entire plasmid previously linearized with Bam HI or Pst I (Fig. 3). Creation of a unique Hph I site within the o_L1 operator site is demonstrated by the presence of only two bands in lanes 4 and 8. All other Hph I sites were inactivated by the M·Hph I used in the presence of λ repressor.

These experiments (see legend to Fig. 3) consisted of the following five steps: (i) addition of λ repressor followed by M·Hph I, (ii) thermal inactivation of M·Hph I, (iii) RecA-mediated cleavage of λ repressor (*10*), (iv) phenol extraction and ethanol precipitation, and (v) cleavage with Hph I. The additional steps are necessary because λ repressor, unlike *lac* repressor, is not irreversibly inactivated by heating to 75°C. The repressor protein must be cleaved by the RecA protease to prevent dimerization and cooperative binding to DNA, which is stable enough to carry the DNA into the phenol phase during the subsequent extraction. Such phenol extraction is necessary to remove NH_2-terminal repressor fragments that bind specifically to the operator and inhibit Hph I cleavage. As an alternative to treatment with RecA, the reactions can be adjusted to *p*H 10, under which conditions λ repressor undergoes autodigestion (*11*).

We have demonstrated efficient operator-specific cleavage in the *lacO* and λ o_L systems. The approach described, however, is not limited to these two systems. We anticipate that it will be applicable to many other methylases in combination with repressors or any of a wide range of other proteins that form stable, sequence-specific complexes with DNA. The particular choice of methylase and "blocking protein" would be dictated by the specificity and frequency of cleavage desired. For example, *lac* repressor mutants with altered binding specificity or affinity (*12, 13*) would generate modified cleavage patterns.

The sizes of fragments that would be generated from a digest of genomic DNA by means of this method would depend on the blocking protein used to protect restriction sites from methylation. For example, protection of restriction sites in mammalian DNA by *lac* repressor should result in very little cleavage, if any, because statistically a 20-bp recognition sequence would be expected to occur only once in every 1.1×10^9 kb. However, if eukaryotic transcription factors could be used to block methylation, the DNA should be cleaved near the genes under the control of the particular transcription factor used. Other factors that would influence the fragment size include the stability of protein binding to variant sites and the frequency of overlap between restriction sites and such variant binding sites.

The types of blocking proteins that will prove useful for this technique remain to be seen. We anticipate that many proteins involved in the regulation of transcription (for example, repressors and transcription factors) will form DNA-protein complexes stable enough to exclude a modification methylase. In addition, the use of restriction enzymes to block methylation of overlapping sequences is being investigated.

This approach, which we would like to designate "Achilles' heel cleavage" (AC), should facilitate the mapping and manipulation of large DNA molecules. A specific gene, for instance, could be tagged by integration of a mobile genetic element containing a cleavable operator, thus allowing physical localization and isolation by a variety of methods, including the use of an affinity (repressor-coated) column. Furthermore, these rare sites are ideal for use in large cloning vehicles requiring unique restriction sites, such as yeast artificial chromosome vectors (*14*). Our methods create the equivalent of new restriction enzymes of extremely high specificity and provide a powerful new tool for genetic manipulation.

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Insensitivity to Ethylene Conferred by a Dominant Mutation in *Arabidopsis thaliana*

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Ethylene influences a number of developmental processes and responses to stress in higher plants. The molecular basis for the action of ethylene was investigated in mutants of *Arabidopsis thaliana* that have altered responses to ethylene. One mutant line, which has a dominant mutation at a locus designated *etr*, lacks a number of responses to ethylene that are present in the wild-type plant. These include inhibition of cell elongation, promotion of seed germination, enhancement of peroxidase activity, acceleration of leaf senescence, and feedback suppression of ethylene synthesis by ethylene. These diverse responses, which occur in different tissues of *Arabidopsis*, appear to share some common element in their transduction pathways—for example, a single receptor for ethylene. Results of ethylene binding experiments in vivo indicate that this receptor may be affected by the *etr* mutation.

