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The Ethylene Signal Transduction Pathway in Plants

Joseph R. Ecker

Ethylene (C_2H_4), the chemically simplest plant hormone, is among the best-characterized plant growth regulators. It participates in a variety of stress responses and developmental processes. Genetic studies in *Arabidopsis* have defined a number of genes in the ethylene signal transduction pathway. Isolation of two of these genes has revealed that plants sense this gas through a combination of proteins that resemble both prokaryotic and eukaryotic signaling proteins. Ethylene signaling components are likely conserved for responses as diverse as cell elongation, cell fate patterning in the root epidermis, and fruit ripening. Genetic manipulation of these genes will provide agriculture with new tools to prevent or modify ethylene responses in a variety of plants.

The simple gas ethylene is an endogenous regulator of developmental adaptations in higher plants (*1*). Exposure to ethylene can produce a myriad of effects on plant growth, development, and physiology, most notably the ripening of fruits, inhibition of stem and root elongation, promotion of seed germination and flowering, senescence of leaves and flowers, and sex determination. How this simple olefin evokes such a diverse array of physiological processes has been a central question in ethylene research.

The biosynthesis of ethylene is stimulated prior to several developmentally programmed senescence processes and in response to environmental insults such as mechanical trauma and pathogen infection (*2*, *3*). As a result of biochemical analysis, the route of ethylene synthesis (the Yang Cycle) is now largely understood (*4*, *5*). The rate-limiting step is the conversion of *S*-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), which is catalyzed by ACC synthase. The enzyme ACC oxidase converts ACC to ethylene, carbon dioxide, and cyanide. ACC oxidase is constitutively present in most tissues, but its synthesis is increased during fruit ripening in tomato. The genes that encode ACC synthase and ACC oxidase have been cloned and characterized from many plant species (*5*, *6*). ACC synthase is encoded by multigene families in all species examined, and individual gene family members are transcriptionally activated by a variety of inducers. Environmental stresses (physical, chemical, and biological) and hormonal signals, such as auxin, cytokininin, and even ethylene itself, stimulate synthesis of the ACC synthase enzyme, thereby providing a means for autoregulation of its production. Although tremendous progress has been made since 1989, questions still remain regarding the complex regulation of ethylene biosynthetic genes. However, it is clear that genetic manipulation of the ACC synthase and ACC oxidase genes by expression of antisense RNA (*7*) will provide a simple means to control the ripening of fruits in a variety of plants (*4*, *8*).

By contrast, biochemical approaches toward dissection of the mechanisms by

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which plants perceive and respond to ethylene have not been as fruitful. Because ethylene is an olefin, it has been postulated that its receptor may be either a Zn^{2+}- or a Cu^{2+}-containing metalloprotein (*9*, *10*). This proposal is attractive because compounds such as carbon monoxide (CO), a neurotransmitter in animals, are known to complex with transition metals and have ethylene-like effects on plants (*9*, *10*). Unfortunately, attempts to purify the putative metalloprotein receptor for ethylene have been unsuccessful (*10*, *11*). Molecular genetic studies of the reference plant *Arabidopsis thaliana* have provided new insight into how plants perceive and respond to ethylene (*12–14*).

The Triple Response Phenotype

Dicotyledonous seedlings grown in the dark undergo dramatic morphological changes in the presence of ethylene. These aberrations are collectively referred to as the "triple response" and in pea include the inhibition of epicotyl and root elongation, radial swelling of epicotyl and root cells, and the development of a diageotrophic (horizontal) growth habit (*15*). The spectacular effects of ethylene on pea seedling development were the first demonstration that a gas could act as a signaling molecule in any biological system (*16*). Before the development of gas chromatography, this response provided a sensitive bioassay for the presence of ethylene, an important plant growth regulator and environmental pollutant (*1*, *17*).

Haberlandt first suggested that these dramatic morphological changes may be a stress-induced adaptation that allows seedlings to penetrate the soil without damage to the apical meristem [cited in (*18*)]. This early hypothesis has been borne out both by physiological (*19*) and genetic experiments (*20*). Physical obstruction of seedling growth leads to dramatic increases in ethylene biosynthesis, which induces development of the triple response morphology (*19*).

Induction of the triple response relies on the plant's ability to perceive and respond to ethylene, because inhibitors of ethylene perception or biosynthesis and mutations that eliminate all ethylene responses prevent this morphological transformation (*21–24*). Characteristics of the ethylene-evoked triple response in *Arabidopsis* include inhibition of root and hypocotyl elongation, radial swelling of the hypocotyl and root, and exaggeration in the curvature of the apical hook (Fig. 1). Because of its high reproducibility and the ease of screening large numbers of individuals at an early stage of development, the triple response phenotype in *Arabidopsis* provides a simple means to identify mutants that either fail to respond to exogenous ethylene or constitutively display the response in the absence of the hormone. Such screens have allowed the identification of a number of genes that are likely to be involved in the control of ethylene biosynthesis, the perception of ethylene, or the propagation of its stimulus (*13*, *14*, *20*, *23–26*) (Table 1).

