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Possible modulation of Arabidopsis ETR1 N-terminal signaling by CTR1

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The mitogen-activated protein kinase kinase kinase (MAPKKK) constitutive triple-response1 (CTR1) plays a key role in mediating ethylene receptor signaling via its N-terminal interaction with the ethylene receptor C-terminal histidine kinase (HK) domain. Loss-of-function mutations of *CTR1* prevent ethylene receptor signaling, and corresponding *ctr1* mutants show a constitutive ethylene response phenotype. We recently reported in *Plant Physiology* that expression of the truncated ethylene receptor Ethylene Response1 (ETR1) isoforms etr1$^{1-349}$ and dominant ethylene-insensitive etr1-1$^{1-349}$, lacking the C-terminal HK and receiver domains, both suppressed the *ctr1* mutant phenotype. Therefore, the ETR1 N terminus is capable of receptor signaling independent of CTR1. The constitutive ethylene response phenotype is stronger for *ctr1-1* than *ctr1-1* lines expressing the *etr1$^{1-349}$* transgene, so N-terminal signaling by the full-length but not truncated ETR1 is inhibited by ctr1-1. We address possible modulations of ETR1 N-terminal signaling with docking of CTR1 on the ETR1 HK domain.

The gaseous plant hormone ethylene is perceived by an ethylene receptor family of five members in Arabidopsis and regulates many aspects of biological processes.[1-6] The ethylene receptors structurally resemble prokaryotic two-component histidine kinase (HK) modules, which perceive and transduce external signals to trigger an array of responses. Acting directly downstream of the ethylene receptors is the mitogen-activated protein kinase kinase kinase (MAPKKK) constitutive triple-response1 (CTR1) protein, which mediates the receptor signal by docking its N portion to the receptor C-terminal HK domain.[7,8] Mutations to abolish HK activity or delete the HK and receiver domains do not prevent ETR1 receptor signaling, and the biochemical nature of the ethylene receptor signal is currently unknown.[9-12]

Members of the ethylene receptor family act cooperatively as clusters.[13,14] The ETR1 C terminus is the CTR1-docking site for the receptor signal output; receptor signaling by truncated, C-terminus–lacking ETR1 isoforms is conceivably mediated by CTR1 via cooperation with other full-length ethylene receptors or by interaction with a third component.[10,15] Alternatively, the truncated ETR1 isoforms may mediate the receptor signal independent of CTR1.[10] The latter scenario is supported by our recent finding that expression of the transgenes *etr1$^{1-349}$* and dominant ethylene-insensitive *etr1-1$^{1-349}$*, which encode the ETR1 isoforms lacking the HK and receiver domains, each greatly suppressed the *ctr1-1* and *ctr1-2* mutant phenotype.[12] Excess CTR1 N-terminal CTR1$^{7-560}$ prevents the receptor signaling, possibly by titrating out available ethylene receptors, and *CTR1$^{7-560}$* overexpressor shows a constitutive ethylene response phenotype.[8,12] Cooperative ethylene receptor signaling of the truncated etr1$^{1-349}$ and etr1-1$^{1-349}$ isoforms with other family members was inferred from transformation studies: expression of *etr1$^{1-349}$* or *etr1-1$^{1-349}$* suppressed the constitutive ethylene response phenotype of *CTR1$^{7-560}$* overexpressor and restored ethylene insensitivity conferred by a dominant ethylene-insensitive receptor in the *ctr1-1* background.[12]

Of note, the *ctr1-1* and *ctr1-2* mutants show a constitutive ethylene response phenotype throughout development, so the ctr1-1 and ctr1-2 isoforms prevent full-length ETR1 N-terminal signaling. Both etr1$^{1-349}$ and etr1-1$^{1-349}$ do not have the CTR1 docking site, and probably their receptor signaling is not prevented by these ctr1 isoforms. The hypothesis that the full-length ETR1 can mediate N-terminal signaling without CTR1 docking can be addressed in a mutation background with lack of CTR1 or the ETR1-CTR1 association. The ethylene receptors are associated with the endoplasmic reticulum, and the ethylene receptor-CTR1 interaction brings CTR1 to the membrane fraction.[16] The *ctr1-8* mutation results from the G354E substitution, which greatly prevents the ETR1-CTR1 interaction, and the ctr1-8 protein predominantly localizes to the soluble but not membrane fraction. *ctr1-8* could be a strong allele because ctr1-8 protein cannot act directly with ethylene receptors. Interestingly, the *ctr1-8* mutant shows a relatively mild constitutive ethylene response phenotype throughout development (**Fig. 1**). The mild phenotype of *ctr1-8* as compared with *ctr1-1* and *ctr1-2* could imply that ETR1 receptor signaling is mediated without ctr1-8 docking to the ETR1 HK domain. Therefore, ETR1 can still mediate the N-terminal signaling without the association of CTR1 and the HK domain,

