

The role of receptor interactions in regulating ethylene signal transduction

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The phytohormone ethylene is perceived in *Arabidopsis* by a five-member receptor family. Earlier work has demonstrated that the basic functional unit for an ethylene receptor is a disulfide-linked homodimer. We recently reported in *The Journal of Biological Chemistry* that the ethylene-receptor ETR1 physically associates with other ethylene receptors through higher order interactions, suggesting the existence of receptor clusters. Here we consider the implications of such clusters upon the mechanism of ethylene signal transduction. In particular, we consider how such clustering provides a cooperative mechanism, akin to what has been found for the prokaryotic chemoreceptors, by which plant sensitivity to ethylene may be increased. In addition, we consider how the dominant ethylene insensitivity conferred by some receptor mutations, such as *etr1-1*, may also be propagated by interactions among members of the ethylene receptor family.

The plant hormone ethylene regulates growth and development, and is perceived by a five-member family of receptors (ETR1, ERS1, ETR2, ERS2 and EIN4) in *Arabidopsis*.¹ Genetic analysis indicates that ethylene receptors are functionally redundant and negatively regulate ethylene responses through interactions with the Raf-like kinase CTR1.²⁻⁵ The functional unit of an ethylene receptor is a disulfide-linked homodimer, with each homodimer capable of binding one ethylene molecule.^{6,7} However, several observations suggest that propagation of the ethylene signal through the receptors is likely to involve more than

just ethylene-induced changes within individual receptor homodimers. First, *Arabidopsis* is amazingly sensitive to ethylene and can respond to ethylene concentrations as low as 0.2 nL/L,⁸ 300-fold lower than the K_d of the receptors for ethylene, which suggests that some mechanism exists for amplifying the input signal.^{7,9} Second, ethylene-insensitive mutations in the binding sites of the receptors exhibit greater dominance than would be predicted solely from a lesion within one member of the receptor family.¹⁰

In our paper published in *The Journal of Biological Chemistry*,¹¹ we demonstrate that the *Arabidopsis* ethylene receptor ETR1 physically associates with other ethylene receptors through higher order interactions. Such physical interactions suggest that the receptors exist in plants as clusters, and that models for cooperative signaling previously applied to the histidine-kinase-linked chemoreceptors of bacteria may also be applicable to the evolutionarily related ethylene receptors of plants. In bacteria, the highly packed chemoreceptors are found in clusters at one or both poles of the cell.^{12,13} Structural studies indicate that chemoreceptors can associate to form a 'trimer of dimers'^{14,15} and also support the possibility that domain swapping may occur to produce a large interconnected array of receptors.¹⁶ Our studies indicate that ethylene receptors can interact through their cytosolic GAF domains, identifying one possible interface through which conformational changes could be propagated in an ethylene receptor cluster.

A higher-order cooperative mechanism among the ethylene receptors may explain

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the high sensitivity of plants to ethylene. In this model, the ethylene receptors amplify ethylene signaling by lateral signal output. Binding of ethylene to one receptor induces the conformational change of the receptor from a tense state (T) to a relaxed state (R). This conformational change is then propagated to other empty receptors in the cluster due to their physical associations with the receptor in the R state. As a result empty receptors also adopt the relaxed state (R'), resulting in amplification of the initial signal. It should be noted here that mutational evidence supports the unbound state of the receptors (T state) as being the lower energy conformation of the receptors.¹⁷ Thus, according to this model, part of the energy from ligand binding would be used to transmit conformational changes to the neighboring receptors.

An alternative model that may also explain the high sensitivity of ethylene responsiveness in plants, and one that is not necessarily incompatible with the previous model, is a conjugation model.¹⁸ Here it is hypothesized that, due to the physical proximity of the ethylene receptors, that ethylene released from one receptor then binds to another receptor rather than diffusing away. Through this conjugation mechanism, one ethylene molecule could amplify its signal by converting the conformations of multiple ethylene receptors from the ethylene-unbound state (T) to the ethylene-bound state (R). This model is based on several assumptions. One assumption is that a single ethylene molecule can bind ethylene receptors in the same cluster multiple times due to the dynamic binding of ethylene and ethylene receptor. A second assumption is that, after ethylene is released from one ethylene receptor, the recovery time for that receptor to resume the T state is longer than the time required for the released ethylene to bind to and convert another receptor from the T to the R state.

