTR1 demonstrates that the spatiotemporal control of ethylene signaling is essential for stress adaptation. Understanding the mechanisms governing the spatiotemporal control of ethylene signaling elements is crucial for unraveling the system-level regulatory mechanisms that collectively fine-tune ethylene responses to optimize plant growth, development, and stress adaptation.

KEYWORDS
Arabidopsis, CTR1, ethylene, hormone, nuclear trafficking, RAF kinase

INTRODUCTION

Cellular signaling pathways allow organisms to translate developmental and environmental cues into coordinated growth and stress response programs essential for survival.[1,2] Precise spatial and temporal control of signaling components is a major mechanism that facilitates cellular communication, thus coordinating biochemical and physiological responses across tissues and organs. This is achieved through subcellular trafficking or compartmentalization of key signaling proteins, paired with tight temporal regulation of signaling pathway activities, which enables activation or deactivation of localized cellular and biochemical cascades. As a result, disturbances in this spatiotemporal regulation can result in aberrant signaling, frequently leading to diseases such as cancer in humans or impaired responses to environmental stressors in plants.[3–6]

Ethylene is a gaseous plant hormone that regulates diverse developmental processes, including fruit ripening, seedling germination, senescence, and root hair formation, as well as responses to a wide range of environmental cues.[7] This multifaceted role of ethylene in different developmental and stress contexts is possible because plants regulate the timing and patterns of growth and stress response processes through mechanisms that allow for the precise spatiotemporal positioning of key ethylene signaling components. Thus, disruption of ethylene signaling often leads to misregulated developmental

processes, such as flowering time and senescence, and stress responses to various biotic and abiotic cues.[8,9] Elucidating the mechanisms governing ethylene signaling pathways is therefore critical for understanding how plants modulate signaling pathways for survival and better fitness in their surroundings. This requires examination of the mechanisms governing ethylene signaling and response at multiple levels: organismal coordination through hormonal crosstalk; tissue- and cell-specific modulation of signaling pathway inputs and outputs; and precise spatiotemporal regulation of signaling components within subcellular compartments.

This review focuses specifically on emerging evidence revealing molecular mechanisms underlying the subcellular organization of ethylene signaling components and their role in modulating ethylene responses. We highlight the subcellular partitioning and trafficking of key ethylene regulatory proteins and the regulation of ethylene responses effectuated by this structural paradigm. We also discuss the potential expansion of this spatiotemporal regulation of ethylene signaling through Constitutive Triple Response 1 (CTR1), which is suggested to be localized to distinct subcellular compartments via interacting with its organelle-specific proximal proteins. Understanding such spatial-temporal regulation of ethylene signaling in plant cells provides deeper insight into the integrated control of plant growth, development, and stress adaptation.

OVERVIEW OF ETHYLENE SIGNALING PATHWAY

Ethylene is perceived by a family of ethylene receptors that reside in the endoplasmic reticulum (ER) through their transmembrane domains (Figure 1).[7,10] The active state of the receptors relies on the Reversion-To-Ethylene Insensitivity 1 (RTE1) protein, which is primarily localized in the ER and Golgi where it regulates receptor activity and conformation.[7,11,12] Under low ethylene conditions, the RTE1-assisted ethylene receptors activate the Raf-like protein kinase CTR1, leading to phosphorylation of the central regulator Ethylene Insensitive 2 (EIN2) for targeted degradation via the 26S proteasomes (Figure 1).[7,13] This effectively shuts down downstream transcriptional activation for ethylene-responsive genes in the nucleus. However, upon ethylene binding to the receptors, this inhibitory cascade is alleviated through the inactivation of the receptors and CTR1, resulting in the proteolytic cleavage of EIN2 at its C-terminus.[13–15] In rice, the activity of CTR1 is also negatively regulated by Mao Huzi 11 (MHZ11), a GDSL lipase located in the ER membrane.[16] MHZ11 reduces the sterol levels within the ER membrane, consequently leading to a reduced interaction between the ethylene receptors and OsCTR1 as well as OsCTR1 phosphorylation.[16] The existence of a counterpart to MHZ11 in Arabidopsis is currently unknown. The cleaved EIN2 C-terminus (EIN2-CEND) is then freed to translocate into the nucleus, which in turn activates the master transcriptional regulators Ethylene-Insensitive 3 (EIN3) and its ortholog EIN3-Like 1 (EIL1), leading to a cascade of transcriptional activation of ethylene-responsive genes (Figure 1).[7,13–15] EIN2-CEND also indirectly enhances EIN3 activity through translational repression of the mRNA of EIN3-Binding F-Box proteins (EBFs), negative regulators of EIN3/EIL1, at P-bodies. Specifically, the cytosolic EIN2-CEND interacts with the 3′UTR of *EBF* mRNA and subsequently targets them into processing bodies (P-bodies) for temporary storage (Figure 1).[17,18] Intriguingly, OsEIN2 is similarly involved in the suppression of OsEBF translation at P-bodies, but it does not directly bind to the 3′UTR of *EBF* mRNAs. Instead, it requires the involvement of MHZ9, a glycine-tyrosine-phenylalanine domain-containing protein, which binds to the *EBF* mRNA through its N-terminus, facilitating the repression of *OsEBF* mRNA translation.[19] This discrepancy may suggest a different mode of action in regulating ethylene responses across plant species. Besides the nuclear trafficking of EIN2-CEND, ethylene activation also prompts the translocation of CTR1 from the ER to the nucleus, where it further enhances EIN3 levels via interacting with EBFs (Figure 1).[20] The ethylene signaling pathway is generally conceptualized as a linear pathway; however, accumulating understanding paints a more complex picture of the ethylene signaling network as an interconnected and spatiotemporally regulated system, with various components exhibiting distinct subcellular localizations.

