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New perspective in ethylene signaling

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Key steps in understanding ethylene signaling have come from studying Arabidopsis mutants. The mechanisms of receptor signal output are still poorly understood and the discovery of new components has increased the apparent complexity. Not all receptors are equivalent and some appear to have unique functions. There are multiple CTR1-like proteins in tomato, which interact with more than one receptor. Focusing on mutants of the Arabidopsis triple response, which is primarily a growth response, may not uncover the complete range of components involved in developmental responses to ethylene and it is also possible there are real differences between species.

Ethylene regulates many aspects of the plant life cycle and understanding the control of ethylene synthesis and action are important objectives in plant biology.1,2 Most of the components responsible for ethylene perception and signaling have been discovered by molecular genetic studies in the model plant Arabidopsis, using alterations in the “triple response” to ethylene to identify mutants of the perception and signal transduction chain. The model that has emerged involves multiple ethylene receptors bound to a membrane that interact directly with a single downstream negative regulator, CTR1 (Constitutive triple response1) (Fig. 1). In the absence of the gas ethylene responses are repressed and ethylene binding to the receptors releases the repression, leading to changes in gene expression in the nucleus. In the last few years several groups,3-7 including ourselves,3-7 have published results suggesting that the ethylene signaling model should be modified to take account of new information. For example: (1) The multiple ethylene receptors in Arabidopsis were proposed to be redundant, but there is increasing evidence suggesting that not all ethylene receptors are equivalent and that ETR1 may play a predominant and/or additional role in ethylene signaling.8-12 In tomato, different ethylene receptors have also been shown to have specific functions during plant development and ripening.13-15 (2) New receptor-interacting components have been described,3-6,16 and tomato has multiple CTR1-like proteins, which interact specifically with more than one ethylene receptor.17 In this article we discuss the implications of these findings for models of ethylene perception and signaling.

There are five ethylene receptors in Arabidopsis: ETR1, ERS1, ETR2, ERS2 and EIN4,18 and six in tomato: LeETR1-LeETR6.19,20 They share a similar domain structure with an N-terminal transmembrane domain, followed by a GAF domain, and a C-terminal signal output domain related to the bacterial two-component histidine kinases. Some, but not all receptors, have a receiver domain (Fig. 1).

The downstream protein CTR1 (Fig. 1) resembles a Raf-like MAPKKK.21 The ethylene receptors are located in membranes and form both homo- and heterodimers.22 The basic functional unit for ETR1 is a disulfide-linked homodimer, which holds a single Cu(I) near the N-termini.23 Gao et al.22 showed that ETR1 can form heterodimers with ERS1, ETR2, ERS2 and EIN4 in vivo, and suggested that ethylene receptors exist in clusters, as found with histidine kinase-linked chemoreceptors of bacteria, and that such interaction may contribute to ethylene signal output. In the two-component system of bacteria...
output. Moussatche and Klee, however, \( \text{CTR1} \) with other ethylene receptors could suggest that \( \text{ETR1} \) may play a predominant and/or additional role in ethylene signaling.\(^{1,4} \) Furthermore, the tomato ethylene receptors \( \text{NR} \) (\( \text{LeETR3} \)), \( \text{LeETR4} \) & 6 are preferentially expressed in fruit and have been shown to have unique roles during ripening\(^{11,13} \) and Whitelaw et al.\(^{14} \) showed that the specific reduction of \( \text{LeETR1} \) results in delayed abscission, shorter internodes, and altered auxin movement. Whether this is an indication of real differences between species, as suggested by Klee, or, alternatively, that the functions of the Arabidopsis ethylene receptors have not been fully explored, remains an open question.

The C-terminal domains of the Arabidopsis ethylene receptor \( \text{ETR1} \) and \( \text{ERS1} \) have direct protein-protein interactions with the N-terminal of the serine/threonine protein kinase \( \text{CTR1} \), which was found to co-localise with the receptors in the ER membrane.\(^{27,28} \) The C-terminal of \( \text{CTR1} \) has high similarity to the mammalian Raf protein kinases, while its N-terminal functions as a regulatory domain with weak similarity to the Raf proteins.\(^{29} \) There are four \( \text{CTR1} \)-like genes in Arabidopsis (\( \text{CTR1}, \text{EDR1}, \text{At1g18160} \) and \( \text{At1g63660} \) (Fig. 2A) and all four proteins contain the conserved \( N \) - and C-terminal domains (Fig. 2B). A critical conserved G residue is also present in all four proteins (Fig. 2B highlighted with a star). The importance of this is shown by the \( ctr1-8 \) mutation, which alters this G to E, rendering \( \text{CTR1} \) unable to bind \( \text{ETR1} \), and resulting in a constitutive ethylene response in air, a phenotype resembling the \( ctr1 \) null mutation.\(^{30} \) Among the four Arabidopsis proteins only \( \text{CTR1} \) has been implicated in ethylene signaling by mutant analysis and by its direct interactions with the receptors, although a link between ethylene and pathogen response has been suggested for \( \text{EDR1} \).\(^{31} \) The functions of \( \text{At1g18160} \) and \( \text{At1g63660} \) remain to be determined. There are also four \( \text{CTR1} \)-like genes in tomato: \( \text{LeCTR1}, \text{LeCTR2}, \text{LeCTR3} \) and \( \text{LeCTR4} \).\(^{32} \) Phylogenetic tree analysis indicates that \( \text{LeCTR1} \), 3 & 4 are in the same cluster with \( \text{CTR1} \) (Fig. 2A), and in yeast 2-hybrid assays all three proteins interact with the tomato ethylene receptors \( \text{LeETR1}, 2 \) and \( \text{NR} \) (Table 1).\(^{16} \) Mutant complementation studies suggest that \( \text{LeCTR3} \) most closely resembles \( \text{CTR1} \) as it is able to complement the \( ctr1 \) mutation. \( \text{LeCTR1} \) & 4 also partially complement \( ctr1 \), suggesting that several CTRs may mediate ethylene signaling in tomato.\(^{32} \)

