Highly conserved proteins that modify plant ethylene responses

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The plant hormone ethylene controls many aspects of growth and development, including organ abscission, leaf and flower senescence, and, in certain species, fruit ripening. It also has a critical role in mediating many stress responses. The importance of ethylene to many fundamental processes in plants has resulted in a great deal of research on how plants perceive and respond to this hormone. Simple genetic screens have facilitated isolation of many of the genes encoding mediators of ethylene responses, most notably the receptors (1, 2). Plants have small gene families of ethylene receptors; *Arabidopsis thaliana*, for example, has five. These receptors are homologous to bacterial two-component regulators and have protein kinase activities (3, 4). Genetic analyses of the receptor family are consistent with a model in which receptors actively suppress ethylene responses in the absence of the hormone. There is redundancy built into the system: single loss-of-function mutants have no visible phenotype, whereas higher-order loss-of-function mutants exhibit varying degrees of enhanced ethylene responses (5). In contrast, single amino acid changes in the ethylene-binding domain of receptors frequently result in dominant ethylene insensitivity. This insensitivity is believed to be the consequence of an inability to recognize ethylene (6). Mechanistically, little is known about how the receptors bind to ethylene and transduce that information to the downstream signaling components. The only protein that is known to physically interact with the receptors is the RAF-like protein kinase CTR1 (7).

In this issue of PNAS, two articles describe the identification of a new class of protein that genetically interacts with ethylene receptors to negatively regulate ethylene responses. Resnick *et al.* (8) started with a dominant ethylene insensitive *Arabidopsis etr1* receptor mutant and screened for second-site suppressors that restore ethylene responsiveness. They identified a loss-of-function mutation in the *REVERSION-TO-ETHYLENE SENSITIVITY1* (*RTE1*) gene that restores ethylene responses to close to wild-type levels, presumably because of inactivation of dominant *etr1* signaling. In contrast, Barry and Giovannoni (9)

Fig. 1. Phylogenetic analysis of RTE1/GR-related proteins. A nonrooted phylogenetic tree was generated by using the PHYLIP 3.5C suite of programs (<http://evolution.genetics.washington.edu/phylip.html>) using the *Giardia* protein as the out group. Bootstrap percentage supports are indicated. TIGR and SGN identifiers and GenBank accession numbers are as follows: tomato GR (DQ372895), tomato GRL1 (DQ372898), tomato GRL2 (DQ372899), potato GRL1 (DQ372900), potato GRL2 (SGN-U276841), *A. thaliana* RTE1 "AtRTE1" (NP_180177), *Arabidopsis* RTE1-HOMOLOG "AtRTH" (NP_190673), rice 1 (NP_916598), rice 2 (AAV59409), rice 3 (AAO37528), human (NP_115501), mouse (AAH37609), honey bee (XP_393764), fruit fly (NP_723362), *Caenorhabditis elegans* (AAF39886), dog (XP_852505), *Xenopus* (AAH87509), zebrafish (NP_001013334), pufferfish (CAG00266), *Trypanosoma brucei* (EAN77990), *Giardia* (XP_767284), *Trypanosoma cruzi* (XP_804751), *Plasmodium* (NP_703394), *Aquilegia* (TC10814), grape (TC40111), sorghum 1 (TC94016), sorghum 2 (TC97340), barley (TC132972), wheat (TC268045), oilseed rape (TC3567), poplar (TC31211), cotton (TC34712), and spruce (TC2575). Figure courtesy of Cornelius Barry (Boyce Thompson Institute for Plant Research, Ithaca, NY).

characterized a tomato mutant in which fruits do not ripen. Tomato fruit ripening is absolutely dependent on ethylene responses, and this dominant mutant, *Green ripe* (*Gr*), had been previously

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identified as being specifically defective in fruit ethylene responses (10). When they cloned the gene associated with *Gr*, they discovered that the mutation was the consequence of a deletion in the 5' flanking region of the gene. Molecular analysis revealed that the *Gr* deletion causes ectopic expression of *GR*, a phenomenon consistent with a dominant gain-of-function mutation. *GR* is a member of the same family as *RTE1*. Thus, the two groups, using two different genetic screens, identified a critical function for the same family of proteins in ethylene responses of two different plants.