SUBCELLULAR AND TEMPORAL LANDSCAPES OF ETHYLENE SIGNALING COMPONENTS AND REGULATION

Endoplasmic reticulum as a hotspot for ethylene signaling activation and attenuation

The ethylene receptor family is localized to the ER and shares similarities with two component receptors in bacterial signal transduction, which allows bacteria to sense and respond to changes in various environmental conditions.[7,21] The five members of the ethylene receptor family, including Ethylene Response 1 (ETR1), Ethylene Response Sensor 1 (ERS1), ETR2, ERS2, and EIN4, are classified into subfamilies I and II based on the number of transmembrane domains and the homology of the histidine kinase domain.[7] Subfamily I receptors (ETR1 and ERS1) have three transmembrane domains and a conserved histidine kinase domain similar to bacterial histidine kinases. In contrast, subfamily II receptors (ETR2, ERS2, and EIN4) possess four transmembrane domains and lack most or all of the histidine kinase motifs.[22–29] Subsequent biochemical studies in tobacco, Arabidopsis, and rice revealed that while subfamily I members primarily exhibit histidine kinase activity, subfamily II members may possess serine/threonine kinase activity.[25,28,30] The receptors are anchored to the ER through their N-terminal ethylene binding domains, while their C-terminal kinase domains face the cytosol, where they can interact with downstream components such as CTR1. In addition to the receptors, three other key signaling components, CTR1, EIN2, and RTE1, are localized to the ER. CTR1 localizes to the ER through direct interaction with the C-terminal histidine kinase domain of ethylene receptors, as demonstrated by sucrose gradient fractionation and interaction with the ETR1 receptor, despite lacking ER targeting sequences or membrane-spanning regions.[31,32] In contrast, the EIN2 N-terminus

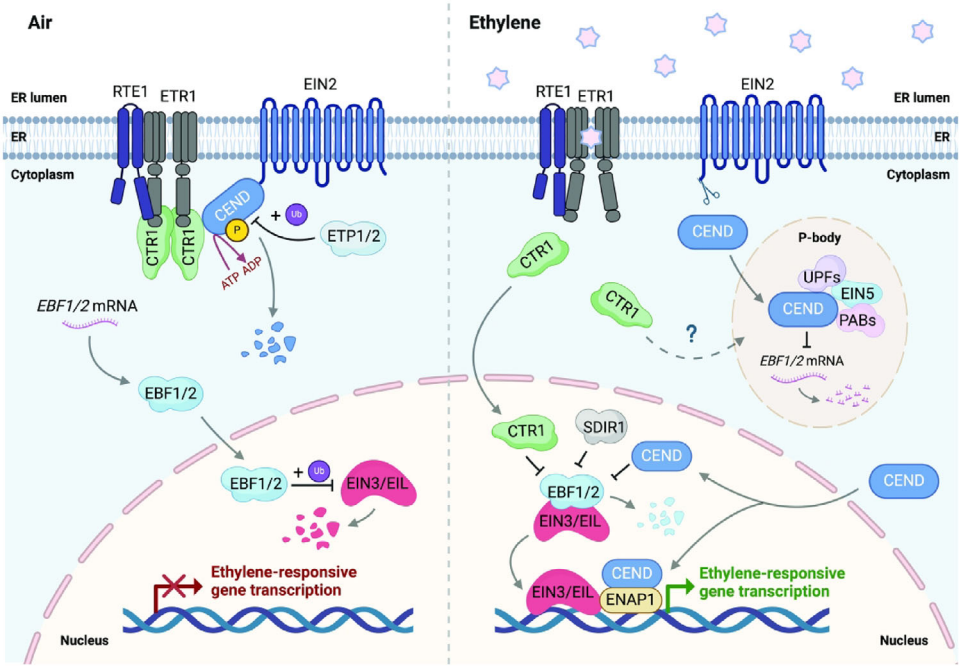

FIGURE 1 Current model for spatiotemporal regulation of ethylene signaling pathway in *Arabidopsis*. In the absence of ethylene (left panel), RTE1-assisted active forms of ER-localized ethylene receptors activate CTR1, a Raf-like Ser/Thr protein kinase. The inactive CTR1 deactivates EIN2 by phosphorylating its C-terminus, leading to degradation via the 26S proteasome-ubiquitin pathway, likely through the recruitment of ETP1/2 F-box proteins. The levels of EIN3/EIL proteins, master transcription factors in the nucleus, are maintained low via the direct interaction with EBF1/2 F-box proteins, which mark them for proteasomal degradation. In the presence of ethylene (right panel), the ethylene-bound receptors and CTR1 become inactivated. Inactive CTR1 no longer phosphorylates EIN2, resulting in the proteolytic cleavage of EIN2 at the C-terminus by an unknown mechanism. The cleaved C-terminal fragment of EIN2 (CEND) is then released to the cytoplasm and subsequently translocates into the nucleus, where it stabilizes EIN3, likely influencing EIN3/EBF complex formation. EIN3 interaction with ENAP1 enhances the DNA binding accessibility of EIN3, thus promoting downstream gene expression. Cytosolic CEND also binds to the 3′UTR of EBF mRNAs, targeting them to P-bodies, where it represses their translation in conjunction with other P-body proteins, including UPF proteins, EIN5, and PABs. Similar to EIN2, inactivated CTR1 is released from the ER and translocates into the nucleus, where it stabilizes EIN3/EIL via interacting with EBFs. The underlying mechanism for CTR1-mediated suppression of EBFs is unknown. CTR1 targeting P-bodies is a possibility given its proximal interaction with the CCR4-NOT complex in found P-bodies, but further investigation is required. In addition to EBFs, SDIR1 E3 ligase provides additional negative regulation on EIN3/EIL stability. Poly (A)-Binding proteins (PABs); EIN2 Targeting Protein 1 and 2 (ETP1/2)

