Ethylene Insensitivity Conferred by *Arabidopsis ERS* Gene

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ERS (*ethylene response sensor*), a gene in the *Arabidopsis thaliana* ethylene hormone-response pathway, was uncovered by cross-hybridization with the *Arabidopsis ETR1* gene. The deduced ERS protein has sequence similarity with the amino-terminal domain and putative histidine protein kinase domain of ETR1, but it does not have a receiver domain as found in ETR1. A missense mutation identical to the dominant *etr1-4* mutation was introduced into the *ERS* gene. The altered *ERS* gene conferred dominant ethylene insensitivity to wild-type *Arabidopsis*. Double-mutant analysis indicates that *ERS* acts upstream of the *CTR1* protein kinase gene in the ethylene-response pathway.

The simple gas ethylene affects many aspects of plant growth and development, including seed germination, flowering, senescence, abscission, and fruit ripening (*1*). In order to dissect the ethylene signal transduction pathway, a number of ethylene response mutants have been isolated in *Arabidopsis thaliana* (*2*, *3*, *4*), and two of the genes, *ETR1* and *CTR1*, have been cloned. All available *etr1* mutants display dominant ethylene insensitivity, and mutant analysis suggests that *ETR1* might be an ethylene receptor (*2*, *5*). The predicted ETR1 protein has a novel NH_2-terminal domain and a COOH-terminal region that is similar to the histidine protein kinase domain and the receiver domain of bacterial two-component regulators (*5*). In prokaryotes, a large family of these regulators controls a variety of adaptive responses to different environmental signals (*6*). The loss-of-function *ctr1* mutants exhibit constitutive ethylene responses, which indicates that *CTR1* acts as a negative regulator of ethylene responses (*3*, *7*). The predicted CTR1 protein is a serine-threonine protein kinase that is most closely related to the Raf protein kinase family (*7*). Double-mutant analysis indicates that *CTR1* acts downstream of *ETR1* (*7*). Thus, the ethylene signal transduction pathway in *Arabidopsis* appears to have features of the bacterial two-component system as well as of the mitogen-activated protein kinase pathway in animals. A similar combination of signaling components was recently postulated for the osmolarity sensing pathway in the yeast *Saccharomyces cerevisiae* (*8*).

The identity of the *Arabidopsis ETR1* gene has raised the question of whether there are other two-component regulators in plants. The *ETR1* gene hybridizes to several fragments on *Arabidopsis* genomic DNA blots at low stringency, which suggests that a family of similar genes exists in *Arabidopsis* (*5*). Using an *ETR1* complementary DNA (cDNA) as a probe to screen an *Arabidopsis* genomic DNA library at reduced stringency, we isolated a gene that we named *ERS* (*9*). Subsequently, cDNA clones of *ERS* were obtained (*10*). Genomic DNA blot hybridizations indicate that *ERS* is the gene most closely related to *ETR1* in *Arabidopsis* (*11*). The *ERS* gene maps to chromosome 2, nearest to restriction fragment length polymorphism (RFLP) marker λAt429 (*12*).

We determined both the genomic DNA and the cDNA sequences of the *ERS* gene (GenBank accession number U21952) (*13*). A comparison of the two sequences revealed that the *ERS* gene has five introns. The longest cDNA is 2311 base pairs, which is consistent with the length of *ERS* transcripts detected on RNA blots (*11*).

The deduced ERS protein of 613 amino acids (Fig. 1) displays 67% identity with the predicted ETR1 protein, but it is shorter than ETR1 by 125 amino acids. The NH_2-terminal domain (residues 1 to 321) is 75% identical to that of ETR1, and this domain has no significant similarity to any other sequences in the databases. The three hydrophobic regions in which all the *etr1* mutations reside are present in this domain of ERS, and the four amino acids substituted in the *etr1* mutant alleles are conserved. The COOH-terminal region of the ERS protein contains a putative histidine protein kinase domain, which is 58% identical to that of ETR1. The five conserved motifs of histidine protein kinases (*6*) are present in ERS, with the putative autophosphorylation site at His^{353}. ERS does not contain a receiver domain, which accounts for the predicted length difference between the two proteins. All other putative eukaryotic sensors—*ETR1*, *S. cerevisiae SLN1* (*14*), and *Neurospora crassa NIK1* (*15*)—have both a histidine protein kinase–like domain and a receiver-like domain.