So what are *RTE1* and *GR*? Both of these genes encode novel proteins that are highly conserved. *RTE1* encodes a novel protein that is highly conserved throughout eukaryotes, with the exception of fungi, and absent in prokaryotes (Fig. 1). There are two homologous genes in *Arabidopsis* and three in tomato and rice. Computer modeling predicts that they should be integral membrane proteins, as are the ethylene receptors. The *RTE1* sequence contains no known motifs, and no biological function has been assigned to this gene/protein in any species. However, the existence of *RTE1* in a wide range of organisms suggests that it performs an essential conserved function in higher eukaryotes. It is curious that functions should be defined for this novel class of proteins in plants, where there is gene redundancy, as opposed to animals, where there is only a single gene in each species. It is also curious that the only described function of the proteins is to modify the action of a two-component regulator

(the ethylene receptor), but the family is nonexistent in bacteria and fungi, where most two-component regulators exist.

RTE1/GR are somewhat unusual with respect to their functions in ethylene signaling. In *Arabidopsis*, *RTE1* appears to interact specifically with *ETR1*. The dominant *etr1* allele used in the second-site suppressor screen exhibits reduced ethylene sensitivity. The *rte1/etr1* mutant, in contrast, is more sensitive to ethylene than is wild type. The phenotype of the double mutant and single *rte1* mutant is nearly identical to that of the *etr1* loss-of-function mutant, suggesting that *ETR1* function depends on a functional *RTE1*. Surprisingly, *rte1* did not suppress the dominant ethylene insensitivity of either a much stronger *etr1* allele or mutants in any of the other receptor genes tested. Thus, the data are consistent with a specific interaction between *RTE1* and *ETR1*. Because *ETR1* is the most prevalent of the ethylene receptors (11), the results could give *ETR1* the appearance of being the main target of *RTE1*. This seeming specificity for *ETR1* might also be the consequence of differential distribution of *RTE1* and the receptors within or between cells.

The tomato results also indicate specificity in *GR* function. The *Gr* mutant was previously shown to be the consequence of a tissue-specific ethylene insensitivity (10). Whereas seedlings show a normal ethylene response, fruits do not respond to ethylene. Although tissue-specific ethylene responses are not unprecedented (2), they are unusual. What makes *Gr* unusual is that the ethylene-insensitive phenotype is the conse-

quence of constitutive overexpression of the gene product. That is, the gene is aberrantly expressed in all tissues, but only a subset exhibit ethylene insensitivity. As further proof, a constitutively expressed *CaMV35::GR* transgene recreates the *Gr* mutant phenotype but does not lead to a global reduction in ethylene responsiveness. Together, these results suggest tissue-specific modulation of ethylene responses in tomato. Specific *GR*/receptor interactions, such as the *Arabidopsis* *RTE1:ETR1* data suggest, could explain the tissue specificity of the tomato response. There are six ethylene receptor genes in tomato, and their expression varies greatly with different tissue types (12). One receptor, *LeETR4*, is highly expressed in flowers and fruits, and reduced expression of *LeETR4* greatly increases fruit ethylene responses. Thus, *GR* could achieve the appearance of tissue-specific function if it interacts with specific receptors.

The presence of *RTE1/GR* in plant and animal genomes suggests an important conserved function. Because ethylene receptors are not found in animals, it seems likely that these proteins have functions beyond ethylene signal transduction. These proteins clearly have the capacity to alter ethylene signaling *in vivo*, but the actions of *GR* and *RTE1* on the ethylene receptor(s) could be indirect. Because these proteins are integral to membranes, demonstration of direct physical interactions with receptors *in vivo* are challenging but clearly necessary. Regardless of mechanism, the *RTE1/GR* family of proteins likely interacts in some way with the receptor complex.

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