contains twelve predicted transmembrane domains that embed it within the ER membrane, followed by a hydrophilic C-terminal domain. Similarly, the C-terminus of RTE1 contains a transmembrane segment that anchors it to the ER, allowing its N-terminus to stabilize ETR1 receptors.[7]

The juxtaposition of ethylene receptors, RTE1, CTR1, and EIN2 in the contiguous ER membrane facilitates a localized signaling hub that enables efficient control of ethylene signaling activation and attenuation (Figure 1). The atypical ER residence of ethylene receptors differs from the archetypal plasma membrane or nucleus localization of many signaling receptors. This intriguing puzzle regarding the ER-localized receptors potentially links to endosymbiotic theory, whereby internalized bacterial membranes were passed to ancient plant cells, and perhaps ancestral endosymbionts contained signaling pathways that plants co-opted for ethylene perception.[33,34] Alternatively, the ER localization of receptors could be a more recent evolutionary adaptation that confers several key advantages for efficient and targeted ethylene perception and signaling. In this regard, the following are a few potential advantages of receptors being localized in the ER: First, localizing receptors in the ER membrane could enable direct and rapid crosstalk between key signaling components without needing cytosolic intermediates. This spatial proximity between the signaling components could facilitate rapid molecular switching between active and inactive signaling states, demonstrating how subcellular partitioning of proteins serves as an integral facet of signal transduction. Second, the ER-confined receptors may efficiently propagate distinct ethylene signaling cascades, separate from those induced by plasma membrane receptors, allowing for specialized pathways optimized for specific growth regulation or stress responses. Third, considering the limited solubility of ethylene gas in the cytoplasm, the ER may provide an excellent environment that allows proximate access to ethylene ligands, since most ethylene biosynthesis enzymes are cytosolic, potentially constraining ethylene gas availability to intracellular loci.[35] This scenario may seem counterintuitive, given that ethylene molecules must traverse the ER membrane to reach the ethylene-binding pocket of receptors located in the ER lumen; however, the extensive ER

network pervading the cytoplasm could facilitate ethylene perception. The widespread nature of the ER network increases the likelihood of ethylene encountering cytoplasmic regions where local ethylene concentrations are elevated due to the proximity of cytosolic ethylene biosynthesis enzymes. As a result, higher ethylene concentrations would accumulate near the ER membrane, facilitating the diffusion of ethylene through the ER membrane and ultimately enhancing the potential for ethylene perception by the receptors residing within the ER lumen. Lastly, positioning receptors at the ER could enhance ligand specificity by limiting stimulation by non-ethylene stimuli such as other volatile hydrocarbon compounds. This hypothesis could be explored by investigating whether ethylene sensitivity is altered when ethylene receptors are expressed at non-ER sites such as plasma membranes. While mechanistic details of the early signaling pathway for receptor-CTR1 interaction and regulation remain to be fully elucidated, the interplay among the key signaling components at the ER represents an intricate plant environmental and developmental sensing apparatus through a localized subcellular hub. By consolidating key components that initiate or activate ethylene signaling pathways at the ER, plants enable efficient and prompt ethylene perception and response, which demonstrate how plants have strategically designed localized signaling systems to accurately interpret environmental cues.

Nucleus as a crossroad for ethylene signal integration and gene expression control

The nucleus serves as the repository for genetic information and orchestrates the transcription of DNA into mRNA, which is precisely regulated by the spatiotemporal control of proteins involved in the process. In ethylene signaling, ethylene perception induces the accumulation of EIN3/EIL in the nucleus, which subsequently activates Ethylene Response Factors (ERFs) that govern the transcription of numerous ethylene-responsive genes required for adapting to developmental transitions or stresses.[7] Therefore, maintaining the appropriate levels of EIN3/EIL1 proteins in the nucleus is essential for fine-tuned and specific responses to ethylene. Plants achieve this goal through the spatial and temporal control of regulatory proteins that modulate EIN3/EIL levels, including those involved in the same signaling pathway as feedback regulations. At the core of this regulation appears to be EBFs, which function as part of Skp, Cullin, and F-box (SCF) E3 ubiquitin ligase complexes that degrade EIN3/EIL. Beyond the direct protein turnover regulation by EBFs, the modulation of EBF protein abundance is also crucial in modulating EIN3/EIL levels.[7,20,36] Subsequent sections discuss the spatial and temporal regulatory mechanisms that control the levels of EIN3/EIL and EBFs, focusing on the processes facilitated by nuclear-cytoplasmic trafficking of regulatory proteins.