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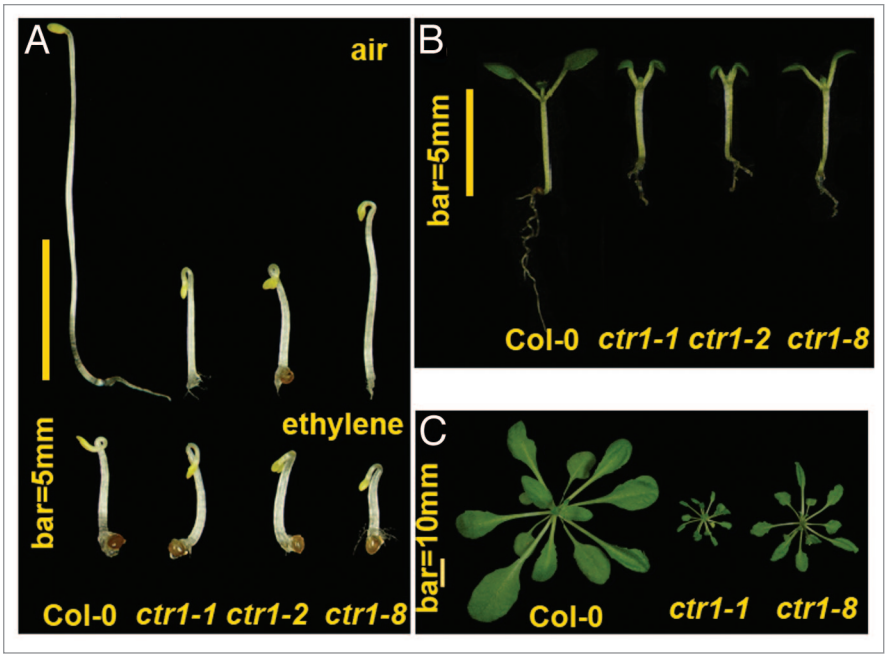

Figure 1. The *ctr1-8* mutant shows a mild constitutive ethylene response phenotype. The constitutive ethylene response phenotypes are shown for wild type (Col-0), *ctr1-1*, *ctr1-2*, and *ctr1-8*. A) Etiolated seedlings of air-grown *ctr1-8* show weaker hypocotyl growth inhibition than *ctr1-1* and *ctr1-2*. Ethylene treatment inhibits the seedling hypocotyl elongation of each genotype to a similar level. B) Light-grown seedlings show stronger growth inhibition in cotyledons and roots for *ctr1-1* and *ctr1-2* than *ctr1-8*. C) At the adult stage, *ctr1-8* produces a much larger rosette than *ctr1-1* and smaller rosette than the wild-type plant.

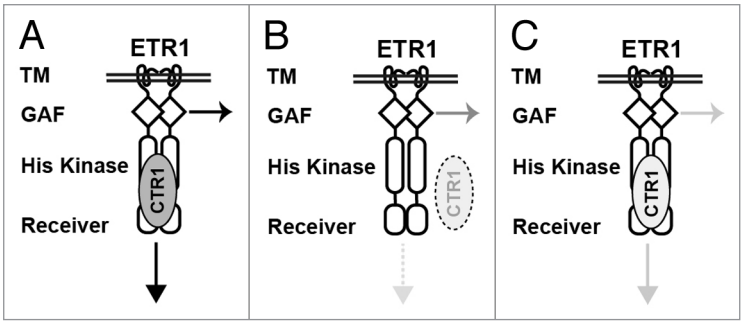

Figure 2. A model for possible modulation of the ETR1 receptor signaling by CTR1. (A) The docking of CTR1 to the ETR1 receptor facilitates receptor signaling by the N terminus and histidine kinase (HK) domain. (B) Without the docking of CTR1 to the ETR1 HK domain, ETR1 receptor signaling is partly mediated by the N terminus but not HK domain. (C) When associated with CTR1 of reduced kinase activity, the ETR1 receptor signaling mediated by N and C termini is attenuated. Arrows indicate ethylene receptor signaling and shading indicate different levels of strength in receptor signal output (arrows) or CTR1 kinase activity (ovals). Dotted lines indicate the absence of receptor signaling (arrow) or ETR1–CTR1 association (oval). TM, transmembrane domain.

and the GAF domain is likely responsible for ETR1 N-terminal signal output (**Fig. 2A and 2B**). A *CTR1* locus-deficient null mutant needs to be identified to support this hypothesis. A very small amount of ctr1-8 may still associate with ETR1 to mediate the receptor signaling via the HK domain; however, we do not know whether the ctr1-8 isoform can mediate the receptor signaling.

The association of CTR1 kinase activity and strength of the receptor signaling is consistent with the difference in degree of the constitutive ethylene response phenotype between *ctr1-1* and *ctr1*^{btk}. ctr1^{btk} has higher kinase activity than ctr1-1, and the *ctr1*^{btk} mutant has a weaker constitutive ethylene response phenotype than does *ctr1-1*.[17] Conceivably, signaling by the ETR1 N terminus, as well as C terminus, can be modulated by alterations in the CTR1 kinase activity: the docking of the ETR1 HK domain with CTR1 with high kinase activity may facilitate strong ETR1 receptor signaling. In contrast, when ETR1 associates with CTR1 with weak kinase activity, ETR1 receptor signaling could be weak (**Fig. 2A and 2C**). CTR1 phosphorylation status could be modulated by a phosphatase or by an ETR1 conformation that is changed on ethylene perception. A recent study showed that HG2 mutations of ETR1 (H353Q and G545A G547A) facilitate ETR1 receptor signaling;[18] we consider the possibility that the ETR1[HG2] isoform could affect CTR1 phosphorylation and alter receptor signaling strength.

Without knowledge of the biochemical nature of the ethylene receptor signal, we face challenges in revealing the underlying mechanism of the ethylene receptor signal output mediated by the ETR1-CTR1 interaction. The current model for ethylene receptor signaling is proposed primarily from genetic studies. Biochemical evidence is lacking for the model that CTR1 mediates ETR1 receptor signaling via the HK domain. CTR1 may be an activator promoting the ETR1 receptor signaling. Or, ETR1 receptor signaling may be mediated by both the GAF and HK domains by different mechanisms. Biochemical and genetic evidence supports that ethylene receptor signaling strength is associated with CTR1 kinase activity.[17] Understanding the regulation of CTR1 kinase activity may advance our knowledge of modulation of ETR1 receptor signaling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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