THE GASEOUS COMPOUND ETHYLENE is an endogenous regulator of growth and development in higher plants. Increases in the level of ethylene influence many developmental processes, from seed germination and seedling growth (*1*) to leaf abscission, organ senescence, and fruit ripening (*2*). A number of environmental stresses including oxygen deficiency, wounding, and pathogen invasion enhance ethylene synthesis (*3*). Stress-induced ethylene elicits adaptive changes in plant development—for example, the formation of adventitious roots and air-conducting channels in water-logged terrestrial plants and rapid stem elongation in submerged semiaquatic plants (*4*). Wounding and pathogen invasion may result in ethylene-mediated acceleration of senescence and abscission of infected organs and in the induction of specific defense proteins such as chitinase, glucan-

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ases, and hydroxyproline-rich glycoproteins (*5*).

Little is known about how plant tissues recognize ethylene and how the recognition signal is transduced to yield the well-characterized responses to ethylene. Work with agonists and antagonists of ethylene action has led to the suggestion that ethylene binds to a transition metal at the receptor site (*6*).

Table 1. Responses to ethylene in wild-type (wt) and ethylene-insensitive (*etr*) *Arabidopsis*. All experiments were performed at least three times with similar results. Organ elongation was measured as the length (millimeters) of organ after 3 days of growth on 1% agar (*9*) in the dark in sealed 1-liter jars containing air or ethylene (1 μl/liter); values are means from ten seedlings ± SE; 100% elongation is 13.2 ± 0.5 (wt), 14.7 ± 0.3 (*etr*) for the hypocotyl, and 2.9 ± 0.8 (wt), 3.4 ± 1.0 (*etr*) for the root. Chlorophyll content was measured as micrograms per gram of fresh weight of rosette leaves and stems of plants incubated for 60 hours in chambers through which either air or ethylene (10 μl/liter) in air was passed at a flow rate of 240 ml/min. The tissue was extracted with 80% acetone and chlorophyll was measured as described (*22*); values are means of four determinations ± SE; 100% chlorophyll is 403 ± 11 (wt), 436 ± 25 (*etr*) for stems, and 811 ± 42 (wt), 944 ± 99 (*etr*) for leaves. For measurement of peroxidase activity, plants were treated as for chlorophyll determinations. Extracts, prepared by grinding tissue in 50 m*M* sodium acetate, *p*H 6.0, were assayed as described (*23*) with guaiacol used as substrate; values are the means of four determinations ± SE; 100% peroxidase activity (micromoles per minute per gram of fresh weight) is 0.57 ± 0.18 (wt), 0.37 ± 0.12 (*etr*) for stems, and 7.9 ± 1.6 (wt), 2.5 ±1.1 (*etr*) for leaves. For measurement of ethylene biosynthesis, excised leaves were treated with air or ethylene (10 μl/liter) in flow-through chambers for 12 hours and placed thereafter in 10-ml glass culture tubes sealed with serum-vial caps. After 30 minutes, 1-ml samples were taken from the head space of the sealed tubes, and ethylene was quantified by gas chromatography (*24*). Ethylene production rates (nanomoles per hour per gram of fresh weight): 100% ethylene biosynthesis is 0.19 (wt), 0.25 (*etr*). For seed germination, seeds (3 to 4 weeks after anthesis) were plated on 1% agar (*9*) and incubated in the dark in air or in ethylene (5 μl/liter). After 5 days, the percentage of seeds that had germinated was recorded; germination in air is 14% (wt), 0% (*etr*).

Parameter	Ethylene response (percent of air control)	
	Wild type	*etr* mutant
Organ elongation		
Root	17	111
Hypocotyl	36	104
Chlorophyll content		
Leaf	35	97
Stem	110	94
Peroxidase activity		
Leaf	303	102
Stem	421	92
Ethylene biosynthesis		
Leaf	15	96
Seed germination	664	100

High-affinity binding sites for ethylene have been detected in plant tissues (*7, 8*). However, no direct connection has been established between ethylene binding and any physiological response. To gain further insight into the mechanism of ethylene action, we isolated mutants with altered responses to ethylene in the cruciferous plant *Arabidopsis thaliana*.