To examine the function of *ERS*, we used a reverse genetic approach. The dominant mutations of the *ETR1* locus lie in the region coding for the NH_2-terminal domain. Because ERS and ETR1 have very similar NH_2-terminal domains, we hypothesized that similar substitutions in the *ERS* gene product might result in a dominant phenotype. Using site-directed mutagenesis, we created a nucleotide change in an *ERS* genomic DNA fragment, resulting in a Phe in place of Ile at residue 62 in the deduced protein sequence (*16*). This substitution is identical to that encoded by the dominant *etr1-4* mutation, which is a strong mutant allele (*5*). A 5-kb DNA fragment containing the altered *ERS* gene was cloned into a plant transformation vector (*17*) that carried a marker for kanamycin resistance. The 5-kb fragment started 2.4 kb upstream of the inferred translation start site of *ERS* and ended approximately 0.4 kb downstream of the polyadenylation site. This construct was transferred to wild-type *Arabidopsis* plants by means of *Agrobacterium*-mediated transformation (*17*). Four independent kanamycin-resistant lines [designated *ers-1(1)* to *ers-1(4)*] were obtained. In addition, we obtained one control line, designated *ers-0*, transformed with the wild-type 5-kb fragment. Each line contained a single transgene insertion (*11*).

We found that the Ile to Phe substitution in the *ERS* transgene conferred dominant ethylene insensitivity. In all four lines, we observed segregation of seedlings that lacked the normal triple response to ethylene (*18*) (Fig. 2A). The triple response consists of inhibition of root and hypocotyl elongation, radial swelling of the hypocotyl, and retention and accentuation of the apical hook. Absence of this response in the transgenic lines cosegregated with kanamycin resistance. In contrast, all seedlings of

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MESCDCFETHVNQDDLLVKYQYISDALIALAYFSIPLELIYFVQKSAFFPYKWVLMQFGAFIILCGATHFINLWMFFMHS  80
KAVAIVMTIAKVSCAVVSCATALMLVHIIPDLLSVKNRELFLKKKADELDREMGLILTQEETGRHVRMLTHGIRRTLDRH 160
TILRTTLVELGKTLCLEECALWMPSQSGLYLQLSHTLSHKIQVGSSVPINLPIINELFNSAQAMHIPHSCPLAKIGPPVG 240
RYSPPEVVSVRVPLLHLSNFQGSDWSDLSGKGYAIMVLILPTDGARKWRDHELELVENVADQVAVALSHAAILEESMHAR 320
DQLMEQNFALDKARQEAEMAVHARNDFLAVMNHEMRTPMHAIISLSSLLLETELSPEQRVMIETILKSSNLVATLISDVL 400
DLSRLEDGSLLLENEPFSLQAIFEEVISLIKPIASVKKLSTNLILSADLPTYAIGDEKRLMQTILNIMGNAVKFTKEGYI 480
SIIASIMKPESLQELPSPEFFPVLSDSHFYLCVQVKDTGCGIHTQDIPLLFTKFVQPRTGTQRNHSGGGLGLALCKRFVG 560
LMGGYMWIESEGLEKGCTASFIIRLGICNGPSSSSGSMALHLAAKSQTRPWNW                            613
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Fig. 1. Deduced amino acid sequence of the *ERS* gene (*21*). The NH_2-terminal domain is in bold, and the five conserved motifs of histidine protein kinases are underlined.

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Table 1. Organ elongation of etiolated seedlings in air and in ethylene.