For over three decades, CTR1 has been firmly established as an ER-tethered negative regulator for ethylene responses.[20] However, emerging evidence reveals a surprising re-localization to the nucleus following ethylene signaling activation[20] (Figure 1). Rather than maintaining its inhibitory role at the ER, nuclear CTR1 counterintuitively promotes EIN3 stabilization, thereby strengthening tolerance against environmental stresses like drought and salinity or influencing balanced plant growth.[20] In the darkness, ethylene treatment significantly inhibits the hypocotyl growth of etiolated seedlings, but this ethylene-mediated growth inhibition is rapidly reversed upon the removal of ethylene.[20,37] Studies showed that the recovery phase of hypocotyl growth is tightly controlled by the temporal regulation of EIN3/EIL levels in the nucleus, which are largely influenced by EBFs.[20,37] The ethylene-induced nuclear translocation of CTR1 contributes to the delay of this growth recovery by increasing EIN3 levels, as evidenced by a CTR1 mutant with constitutive nuclear localization exhibiting slower growth recovery compared to seedlings expressing wild-type CTR1.[20] However, this nuclear CTR1-mediated suppression of growth recovery becomes advantageous during drought and salinity stress, where EIN3 activation is pivotal for inducing downstream stress-response genes.[20] The nuclear translocation of CTR1 likely strengthens EIN2-mediated ethylene responses by further increasing EIN3/EIL levels through direct interaction with EBFs (Figure 1). While the molecular mechanisms by which CTR1 suppresses EBF function remain unknown, possibilities include attenuation of EBF E3 ubiquitin ligase activity or disruption of EIN3-EBF complex assembly. The transition of CTR1 from a negative to a positive regulator may seem peculiar; however, such multifunctional roles are common among various signaling proteins with some exhibiting context-dependent activities that are antagonistic within the same pathway. For example, the signaling modulator AGAP3, an NMDA receptor-interacting protein, plays dual functions in human memory control, depending on the perception of different signals.[38] The dynamic regulatory switch that transforms CTR1 from its canonical ER inhibitory site to an ancillary nuclear coactivator role in the nucleus enables more favorable signal transduction trajectories, which enhance adaptive responses to prevailing environmental conditions.

Proteolysis and subsequent subcellular trafficking of EIN2 from the ER to the nucleus are another layer of spatiotemporal control to orchestrate ethylene responses. At the ER, EIN2 is cleaved at the C-terminus in a CTR1-dependent manner, releasing the C-terminus (EIN2-CEND) to the nucleus upon CTR1 inactivation. Intriguingly, EIN2 shuttling to the nucleus is inhibited by phosphorylation mediated by the target of rapamycin (TOR).[39] Notably, this TOR-mediated phosphorylation occurs at a different site from where CTR1 phosphorylates EIN2, suggesting the presence of multiple regulatory mechanisms governing the nucleocytoplasmic trafficking of EIN2.[39] Upon entering the nucleus, EIN2-CEND activates EIN3/EIL, leading to the transactional activation of ethylene-responsive genes (Figure 1).[7,13–15] The underlying mechanisms by which nuclear EIN2-CEND regulates EIN3/EIL function/activity are currently unknown, but modulating the complex formation between EIN3/EIL and EBFs could be a likely possibility. Beyond bridging communication between the ER and nucleus, EIN2-CEND also promotes EIN3-mediated gene expression by enhancing the DNA-binding ability of EIN3/EIL. Specifically, EIN2-CEND forms a complex with the histone-binding protein, EIN2 Nuclear-Associated Protein 1 (ENAP1), which promotes H3K14Ac and H3K23Ac histone acetylation at target promoters (Figure 1). This alteration in chromatic architecture enables subsequent enhancement of EIN3 accessibility

to DNA and activation of EIN3-dependent transcription.[40] Similar to CTR1 trafficking, precise control of EIN2-CEND nuclear translocation and its interactions with nuclear proteins likely play a crucial role in facilitating tailored ethylene responses that fulfill specific environmental and developmental needs.

While the nucleocytoplasmic trafficking of CTR1 and EIN2 in the ethylene signaling pathway is an exciting discovery, the nuclear-cytoplasmic shuttling of proteins is a well-established phenomenon across many cellular pathways. For example, phytochromes such as PhyA and PhyB translocate between the nucleus and cytoplasm in response to light signals, thereby regulating processes like photomorphogenesis, seed germination, and flowering.[41–44] Additionally, members of the AUXIN RESPONSE FACTOR (ARF) family, including ARF7 and ARF19, shuttle between the cytoplasm and nucleus in response to auxin, modulating auxin-responsive gene expression and influencing plant growth, organogenesis, and tropic responses.[45]

Despite this established concept of nucleocytoplasmic trafficking, the mechanism regulating the nuclear export of EIN2-CEND and CTR1 to the cytoplasm remains elusive. Time-lapse imaging studies revealed that the GFP fluorescence of nuclear-localized GFP-CTR1 becomes undetectable within 60 min after removing ethylene, suggesting that either degradation or export from the nucleus occurs.[20] Elucidating the export mechanism, such as identifying the responsible nucleoporin proteins that regulate their nucleocytoplasmic trafficking or unraveling the degradation mechanism in the nucleus, could provide valuable insights into the dynamic regulation governing the ethylene signaling pathway.