We identified ethylene-insensitive mutants by taking advantage of the ethylene-mediated inhibition of hypocotyl elongation in dark-grown seedlings. Populations of the M_2 generation from mutagenized seed (*9*) of *A. thaliana* (Columbia wild type) were plated on a minimal medium solidified with 1% agar (*9*) and placed in a chamber through which ethylene (5 μl/liter) in air was circulated. Seedlings that had grown more than 1 cm after 4 days were selected as potential ethylene-insensitive mutants. A screen of 75,000 seedlings yielded three mutant lines that showed heritable insensitivity to ethylene. We have designated the mutation that causes ethylene insensitivity in one of these lines as *etr* to indicate that it is a mutant allele of the *ETR* gene (*10*). Dose-response curves for the effect of ethylene on hypocotyl elongation in the wild-type and the *etr* mutant line are shown in Fig. 1. While elongation of wild-type hypocotyls was inhibited by 70% with ethylene at 1 μl/liter, hypocotyl elongation of the mutant line was unaffected by concentrations of up to 100 μl/liter. Growth of the primary root was also inhibited by ethylene in the wild-type but not in the *etr* mutant (Table 1).

The genetic basis of the ethylene insensitivity was examined in the *etr* mutant. Segregation of ethylene insensitivity in the M_3 progeny of the original isolate was consistent with a 3:1 ratio (38 insensitive, 13 sensitive; $\chi^2 = 0.01$, $P > 0.9$), indicating that the initial ethylene-insensitive plant was heterozygous for a dominant mutation. Ethylene-insensitive M_3 plants were backcrossed to the wild-type plant, ethylene-insensitive F_1 plants from these crosses were allowed to self-fertilize, and several mutant lines homozygous for the *etr* mutation were identified in the resulting F_2 generation. Crosses of these lines to the wild type resulted in ethylene-insensitive F_1 progeny. Segregation of ethylene insensitivity in the F_2 progeny was also consistent with a 3:1 ratio (53 insensitive, 20 sensitive; $\chi^2 = 0.29$, $P > 0.5$), confirming that ethylene insensitivity was due to a dominant mutation. To obtain a chromosomal location for the *etr* mutation, we crossed a plant homozygous for *etr* onto a line (W-100) carrying ten phenotypic markers in the Landsberg erecta background (*11*). Linkage analysis of 176 F_2 plants indicated that the *etr* mutation is located near the *ap-1* locus (percent recombination, 5.5 ± 8.0) on chromosome 1 (*12*).

Plants homozygous for the *etr* mutation are similar in appearance to their wild-type counterparts, indicating that the mutation does not interfere with major developmental processes. Rosette leaves of the mutant were, on average, 25% larger in area than wild-type leaves. In addition, mutant plants were delayed by 1 to 2 weeks in bolting (flowering) and senescence of rosette leaves

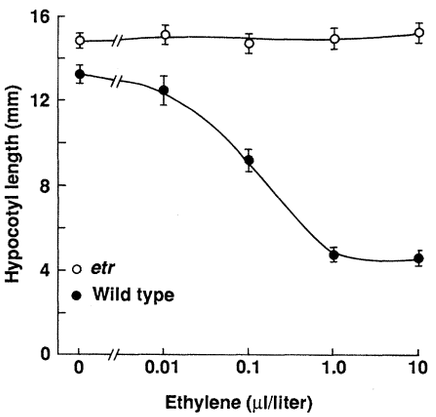

Fig. 1. The effect of increasing ethylene concentrations on hypocotyl elongation of dark-grown wild-type (●) and *etr* (○) *Arabidopsis* seedlings. Seeds were germinated on 1% agar (*9*) in sealed 1-liter containers into which ethylene was injected to give the concentrations indicated. The jars were opened every 12 hours, flushed with air, resealed, and then reinjected with ethylene. Hypocotyl length was measured after 3 days of growth. Each point represents the mean of ten measurements ± SE. In a separate experiment, ethylene at 100 μl/liter had no effect on the elongation of mutant hypocotyls.