Organ elongation and response	Strain						
	No-0	*ers-1(1)*	*ers-1(2)*	*ers-1(3)*	*ers-1(4)*	*etr1-1**	*etr1-2*
Hypocotyl (air) (cm)	1.04 ± 0.17	1.17 ± 0.07	1.10 ± 0.19	1.18 ± 0.06	1.13 ± 0.15	1.15 ± 0.15	0.95 ± 0.08
Hypocotyl (ethylene) (cm)	0.40 ± 0.07	1.19 ± 0.15	1.06 ± 0.13	1.03 ± 0.11	1.11 ± 0.10	1.32 ± 0.11	0.71 ± 0.09
Hypocotyl response (%)	38	102	96	87	98	115	75
Root (air) (cm)	0.31 ± 0.13	0.51 ± 0.15	0.39 ± 0.09	0.39 ± 0.10	0.38 ± 0.10	0.39 ± 0.15	0.25 ± 0.06
Root (ethylene) (cm)	0.04 ± 0.02	0.47 ± 0.16	0.31 ± 0.10	0.37 ± 0.09	0.37 ± 0.10	0.38 ± 0.13	0.13 ± 0.03
Root response (%)	13	92	79	95	97	97	52

*Homozygous *etr1-1* transformed No-0 (*5*).

the control line (*ers-0*) displayed the normal triple response (Fig. 2A).

To analyze the extent of ethylene insensitivity of the *ers-1* lines, we examined the inhibitory effect of ethylene on root and hypocotyl elongation in etiolated seedlings (Table 1). The same responses were analyzed in several other genotypes, including *ers-0* (*11*), wild-type [ecotype Nossen (No-0)], a strong ethylene-insensitive *etr1-1* transgenic line (*5*), and a weak ethylene-insensitive *etr1-2* mutant (Table 1). Comparison of the ethylene responses of all these genotypes revealed a strong ethylene-insensitive phenotype in the *ers-1* lines. Because all four *ers-1* lines were similar in phenotype, the *ers-1(1)* line was chosen for analysis at other developmental stages.

Older *ers-1* plants displayed ethylene insensitivity as well. At 2 weeks after germination, light-grown wild-type and *ers-0* (*11*) plants grown in the constant presence of ethylene (*18*) were short and had compressed leaves, whereas the ethylene-treated *ers-1* plants were unaffected (Fig. 2B). Leaf senescence, a typical response of mature wild-type plants to ethylene, was observed in wild-type and *ers-0* plants but not in the *ers-1* plants (*11*). In the absence of exogenous ethylene, the leaves of *ers-1* plants were 50% larger than those of wild-type or *ers-0* plants (*11*). The *etr1* mutants also had larger leaves than did wild-type plants (*2*). To analyze the basis for the enlargement in *ers-1* plants, we examined the upper leaf epidermal cells using Nomarski microscopy (Fig. 3) (*19*). The *ers-1* upper leaf epidermis had only about half the cell concentration of that in wild-type leaves (*11*), which implies that the transgenic cells are approximately twice as large as wild-type cells. This indicates that the primary cause of the larger leaves is cell enlargement rather than increased cell number. This enlargement is likely to be due to the cell's insensitivity to endogenous ethylene, because ethylene can inhibit cell expansion in other species (*20*). The opposite phenotype is observed in *ctr1* mutants, in which reduced leaf size is due at least in part to inhibition of cell expansion (*7*).

The altered *ERS* gene thus blocked a range of responses to either exogenous or endogenous ethylene. That *ctr1-1* is epistatic to *ers-1* was shown by double-mutant analysis (*11*), indicating that *ERS* acts upstream of *CTR1* in the ethylene signal transduction pathway, just as does *ETR1*. The sequence homology and the mutant phenotype suggest that ERS, like ETR1, might be a sensor of ethylene. Therefore, ethylene sensing in *Arabidopsis* could involve redundant functions of ERS and ETR1, which would help to explain the failure to find recessive mutants of either gene. Alternatively, ETR1 and ERS might act sequentially or as subunits in an ethylene-sensing complex.