\( \text{LeCTR2} \) is more similar to \( \text{EDR1} \) than to the other \( \text{LeCTR} \)s (Fig. 2A). It has the same number, size and position of exons as \( \text{EDR1} \) with homology in both the N-terminal and the C-terminal domains.\(^{6} \) Lin et al.\(^{6} \) showed it was able to interact with the subfamily I ethylene receptors \( \text{LeETR1} \) (and \( \text{LeETR2} \)) but not with \( \text{NR} \) in yeast and in vitro. Overexpression of the \( \text{LeCTR2} \) N-terminus in tomato resulted in enhanced hypersensitive response to the fungal pathogen \( \text{Botrytis cinerea} \), and the transgenic plants also displayed abnormal development, but without any effect on the classical ripening response to ethylene. These observations raise important questions: (1) Do multiple CTRs interact with ethylene receptors in vivo, are the selective interactions of CTRs with the receptors
Figure 2. Analysis of CTR1-like sequences from Arabidopsis and tomato. (A) Phylogenetic tree produced in DNA Star software using the full-length protein sequences of CTRs, and the human B-raf was used as an outgroup. (B) Alignment of the N- and C-termini of Arabidopsis CTR1-like proteins. Consensus sequences are shown in red. The conserved amino acid residue Glycine*34 (G) (circled and marked with *) in the CTR1 N-terminus, shown to be important for CTR1 function and discussed in the text, is conserved all CTR1-like proteins.
significant in ethylene signaling, and are there differences between species? (2) Do some CTR1-like proteins interact with other hormone receptors, such as cytokinin receptors? (3) Do different CTRs interact with each other? Dimerization is a common mechanism for regulating phosphorylation by protein kinases. The Raf protein can form “side by side” dimers through the kinase domains, thereby activating kinase activity even if one partner is catalytically “dead”. Further investigation of the interacting partners of CTR-like proteins should shed more light on ethylene signaling.

Conformational changes in the ethylene receptors caused by ethylene binding probably hold the key to understanding ethylene signaling. The Arabidopsis RTE1 (REVERSION-TO-ETHYLENE SENSITIVITY) has been shown to regulate ETR1 functions specifically and to be co-localised with ETR1 in the ER. By double mutant analysis of the rct1 loss-of-function mutation and 11 etr1 ethylene-binding domain mis-sense mutations, Resnick et al. found that the ethylene binding domain of ETR1 is the target for RTE1 action and proposed that it regulates the conformational changes of the ETR1 receptor. A newly discovered protein SITPR1 binds the tomato NR and LeETR1 receptors in yeast and in vitro. A related protein AtTRP1 in Arabidopsis also interacts with the ERS1 ethylene receptor2 (Fig. 1). Overexpression of SITPR1 in tomato and AtTRP1 in Arabidopsis plants caused a variety of phenotypes suggesting altered hormone responses related to ethylene and auxin. It was postulated that SITPR1 might be involved in receptor degradation, or might interfere with the association of CTR1-like proteins with receptors (Fig. 1), and the mechanism of SITPR1/AtTRP1 action warrants further investigation. Protein degradation plays an important role in modulating ethylene synthesis and signal transduction. Ethylene-induced ethylene receptor degradation has been reported in both Arabidopsis and tomato. Chen et al. showed that the Arabidopsis ETR2 receptor is degraded following treatment with ethylene at concentrations above 1 µl/litre in the wild type background and 0.2 µl/litre in the ctr1-2 background by the physical association of ethylene with the receptor through a proteasome-dependent pathway. Kevany et al. showed that the tomato ethylene receptors LeETR4 & 6 are subjected to degradation in the presence of ethylene and that the process depends on the 26S proteasome.

In order to understand ethylene signaling we need more information about ethylene-induced conformational changes in the receptors, the relationship to one or more CTR(s), the precise role of the possible phosphorelay cascade to downstream components, and how recruitment of receptor degrading machinery is regulated.

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