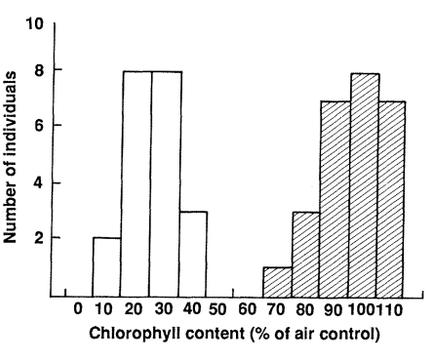

Fig. 2. The cosegregation of insensitivity to ethylene-induced chlorophyll loss with insensitivity to ethylene-mediated inhibition of hypocotyl elongation. A plant heterozygous for the *etr* mutation was self-fertilized, and the progeny were separated into ethylene-sensitive (open bars) and ethylene-insensitive (shaded bars) plants on the basis of the hypocotyl elongation response. For the determination of ethylene-induced chlorophyll loss, leaf disks (6 mm in diameter) were taken from leaves of 5-week-old plants and incubated on distilled water in the dark for 3 days in air or ethylene (10 μl/liter) in air. Chlorophyll content was determined as described (*19*).

Table 2. In vivo binding of [^{14}C]ethylene in wild-type and ethylene-insensitive (*etr*) *Arabidopsis* was measured by using the isotope displacement assay of Sisler (*8*). Rosette leaves (7 to 10 g) were incubated in sealed 1.5-liter glass chambers for 5 hours in the presence of 0.5 μCi of [^{14}C]ethylene (specific activity 103 mCi/mmol) with or without [^{12}C]ethylene (667 μl/liter). The leaves were then removed from each chamber, aired for 50 seconds, and transferred to a second chamber for 16 hours. A beaker containing 1 ml of mercuric perchlorate was included in the second chamber to trap ethylene emanating from the leaves. Radioactivity associated with trapped ethylene was determined by liquid scintillation spectrometry. Values are expressed as disintegrations per minute per 10 g of fresh weight and are the means of three experiments ± standard deviation. The difference between determinations *a* and *b* reflects the level of saturable binding.

Treatment	[^{14}C]Ethylene trapped (dpm/10 g)	
	Wild type	*etr* mutant
(*a*) [^{14}C]ethylene (0.07 μl/liter)	382 ± 52	135 ± 30
(*b*) [^{14}C]ethylene (0.07 μl/liter) + [^{12}C]ethylene (667 μl/liter)	57 ± 10	76 ± 28
Difference: *a* − *b*	325 ± 53	59 ± 41

relative to the wild type. Seeds carrying the *etr* mutation showed very low germination rates when compared to wild-type seeds germinated under the same conditions (*13*).

Since the *etr* mutation affected the response to ethylene in both root and shoot tissue of the seedling, we examined the effect of the mutation in other tissues and at different developmental stages of the plant. The germination of dormant seeds of the wild-type but not of mutant plants could be induced by ethylene treatment (Table 1). When 6-week-old wild-type plants were placed in a chamber through which ethylene (10 μl/liter) was circulated, leaf senescence, as measured by chlorophyll loss, was observed after 2 to 3 days (Table 1). No ethylene-induced leaf senescence was detected in mutant plants. The lack of ethylene-mediated chlorophyll loss in *etr* mutants is genetically linked to the lack of effect on hypocotyl elongation (Fig. 2). Ethylene treatment also caused a three- to fourfold increase in extractable peroxidase activity (*14*) in the stem and leaves of wild-type but not of mutant plants (Table 1).

Excised leaves from wild-type and *etr* mutants showed similar rates of ethylene production (Table 1). Wound-induced increases in ethylene synthesis in stem tissue were also observed in wild-type and mutant plants (*15*). However, when excised leaves were pretreated with ethylene, endogenous ethylene production was greatly reduced in the wild type but not in the mutant (Table 1). Thus, while ethylene synthesis rates are not significantly altered by the *etr* mutation, feedback regulation by ethylene (*3*) does not occur in these plants.

