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Molecular Analysis of Ethylene-Insensitive Mutants in Arabidopsis

The subject of the work funded by DOE has been the biochemical basis of ethylene reception. The work follows the isolation of a mutation in an Arabidopsis thaliana gene called ETR. The phenotype of etr mutations is a loss of the ability to respond to ethylene in a number of different assays of response to this plant hormone, such as hypocotyl elongation, peroxidase induction, and leaf senescence. In addition, binding of labeled ethylene to leaves of the mutant is about 5-fold lower than bonding to wild-type. This led to the conclusion that the Arabidopsis ETR gene codes either for the ethylene receptor, or for a component of the cellular machinery specifically necessary for the action of this receptor. One peculiarity of the etr mutation is that it is partially dominant: one copy of the mutant gene heterozygous with one copy of the wild-type allele gives a partially ethylene-resistant phenotype. Specifically, the hypocotyl elongation response is absent in heterozygotes, as is ethylene-dependent leaf senescence. Homozygotes for the mutant copy have no root growth response to ethylene, and no germination or ethylene production responses to the hormone, either.

We have cloned the region of the Arabidopsis genome that contains the ETR gene by chromosome walking. Overlapping cosmid and yeast artificial chromosome (YAC) clones have been isolated, starting from a genomic position defined by an RFLP marker that maps very near the ETR gene. The cloned region is over 100 kilobase pairs, and is known to contain the ETR gene by genetic criteria: recombinants between ETR and flanking genes have been made, and the points of recombination between the flanking genes and ETR have been shown by RFLP analysis to be in the region

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walked over. Cosmid-cloned segments of genomic DNA representing much of the walk were isolated from dominant mutant etr plants were transformed into wild-type Arabidopsis plants, and some of the transformants were resistant to ethylene in the hypocotyl elongation assay. This is already a useful result, because it means that we can take wild-type plants and make them at least partially ethylene-resistant by standard procedures.

The remarkable results are only developing now: we have started sequencing the two genes that are found in those cosmid clones that confer hormone resistance. One of them has a derived amino acid sequence with three recognizable protein motifs. One is a clear hydrophobic transmembrane domain, indicating that the gene product is a transmembrane protein. On the cytoplasmic (carboxy-terminal) side there is a serine-threonine protein kinase domain, showing high homology with the conserved regions exhibited by proteins with this activity. Outside the membrane is a leucine-rich repeat of the type shown in yeast, Drosophila and mammals to be a region of interaction with other proteins (in the yeast adenyl cyclase protein, this region apparently interacts with the RAS protein). While this is a new type of protein kinase, and the first representative of what we now know to be a family of genes coding for similar kinases, the DNA sequence of the new kinase gene shows no differences between wild type and etr mutants. It is thus most likely not the ETR gene (unless the dominant mutation is due to an alteration in a region upstream of the gene, which is not yet sequenced).

The second gene is thus our current candidate. The sequence information so far available shows this one to code for a protein with a zinc-finger, an attractive possibility for an ethylene-binding protein, since studies of ethylene binding to plant material indicate that a transition metal is involved in the binding. We have not found a sequence difference between the zinc-finger genes from wildtype and etr plants yet, though, so there is no evidence that this is the ETR gene. We continue to

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sequence, and to look for new transcripts that derive from the 10 kilobase-pair region that gives the positive transformation results. We are also more finely defining the genetic region that contains ETR by obtaining more lines with meiotic recombination points between ETR and the flanking markers.

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