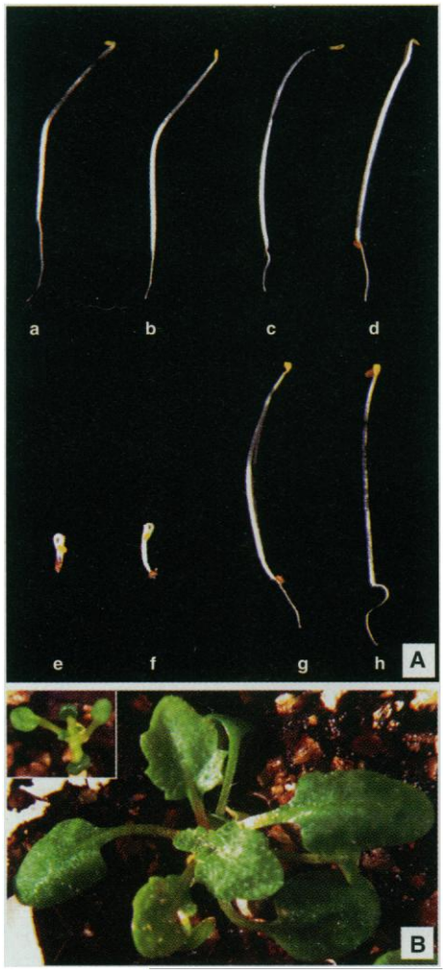

Fig. 2. Ethylene insensitivity of *ers-1* plants. (**A**) The triple response of 3-day-old seedlings. Etiolated seedlings were treated without ethylene (a through d) or with ethylene (e through h). a and e, wild-type (No-0) plants; b and f, *ers-0* plants; c and g, *ers-1* plants; and d and h, *etr1-1*–transformed No-0 plants. (**B**) Absence of growth inhibition by ethylene in the *ers-1* plant. The wild-type (No-0) plant (top left) and the transgenic *ers-1* plant (shown at the same magnification) were 2 weeks old and were grown under constant ethylene concentration (10 μl/liter).

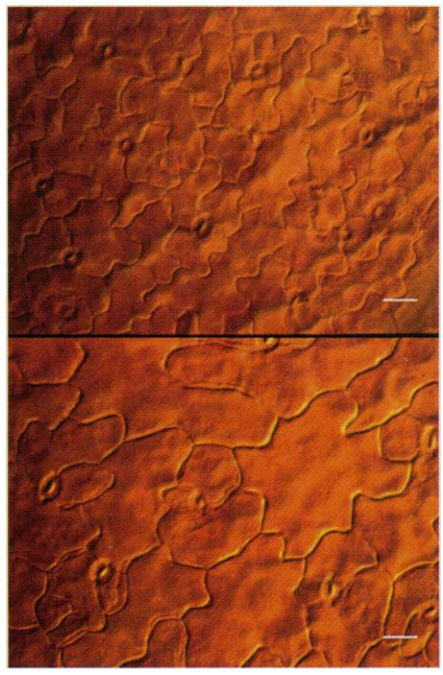

Fig. 3. Examination of cell expansion in upper leaf epidermal cells. Shown are Nomarski microscopy images of epidermal cells of the wild-type plant (**top**) and of the *ers-1* plant (**bottom**). Scale bars, 20 μm.

REFERENCES AND NOTES

1. F. B. Abeles, P. W. Morgan, M. E. Saltveit Jr., *Ethylene in Plant Biology* (Academic Press, New York, ed. 2, 1992).
2. A. B. Bleecker, M. A. Estelle, C. Somerville, H. Kende, *Science* **241**, 1086 (1988).
3. P. Guzmán and J. R. Ecker, *Plant Cell* **2**, 513 (1990).
4. N. V. J. Harpham *et al.*, *Ann. Bot.* **68**, 55 (1991); D.