The results summarized in Table 1 indicate that a variety of ethylene responses, occurring in different tissues and at different stages in the life cycle of the wild-type *Arabidopsis* plant, are affected by the *etr* mutation and, therefore, must share some common element in their signal transduction pathways. It can also be argued that the lesion produced by the *etr* mutation must occur early in the signal transduction chain because the biochemical bases for the various responses are presumably different. It is interesting that the apparent elimination of detectable ethylene responses does not drastically alter the growth and development of the plant. Although we cannot rule out the possibility that there are ethylene-mediated processes in *Arabidopsis* that are not affected by the *etr* mutation and that may be required for normal development, the ethylene responses examined in the above experiments represent many of the effects by which ethylene has been recognized as a regulator of plant growth and development (*1, 2, 4*).

Because all of the ethylene responses that were examined are affected by the *etr* mutation, a single receptor for ethylene may be present in all tissues of *Arabidopsis*, and it is possible that the mutation directly affects this receptor. As a first step toward testing this hypothesis, we determined the capacity of wild-type and mutant plants to bind [^{14}C]ethylene in vivo by using the isotope displacement assay of Sisler (*8*). The results indicate that saturable binding of ethylene in *etr* mutants is one-fifth of that found in wild-type plants (Table 2). While the in vivo binding assay does not provide a direct measure for ethylene binding to a receptor, our data are nevertheless consistent with the possibility that the receptor function in the mutant is impaired.

Any hypothesis regarding the biochemical basis for the mutant phenotype must take into account the dominant nature of the mutation. Dominance could be explained by models involving multimeric receptor complexes containing identical subunits of the *ETR* gene product (*16*). Alternatively, dominance could be explained by models in which the product of the *ETR* gene functions, in the absence of ethylene, to suppress the expression of ethylene responses. In wild-type plants, ethylene would inhibit the function of this gene product, while in the mutant ethylene would be ineffective. Biochemically defined mutations of this type, identified in bacteria and yeast (*17*), occur in genes involved in the regulation of transcription. There is evidence that differential gene expression is associated with a number of ethylene responses in plants (*5, 18*). The possibility that ethylene could interact directly with transcriptional regulatory proteins may therefore be considered. Ethylene binding in plants is thought to involve interaction with a transition metal complex (*6*). There is evidence that a number of eukaryotic DNA-binding proteins contain transition metal complexes that are important in the molecular structure of the DNA-binding regions (*19*).

Cloning of the *ETR* gene would facilitate the testing of hypotheses concerning the nature of its gene product. Having a relatively small genome and little repetitive DNA (*20*), *Arabidopsis* is particularly suited for the cloning of genes based on the genetic segregation of mutant phenotypes. The pending development of methods for chromosome walking and transposon tagging (*21*) in *Arabidopsis* is expected to open the possibility that genes, such as *ETR*, involved in plant hormone action will soon be cloned.

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10. The convention for genetic nomenclature recommended at the Third International *Arabidopsis* Meeting (East Lansing, MI, April 1987) is (i) genotypes are italicized or underlined; (ii) the wild-type genotype is capitalized; (iii) the genotype of mutant alleles is lower case; (iv) alleles are specified by a dash followed by a number, and if no allele is specified, it is assumed to be number 1; (v) all gene symbols should be in three letters; and (vi) phenotypes are designated by the gene symbol, which is not underlined but has the first letter capitalized.