The modulation of EIN3/EIL levels also occurs through the crosstalk with other cellular signaling pathways, such as light signaling. In the darkness, Constitutive Photomorphogenic 1 (COP1), an E3 ligase acting as a central repressor of photomorphogenesis, predominantly resides in the nucleus, where it facilitates the degradation of EBFs, promoting ethylene responses by increasing EIN3 levels.[36] Upon illumination, photoreceptors prompt the translocation of COP1 back to the cytosol, thus mitigating its negative influence on EBFs. This translocation of COP1 from the nucleus to the cytosol leads to the enhanced degradation of EIN3/EIL by EBFs and the consequent attenuation of ethylene signaling.[36,46] Concurrently, light perception induces the nuclear import of the photoreceptor phytochrome B, which directly interacts with EIN3 and EBFs, leading to EIN3 degradation.[42] This coordinated crosstalk mechanism between ethylene-light signaling allows for the rapid dampening of ethylene signaling when plants are exposed to light, particularly following seedling emergence from the soil, through the spatial and temporal distribution of regulatory proteins in both pathways. The levels of EBFs are also temporally regulated by another Really Interesting New Gene (RING)-type E3 ligase, Salt- and Drought-Induced Ring Finger 1 (SDIR1).[47] SDIR1 specifically ubiquitinates EBF1/2, marking them for degradation by the 26S proteasome. When temperatures rise from 22°C to 28°C, SDIR1 expression is upregulated, leading to a reduction in EBF1/2 protein levels and consequently increasing the stability and accumulation of EIN3.[47]

P-body as the dynamic transit for the spatiotemporal modulation of ethylene signaling

Processing bodies (P-bodies) are biological condensates that play a role as cytoplasmic mRNA regulation hubs.[48] It controls mRNA fate post-transcriptionally through the regulation of mRNA decay and translational repression.[48] Assembled via phase separation of proteins and RNAs, these dynamic granules contain various proteins involved in mRNA turnover, and their number and size increase during stress, such as drought, salinity, and oxidative damage, to modulate associated stress response pathways.[48] One such pathway is ethylene signaling, where P-bodies enable tight temporal mRNA control, which is crucial for ethylene responses.[7,17,18] In support of this, mutants lacking nonsense-mediated decay factors (UPFs), which are known to suppress mRNA translation by targeting them to P-bodies, exhibit constitutive ethylene responses.[17,18] Further evidence for the intersection between P-body and ethylene signaling lies in the P-body localization of EIN2-CEND (Figure 1). The cytosolic EIN2-CEND influences ethylene signaling not only by moving into the nucleus to activate EIN3-mediated transcriptional regulation but also by translational repression of EBF mRNA in P-bodies. Specifically, EIN2-CEND interacts with the 3′UTR of *EBF1/2* mRNA in the cytoplasm, subsequently recruiting UPF proteins that bind to the *EBF2* 3′UTR and target these mRNAs to P-bodies for temporary sequestration. This P-body sequestration of *EBF* mRNAs by EIN2-CEND and UPF proteins leads to the activation of ethylene signaling as it consequently increases EIN3/EIL levels in the nucleus.[17,18] Rice OsEIN2 plays a similar role in suppressing the translation of OsEBF mRNA at P-bodies, but it requires an additional component MHZ9 to facilitate this process.[19] Although the mechanism governing EIN2-CEND partitioning between the nucleus and P-bodies is not fully understood, this dual localization enables the simultaneous regulation of both transcriptional and post-transcriptional processes, thereby enhancing the ability of plants to fine-tune their ethylene response. Concurrently, another ethylene signaling component, EIN5 (also known as XRN4), participates in this translational regulation of EBFs through its 3′-to-5′ exoribonuclease activity, which leads to the degradation of mRNAs.[17,18]

EXPLORING NEW TERRITORIES FOR SPATIOTEMPORAL CONTROL OF ETHYLENE SIGNALING THROUGH CTR1

Emerging evidence demonstrates that CTR1 localizes to multiple subcellular compartments, including the ER, cytosol, and nucleus (Figure 2).[20,49,50] This raises the possibility that ethylene signaling and pathway crosstalk may also be governed through multi-compartment CTR1 distribution, which expands the spatiotemporal control complexity of ethylene signaling through interacting with partner proteins in different subcellular locations. Supporting this, nuclear CTR1 enhances ethylene responses by stabilizing EIN3 through direct interaction with EBFs.[20] Additionally, a recent proteomics study identified putative