Van der Straeten, D. A. Djudzman, W. Van Caeneghem, J. Smalle, M. Van Montagu, *Plant Physiol.* **102**, 401 (1993); G. Roman, B. Lubarsky, J. J. Keiber, M. Rothenberg, J. R. Ecker, *Genetics* **139**, 1393 (1995).
5. C. Chang, S. F. Kwok, A. B. Bleecker, E. M. Meyerowitz, *Science* **262**, 539 (1993).
6. J. B. Stock, A. M. Stock, J. M. Mottonen, *Nature* **344**, 395 (1990); J. S. Parkinson and E. C. Kofoid, *Annu. Rev. Genet.* **26**, 71 (1992).
7. J. J. Kieber, M. Rothenberg, G. Roman, K. A. Feldmann, J. R. Ecker, *Cell* **72**, 427 (1993).
8. T. Maeda, S. M. Wurgler-Murphy, H. Saito, *Nature* **369**, 242 (1994).
9. Genomic DNA isolated from *Arabidopsis* (ecotype Columbia) was partially digested with Sau 3A, then partially filled in, ligated to Xho I half-site arms of λ GEM11 (Promega), and packaged in vitro. We screened this library representing four to five genomes using the *ETR1* C5 cDNA (encoding residues 270 to 738) as a probe. We obtained 10 positive clones using a wash of 2× standard saline phosphate EDTA [SSPE; 1× is 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA] at 57°C. Five of them were reisolations of the *ETR1* gene, and the other five represented a newly discovered gene.
10. We screened approximately 1×10^5 plaque forming units of an *Arabidopsis* flower cDNA library in lambda ZAPII [D. Weigel, J. Alvarez, D. R. Smyth, M. F. Yanofsky, E. M. Meyerowitz, *Cell* **69**, 843 (1992)] at high stringency (65°C, 0.1 × SSPE), using a 1.2-kb Sac I–Sph I *ERS* genomic DNA fragment as a probe. Nine positive clones were obtained, and they were converted to plasmids by in vitro excision according to the manufacturer's instructions (Stratagene). They represented five cDNAs starting at different 5′ positions.
11. J. Hua, C. Chang, Q. Sun, E. M. Meyerowitz, unpublished data.
12. An *ERS* genomic DNA fragment was mapped by restriction fragment length polymorphism analysis in a Columbia-Landsberg mapping population, and linkage analysis was done with the MAPMAKER program [C. Chang, J. L. Bowman, A. W. DeJohn, E. S. Lander, E. M. Meyerowitz, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6856 (1988)].
13. We determined nucleotide sequences by the dideoxynucleotide chain termination method using a Sequenase II kit (U.S. Biochemical) according to the manufacturer's instructions.
14. I. M. Ota and A. Varshavsky, *Science* **262**, 566 (1993).
15. R. V. Swanson, L. A. Alex, M. I. Simon, *Trends Biochem.* **19**, 485 (1994).
16. A full-length *ERS* gene was cloned into the Hind III and Sac I sites of plasmid pGEM7Z(+) (Promega) by ligation of a 4-kb Hind III fragment and a 2-kb Hind III–Sac I fragment. In vitro mutagenesis was done with the use of a Muta-Gene Phagemid in vitro mutagenesis kit (Bio-Rad). A primer (5′-GGAGCCTTTTTCATTCTC-3′) containing the desired A to T change was used.
17. A 5-kb Kpn I genomic DNA fragment was cloned into the Kpn I site of pCGN1547 [K. E. McBride and K. R. Summerfelt, *Plant Mol. Biol.* **14**, 269 (1990)]. *Agrobacterium tumefaciens* strain ASE [R. T. Fraley *et al.*, *Biotechnology* **3**, 629 (1985)] carrying constructs in pCGN1547 was used to transform roots of *Arabidopsis* (ecotype No-0) according to standard protocols [D. Valvekens, M. Van Montagu, M. Van Lijsebettens, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5536 (1988); L. Marton and J. Browse, *Plant Cell Rep.* **10**, 235 (1991)].
18. We performed incubations in ethylene by placing plates or pots in a sealed chamber with an ethylene flow giving a concentration of approximately 10 μl/liter.
19. The last two rosette leaves produced by the plants before bolting were excised, prepared, and examined as described (*7*). Ten representative areas from the upper leaf epidermis per leaf were photographed. Epidermal cell numbers in each photograph were counted, and the cell concentration was deduced. Seven leaves each from *ers-1* and No-0 plants were analyzed.
20. G. L. Funke, F. DeCoeyer, A. DeDecker, J. Maton, *Biol. Jaarb.* **5**, 335 (1938); O. M. Van Andel and D. R. Verkerke, *J. Exp. Bot.* **29**, 638 (1978).
21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. We thank E. Koh for carrying out plant transformations; L. Medrano for RFLP mapping; and X. Chen, S. Jacobsen, B. Krizek, J. Levin, Z. Liu, J.-L. Reichmann, M. Running, R. Sablowski, H. Sakai, and B. Williams for critical comments on the manuscript. Funded by U.S. Department of Energy grant FG03-88ER13873 to E.M.M.