11. The W-100 line carries ten homozygous recessive mutations with visible phenotypes, two on each chromosome. The *ap-1* (apetala) locus has been mapped to position 103.5 on chromosome 1 [M. Koornneef *et al.*, *J. Hered.* **74**, 265 (1983)]. Seeds of W-100 were kindly supplied by M. Koornneef, Department of Genetics, Agricultural University, Wageningen, The Netherlands.
12. Values given for linkage analysis are percent recombination ± the standard error as calculated with maximum likelihood formulas. Calculations were performed with the Linkage-1 computer program [K. A. Suiter, J. F. Wendel, J. S. Case, *J. Hered.* **74**, 203 (1983)].
13. Freshly harvested seeds of the wild type showed >95% germination if imbibed seeds were preincubated at 4°C for 3 days. The *etr* mutant seeds did not germinate under these conditions. The mutant line showed >90% germination if seeds were treated with 5 μM gibberellic acid (GA_3) during the 3-day preincubation.
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Isotypic Exclusion of γδ T Cell Receptors in Transgenic Mice Bearing a Rearranged β-Chain Gene

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The rearrangement of T cell antigen receptor β- and γ-chain gene segments was studied in transgenic mice that bear a functional β-chain gene. Virtually all CD3-positive T cells derived from transgenic mice express β chains containing the transgene-encoded $V_\beta 8.2$ variable region on their surfaces and do not express endogeneous β-chain variable regions. Expression of endogenous V_β genes is inhibited at the level of somatic recombination during thymic ontogeny. Furthermore, rearrangements of the TCR γ-chain genes are also markedly inhibited in these transgenic animals. Hence expression of the TCR β transgene has led to allelic exclusion of αβ receptors and isotypic exclusion of γδ T cell receptors.

THE DIVERSITY OF THE T CELL RECEPTOR (TCR) for antigen is generated during intrathymic development by a regulated process involving somatic recombination of variable (V), diversity (D), and joining (J) gene segments. Findings that individual T cells can express on their surface either a γδ or an αβ receptor (*1*, *2*) and that the γ and δ genes rearrange early in ontogeny, followed by β and then α (*3*), have led to models in which products of TCR rearrangement determine not only the antigenic specificity of the T cell but also the lineage to which it will belong. Although recombination of TCR gene segments involves a mechanism whose features appear similar to those of the immunoglobulin (Ig) system (*4*), the signals that sequentially activate each locus to begin recombination and those that terminate these events are unknown.

Each T cell expresses only one functional idiotypic receptor (*2*), and therefore mechanisms must exist to exclude further TCR gene rearrangements once a functional receptor is produced, a phenomenon referred to as allelic exclusion. Allelic exclusion in B cells has been studied by introducing functional heavy or light chain Ig genes into the germline of mice and defining the effects of the transgene on endogenous Ig gene rearrangements. Expression of injected μ heavy chain genes significantly decreases the expression of endogenous heavy chain loci by inhibiting V-to-DJ and D-to-J recombination events (*5*). Several lines of evidence, including the characterization of transgenic mice bearing a productive κ-chain gene, indicate that allelic exclusion of light chain loci is mediated by intact Ig (*6*).

To study the mechanisms by which productively rearranged TCR genes regulate developmental events during T cell ontoge-

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Table 1. Phenotype of peripheral T cells and thymocytes by FACS analysis. Screening of purified peripheral blood T cells showed that all animals were heterozygous H-2^b × H-2^s and homozygous for the SJL endogenous TCR β locus. Lymph node T cells were purified by passage over nylon wool. Primary antibodies were KJ23a (*20*), 2C11 (*21*), F23.1 (*9*), GK1.5 (*22*), and 53.6.72 (*23*). RR-4.7 is a rat monoclonal antibody specific for $V_\beta 6$ (*24*). T cells and thymocytes were stained and analyzed as previously described (*20*). FACS analysis performed on other mice from the TCRE-1 and TCRE-2 lines confirms the data shown here.

Determinant	Antibody	Peripheral T cells (%)		Thymocytes (%)	
		Control	TCRE-1	Control	TCRE-1
CD3	2C11	99	94		
$V_\beta 8$	F23.1	0	97	0.06	76
$V_\beta 17a$	KJ23a	5.45	0.05	3.28	0.2
$V_\beta 6$	RR-4.7	12.12	0.34		
CD4	GK1.5	58.78	41.7		
CD8	53.6.72	36.93	54.78		