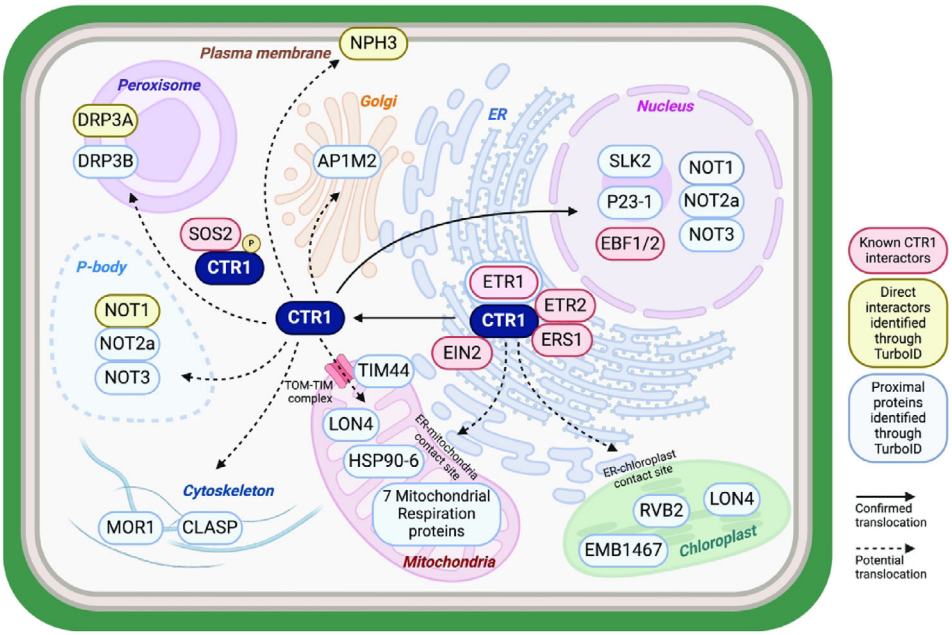

FIGURE 2 Proximal protein Interactome of CTR1 in different subcellular compartment. This model recapitulates and modifies recently published works showing the CTR1 proximal interactome in tobacco. Only a few proteins have been validated as direct CTR1-interacting partners (pink circles), including three ethylene receptors (ETR1, ETR2, and ERS1) and EIN2, all of which reside at the ER. Additionally, CTR1 interacts with EBFs in the nucleus and the protein kinase SOS2 in the cytoplasm. A TurboID-based proximal protein labeling study identified proteins in close proximity to CTR1, which are distributed across several subcellular compartments, including the nucleus, Golgi apparatus, peroxisomes, P-bodies, cytoskeleton, mitochondria, chloroplasts, and plasma membrane. The identified CTR1-proximal proteins were labeled by their known subcellular localizations in the figure (yellow circles). Among these, direct physical interactions with CTR1 were validated for NPH3, DRP3A, and NOT1 (blue circles). Solid arrows indicate the known translocation of CTR1 from the ER upon activation; dotted arrows represent potential subcellular translocations from the ER or cytoplasm to other organelles, based on the locations of the CTR1-proximal proteins. Dynamin-related protein 3A-like (DRP3A); Dynamin-related protein 3B-like (DRP3B); AP-1 complex subunit mu-2 (AP1M2); Protein MOR1-like (MOR1); CLIP-associated protein-like (CLASP); Heat shock protein 90-6, mitochondrial-like (HSP90-6); RuvB-like helicase (RVB2); NADH dehydrogenase (EMB1467); Probable transcriptional regulator SLK2 (SLK2); Co-chaperone protein p23 (P23-1).

CTR1 associations with proteins at various distinct subcellular localizations, including the plasma membrane, mitochondria, and P-bodies (Figure 2).[49] By expressing a CTR1-TurboID fusion for proximity-based biotinylation, the local CTR1 protein interactome was mapped, revealing known (e.g., ETR1 and EIN2) and novel candidates.[49] The diversity of these potential binding proteins implies a prospective control for ethylene signaling mediated through CTR1 trafficking between specialized subcellular niches. In this section, we discuss the potential and speculative roles of CTR1 localization at distinct subcellular sites, specifically the plasma membrane, nucleus, mitochondria, and P-bodies, based on the proteomics study and other independent works. Association with organelle-specific proteomes may confer distinct regulation of the CTR1 signaling modality, expanding regulatory mechanisms for ethylene signaling and perhaps the crosstalk of ethylene with other cellular processes.

Processing bodies

The understanding of P-bodies and their role in ethylene signaling has just begun to emerge through the discovery of the translational repression of EBF mRNA by known ethylene signaling components such as EIN2 and EIN5.[17,18] However, the role of CTR1 has not been linked to the P-bodies. Intriguingly, a group of three proximal CTR1 partner proteins belongs to the evolutionarily conserved Carbon Catabolite Repression–Negative On TATA-less (CCR4-NOT) transcriptional regulatory complex, consisting of at least 9 conserved canonical subunits with different functions (Figure 2).[49] Among these multi-subunits of the CCR4-NOT complex, CTR1 forms a proximal interaction with three subunits, NOT1, NOT2, and NOT3. The CCR4-NOT complex plays a major role in regulating mRNA dynamics from synthesis to degradation in eukaryotic cells.[51] In the nucleus, CCR4-NOT is involved in regulating transcription through the interaction with transcription factors and RNA Polymerase II.[52] In the cytoplasm, CCR4-NOT forms mRNA deadenylase complexes associating with the 3′ UTRs of specific mRNAs in P-bodies, where it triggers the removal of poly(A) tails along with UPF proteins, eliciting transcript degradation or storage.[53] In ethylene signaling, the EIN2-CEND fragment engages UPF proteins in P-bodies to repress *EBF1/2* mRNA translation, amplifying pathway output.[17,18] Given this and evidence supporting the interaction of CTR1 with CCR4-NOT subunits, cytosolic CTR1 may also localize to P-bodies by complexing with CCR4-NOT, which enables it to promote targeted mRNA decay or translational repression. One of the putative targets for P-body-localized CTR1 is EBF mRNAs, whose translation is