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Convergent Domestication of Cereal Crops by Independent Mutations at Corresponding Genetic Loci

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Independent domestication of sorghum, rice, and maize involved convergent selection for large seeds, reduced disarticulation of the mature inflorescence, and daylength-insensitive flowering. These similar phenotypes are largely determined by a small number of quantitative trait loci (QTLs) that correspond closely in the three taxa. The correspondence of these QTLs transcends 65 million years of reproductive isolation. This finding supports models of quantitative inheritance that invoke relatively few genes, obviates difficulties in map-based cloning of QTLs, and impels the comparative mapping of complex phenotypes across large evolutionary distances, such as those that separate humans from rodents and domesticated mammals.

Most calories consumed by humans and livestock derive from the major cereals: rice, wheat, maize, millet, and sorghum. The cereals are members of the grass family (Poaceae) and were each domesticated from their wild relatives between 7000 and 12,000 years ago (*1*), quite recently in human history. Independent domestication of the four major cereal complexes—in Africa (sorghum and millet), Asia (rice) [but see (*1*)], the Near East (wheat, barley, oats, and rye) and America (maize)—produced similar results: In all cases, small-seeded wild grasses with natural seed dispersal were converted into large-grained symbionts that depended on farmers to harvest and sow their seed (*1*).

Although conservation of gene order along the chromosomes is well known to transgress species boundaries, the extent of correspondence in the QTLs that account for variation in complex phenotypes has been a point of conjecture. Comparative maps reveal a common order of genes and monogenic phenotypes over large chromosomal tracts in grasses after 65 million years (My) of divergence (*2*) and in mammals after 100 My of divergence (*3*). However, models for quantitative inheritance that invoke many genes, each with only tiny effects (*4*), would predict little correspondence of QTLs across taxa. Recent QTL mapping studies suggested a simpler basis for quantitative inheritance (*5*), although such studies detected only a subset of QTLs with relatively large effects (*6*). Correspondence of QTLs in congeneric plant species has been suggested (*7*), but the promiscuity of plants carries the possibility of recent genetic exchange between species.

If QTLs in disparate taxa mapped to corresponding locations more often than would be expected by chance, such a finding would strongly suggest that corresponding genes were involved in the evolution of the relevant phenotypes. To investigate this hypothesis, we assessed correspondence between QTLs that affect seed mass and disarticulation of the mature inflorescence (shattering) in crosses between cultivated and wild sorghum species, between cultivated and wild maize species, and between divergent subspecies of cultivated rice (*8*). Correspondence among short-day flowering mutations was evaluated in these and additional taxa. Use of interspecific (sorghum, maize) or subspecific (rice) crosses maximized segregation for allelic variants at both QTLs and DNA markers.

We studied three traits that were inde-

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