Models for cooperativity need to also explain the dominant ethylene insensitivity of various mutant receptors such as *etr1-1*, in which a missense mutation results in a receptor incapable of binding ethylene. Several studies indicate that the *etr1-1* mutant receptor acts cooperatively to affect the signal output from other wild-type receptors (i.e., the presence of the *etr1-1* receptor in its T state increases the likelihood of other

receptors adopting the T state).^{10,11} This observation can be most readily explained if the dominant ethylene-insensitive mutations result in a receptor that requires more energy to undergo the T to R transition than do the wild-type receptors. For example, the *etr1-1* mutation may increase the stability of the T form (a T' state). There is evidence to support this possibility. The *etr1-1* missense mutation results in a receptor unable to chelate a copper cofactor necessary for ethylene binding,¹⁹ but the effects of this mutation on signaling are different from wild-type receptors that lack their copper cofactor. The *etr1-1* mutant receptor appears locked in its T state, whereas wild-type receptors lacking the copper cofactor appear to be in the R state.²⁰ Thus *etr1-1* is truly a gain-of-function mutation that alters the conformation of the receptor in ways not necessarily predicted from just the loss of the copper cofactor.

In conclusion, we have attempted here to provide models that can resolve an apparent contradiction in the cooperative signaling behavior exhibited by ethylene receptors. The high sensitivity of plants to ethylene suggest cooperative changes in which an R state can be propagated within a receptor cluster, but the dominance of the ethylene ethylene-insensitive mutant *etr1-1* suggests that the T state can also be propagated within a receptor cluster. It should be born in mind, however, that ethylene signaling is mediated by multiple signaling components. The ethylene receptors regulate ethylene responses through interaction with and modulation of CTR1 kinase activity. Thus, the total kinase activity of CTR1 represents the signal output from the receptors. This situation is very similar to that of the bacterial chemoreceptors, which regulate the activity of an associated histidine kinase, and, as with the chemoreceptors, the stoichiometry of CTR1 interactions with the ethylene receptors and the means by which its kinase activity is regulated are important for the elucidation of the mechanism of ethylene signal transduction.

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References

- Schaller GE, Kieber JJ. Ethylene. The Arabidopsis Book 2002; 1-18.
- Hua J, Meyerowitz EM. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 1998; 94:261-71.
- 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; 72:427-41.
- Gao ZY, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, et al. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of Arabidopsis through participation in ethylene receptor signaling complexes. J Biol Chem 2003; 278:34725-32.
- Clark KL, Larsen PB, Wang XX, Chang C. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. Proc Natl Acad Sci USA 1998; 95:5401-6.
- Schaller GE, Ladd AN, Lanahan MB, Spanbauer JM, Bleecker AB. The ethylene response mediator ETR1 from Arabidopsis forms a disulfide-linked dimer. J Biol Chem 1995; 270:12526-30.
- O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, Klee HJ, et al. Ethylene-binding activity, gene expression levels and receptor system output for ethylene receptor family members from Arabidopsis and tomato. Plant J 2005; 41:651-9.
- 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; 136:2921-7.
- Schaller GE, Bleecker AB. Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. Science 1995; 270:1809-11.
- Gamble RL, Qu X, Schaller GE. Mutational analysis of the ethylene receptor ETR1. Role of the histidine kinase domain in dominant ethylene insensitivity. Plant Physiol 2002; 128:1428-38.
- Gao Z, Wen CK, Binder BM, Chen YF, Chang J, Chiang YH, et al. Heteromeric interactions among ethylene receptors mediate signaling in Arabidopsis. J Biol Chem 2008; 283:23801-10.
- Maddock JR, Shapiro L. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science 1993; 259:1717-23.
- Gestwicki JE, Lamanna AC, Harshey RM, McCarter LL, Kiessling LL, Adler J. Evolutionary conservation of methyl-accepting chemotaxis protein location in Bacteria and Archaea. J Bacteriol 2000; 182:6499-502.
- Kim KK, Yokota H, Kim SH. Four-helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor. Nature 1999; 400:787-92.
- Francis NR, Wolanin PM, Stock JB, Derosier DJ, Thomas DR. Three-dimensional structure and organization of a receptor/signaling complex. Proc Natl Acad Sci USA 2004; 101:17480-5.
- Wolanin PM, Stock JB. Bacterial chemosensing: cooperative molecular logic. Curr Biol 2004; 14:486-7.
- Wang W, Esch JJ, Shiu SH, Agula H, Binder BM, Chang C, et al. Identification of important regions for ethylene binding and signaling in the transmembrane domain of the ETR1 ethylene receptor of Arabidopsis. Plant Cell 2006; 18:3429-42.
- Lagerholm BC, Thompson NL. Theory for ligand rebinding at cell membrane surfaces. Biophys J 1998; 74:1215-28.
- Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science 1999; 283:996-8.
- Woeste KE, Kieber JJ. A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype. Plant Cell 2000; 12:443-55.