inhibited by EIN2-CEND in P-bodies. This hypothesis is based on the nuclear role of CTR1 in suppressing EBF functions to increase EIN3, conferring improved salinity and drought tolerance. Such stresses are known to expand P-bodies (e.g., size and numbers) to promote stress adaptation via post-transcriptional regulation of stress-response transcripts. With the indications of CTR1 P-body localization through the CCR4-NOT scaffold, a model emerges wherein differential partitioning allows CTR1 to concomitantly stimulate ethylene responses by targeting EBFs in two distinct cellular compartments. Specifically, coordinated repression of EBF mRNA translation by P-body-localized CTR1 may coincide with selective moderation of EBF E3 ligase activity by nuclear CTR1 counterpart. Building on this, nuclear CTR1 may interact with the nuclear population of the CCR4-NOT complex, directly participating in transcriptional regulation via repression or chromatin modification. The association of CTR1 with this multifunctional nuclear and cytoplasmic gene expression machinery allows for coordinated spatiotemporal control across cellular compartments.

Mitochondria

Another notable group of CTR1 proximal proteins includes seven mitochondrial respiration components, such as enzymes involved in the tricarboxylic acid cycle, including succinate dehydrogenase subunits, a pyruvate dehydrogenase subunit, NADH dehydrogenase, dihydrolipoyl dehydrogenase, ATP synthase subunit, and malic enzymes (NAD-ME) (Figure 2).[49] Among these, the direct interaction between NAD-ME and CTR1 was confirmed using Bimolecular Fluorescence Complementation (BiFC), suggesting that CTR1 may be localized within the mitochondria.[49] However, it remains puzzling how CTR1, which lacks mitochondrial targeting signals, localizes to the mitochondrial matrix and forms complexes with these mitochondrial partner proteins. Nevertheless, an association of CTR1 with the Translocase of the outer mitochondria membrane 44-2 (TIM44-2), a component of the TOM-Translocase of the Inner mitochondria membrane (TIM) import component, suggests a prospective CTR1 mitochondrial import mechanism (Figure 2).[49,54] One possible mechanism is that CTR1 undergoes "piggybacking" onto interacting proteins destined for mitochondria, gaining access through the TOM-TIM import machinery. Piggyback import allows specific proteins lacking an intrinsic targeting sequence to achieve translocation into cellular organelles by associating with partner proteins that contain an organelle targeting signal.[55] Significantly, mammalian Raf kinases, which share structural similarity with CTR1, interact with import proteins like hTOM and hTIM44 and are found to be localized within mitochondria.[56] This raises the prospect that CTR1 plays an analogous conserved role governing mitochondrial activity, akin to Raf. While further experimental verification is needed, these preliminary CTR1-mitochondria interactions provide initial evidence that CTR1 partakes in mitochondrial regulation and links to ethylene-mitochondria crosstalk. In fact, functional connections exist between ethylene and mitochondria. Emerging research has revealed that ethylene signaling integrates with retrograde responses from stressed mitochondria.[57,58] Impeding mitochondrial translation triggers the mitochondrial Unfold Protein Response, resulting in stimulated ethylene synthesis and substantially higher expression of ethylene genes. This positions ethylene as a key signal to reshape nuclear gene expression when mitochondria face stressors like proteotoxicity.[57] Reinforcing this, ethylene and mitochondrial ROS cooperatively prompt retrograde signaling during seed dormancy release. The effects of ethylene on seed germination necessitate ROS generation through electron transport.[58] Seed responses involved in a mitochondria retrograde response via ROS production also demand activating canonical ethylene signaling.[58] Intriguingly, the Lon protease (Lon4), another protein proximal to CTR1 in mitochondria (Figure 2), also plays a pivotal role in mitochondrial stress responses, such as clearing proteins damaged by oxidative stress.[59]. The Lon protease is a highly conserved ATP-dependent serine-lysine protease that is involved in maintaining mitochondrial homeostasis and protein quality control.[60] Together, these works reveal the integration of ethylene signaling with mitochondrial stress pathways, including coordinated ethylene-ROS activation of mitochondrial retrograde signaling. The spatiotemporal CTR1-mitochondria associations may constitute part of these crosstalk mechanisms connecting ethylene and mitochondria in a developmentally or stress-coordinated manner.

Plasma membrane

The plasma membrane is another potential site for CTR1 subcellular localization, given the proximal and direct interaction between CTR1 and Non-Phototropic Hypocotyl 3 (NPH3), a central mediator of phototropism that associates with the plasma membrane in the darkness (Figure 2).[61–63] NPH3 interacts with the blue light photoreceptors, Phototropins (Phot1 and Phot2), in the plasma membrane. Blue light activation of these photoreceptors trigger phosphorylation of NPH3, shifting its localization from the plasma membrane to cytosolic condensates and initiating phototropic signaling.[64] Co-immunoprecipitation and BiFC assays further showed that CTR1 interacts with NPH3 at the plasma membrane and within condensate-like cytosolic structures.[65] This suggests the possible integration of light and ethylene signaling cascades, which converge at CTR1 as a crosstalk point. The direct regulation of blue light responses by CTR1 remains undetermined, but several studies have already demonstrated the crosstalk between ethylene and blue light signaling, which governs various developmental processes. For instance, ethylene and blue light are involved in regulating stomata opening.[66–69] They also converge to regulate hypocotyl elongation, where blue light antagonizes ethylene-mediated hypocotyl elongation in seedlings, thus determining photomorphogenic versus skotomorphogenic growth.[70–73] Given these functional links between ethylene and light signaling, it is plausible that CTR1 could competitively or synergistically phosphorylate NPH3 along with PHOT at the plasma membrane, therefore regulating the subcellular location of NPH3. In support of CTR1 plasma membrane trafficking, a recent study showed that CTR1 interacts with Salt Overly Sensitive 2 (SOS2), which is a key player in the SOS pathway that maintains ion homeostasis and salt stress.[50] SOS2, a serine/threonine

protein kinase, forms a complex with SOS3 in the presence of calcium, which in turn translocates to the plasma membrane and activates SOS1 to export excess sodium ions.[74] The interaction of CTR1 with SOS2 leads to the inhibition of CTR1 kinase activity, which in turn enhances ethylene responses under salt stress conditions. Intriguingly, SOS2 resides not only in the cytoplasm but also in the plasma membrane and nucleus, implying a potential interplay of CTR1 with SOS2 at all three locations.[50,74] Corroborating this, increased nuclear localization of CTR1 reinforces tolerance to high salinity regardless of its kinase activity.[20] Potential CTR1-SOS2 interactions at various subcellular localizations could facilitate prompt, robust adaptation to salt stress by enabling crosstalk between the SOS and ethylene pathways. Plants may leverage the subcellular dynamics of CTR1 to mount optimized, integrated responses to environmental stresses.

In addition to the plasma membrane, cytosol, P-body, and nucleus, CTR1 may localize to other compartments, including peroxisomes, Golgi, chloroplasts, and cytoskeleton, potentially through interactions with organelle-tethered or targeted proteins (Figure 2). Mirroring its mammalian ortholog Raf kinases, the multifaceted localization of CTR1 across subcellular sites could enable diverse functional roles tailored to different cellular processes. Such mutable distribution is a common characteristic of cellular signaling proteins, facilitating selective pathway modulation and precise control of response networks. By dynamically trafficking CTR1 to distinct interactomes within the cell, plants can strategically position it to act as a signaling hub, assimilating environmental cues into tailored downstream responses. Stimuli-induced shifts in CTR1 localization may also allow it to selectively alter interacting partners and activities at different intracellular sites. Further elucidating the interplay between CTR1 subcellular distribution, binding partners, pathway control, and post-translational regulation remains an open frontier. Gaining mechanistic insight into such localization-function dynamics will unveil key principles underlying how the re-positioning of signaling proteins enables plants to propagate finely tuned signals for adapting growth, development, and stress tolerance in a fluctuating environment.

CONCLUSIONS AND PROSPECTIVE

Subcellular compartmentalization, dynamic trafficking between cellular locations, and temporal control of signaling events are key regulatory mechanisms governing cellular signaling pathways. In plants, ethylene signaling is precisely controlled to enable optimal stress or developmental responses. This tight regulation is mostly possible due to the distinct subcellular localization and trafficking of key signaling molecules like CTR1 and EIN2, which allows for tailored modulation of pathway activity, interaction landscapes, and crosstalk with other cellular pathways. In particular, the translocation of CTR1 between the ER and other cellular compartments likely adds significant regulatory complexity to the existing ethylene pathway, which could explain the versatility of ethylene that enables plants to adapt to diverse developmental and stress cues. It is also possible that CTR1 may localize to multiple subcellular locations independently of ethylene, which grants further control over its signaling functions. Within discrete subcellular compartments, CTR1 could encounter distinct interacting partners, like EBFs in the nucleus, driving localized signaling roles and crosstalk with other essential cellular pathways key to plant health and survival.

Along with further validation of the subcellular trafficking of CTR1 to various subcellular locations, several key questions remain to be answered. What molecular signals or components direct CTR1 trafficking between compartments? How do plants partition CTR1 among different subcellular sites? How do compartmentalized protein interactions translate into specific downstream outputs? How could modulating CTR1 trafficking affect stress resilience or developmental processes? How might we leverage this understanding to beneficially modulate ethylene signaling in crops? Addressing these unknowns through advanced imaging, protein interaction studies, and genetic manipulations will provide a more comprehensive picture of the dynamic signaling hubs of CTR1 within the ethylene network. The recent identification of a small molecule that inhibits CTR1 activity could also provide an additional valuable tool to further dissect the subcellular-specific role of CTR1 in plants.[75] In addition to addressing the aforementioned questions, investigating ethylene signaling in Arabidopsis and its comparative analysis with other plant species, including economically important crop plants such as rice, also holds significant potential for novel discoveries that broaden our understanding of this critical pathway across the plant kingdom. Comparative studies could reveal conserved mechanisms as well as species-specific adaptations, shedding light on the evolutionary trajectories of ethylene signaling components and their functional diversification. This expanded knowledge would ultimately empower the targeted engineering of crops with enhanced adaptability and stress resilience to climate change through the precise modulation of ethylene responses.

AUTHOR CONTRIBUTIONS

Both Y.C. and G.M.Y. contributed to the writing of this manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Gyeong Mee Yoon https://orcid.org/0000-0003-3936-0700

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