Constitutive Response Mutants in *Arabidopsis*

Plant mutants that produce significantly increased amounts of ethylene have been isolated in *Arabidopsis* (*13*, *24*). The ethylene overproduction (Eto^-) mutants *eto1*, *eto2*, and *eto3* all display the triple response in the absence of exogenously added ethylene (Table 1). Treatment of Eto^- seedlings with inhibitors of ethylene biosynthesis or antagonists of ethylene action abrogates the constitutive triple response phenotype, indicating that the Eto^- mutants are defective in ethylene biosynthesis (*13*, *24*). Unlike Eto^- seedlings, the constitutive triple response phenotype displayed by one mutant, *constitutive triple response 1* (*ctr1*) (Fig. 1), cannot be reversed by inhibitors of ethylene biosynthesis or action, suggesting that this mutant is defective in ethylene signal transduction (*13*). The growth habit of mature *ctr1* plants is also altered dramatically, with compact and epinastic (downward curled) rosette leaves that resemble those of wild-type plants grown in ethylene, a phenocopy of the *ctr1* morphology (Fig. 1) (*13*). The reduced size of *ctr1* plants may be accounted for, at least in part, by a dramatic decrease in cell size (*13*) and may also underlie many of the other *ctr1* phenotypes such as the short hypocotyl and root, compact inflorescence, and a reduced root system. Because ethylene can inhibit DNA synthesis and subsequent cell division in etiolated pea seedlings (*27*), a reduction in cell number may also contribute to the Ctr^- phenotype. Less well understood effects of *ctr1* include a delay in flowering time, abnormal time of maturation of sex organs, and reduced viability of female gametophytes (*28*). In addition to its influences on plant morphology, the *ctr1* mutation causes constitutive activation of all known ethylene-responsive genes (*13*). All alleles of *ctr1* are recessive (loss-of-function) mutations, and thus at least one component of the ethylene response pathway may serve as a negative regulator of ethylene responses in *Arabidopsis*.

Ethylene-Insensitive Mutants in *Arabidopsis*

Mutant plants that show insensitivity to the effects of ethylene gas have also been identified in *Arabidopsis* (Fig. 1) (*6*). Mu-

Fig. 1. (Top panel) Phenotypes of wild-type, *ctr1*, and *ein2* ethylene response mutants in *Arabidopsis*. Surface-sterilized seedlings were germinated and allowed to grow in the dark for 3 days in the presence of either hydrocarbon-free air or 10 μl of ethylene per liter of air. Wild-type seedlings grown in hydrocarbon-free air developed a thin and elongated hypocotyl and root; in the presence of ethylene, they showed the triple response phenotype: short root and hypocotyl and exaggeration of the apical hook. *ein2* seedlings displayed an insensitive seedling phenotype; no triple response was elicited by ethylene, whereas the *ctr1* mutant seedlings displayed a constitutive triple response even in the absence of ethylene (Air). (Bottom panel) Phenotypes of wild-type, *ctr1*, and *ein2* adult plants. Seeds were sown in soil and grown under continuous light for 18 days in hydrocarbon-free air or in the presence of 1 μl of ethylene per liter of air. Representative seedlings and adult plants are shown.

tant (tall) seedlings are readily identified protruding above the "lawn" of wild-type (short) seedlings when mutagenized populations are plated in the dark in the presence of ethylene. Eight ethylene-insensitive (*ein/etr/eti/ain*) loci have been characterized genetically: *etr1* (*14, 23*), *ein2* (*24, 26*), *ein3* (*13, 26*), *ain1* (*25*), *ein4*, *ein5*, *ein6*, and *ein7* (*26*). Each of the mutant seedlings shows varying degrees of insensitivity to ethylene (Table 1) as defined by a complete deficiency or reduction in the magnitude of the triple response (*26*). *etr1*, the first characterized ethylene-insensitive mutant, is inherited as a single gene, dominant mutation (*23*). *etr1* was identified on the basis of its high degree of insensitivity to ethylene-mediated inhibition of hypocotyl elongation in etiolated seedlings. This mutant is also defective in a number of other ethylene responses, including promotion of seed germination, enhancement of peroxidase activity, acceleration of senescence of detached leaves, and negative feedback of ethylene biosynthesis (*23*). Furthermore, the ability of ethylene to induce the transcription of target genes is blocked in *etr1* plants (*29, 30*). Notably, *etr1* plants bind only one-fifth as much ethylene as wild-type plants (*23*). The pleiotropic effects of *etr1* suggest that the wild-type gene may encode an ethylene receptor or act at an early step in the signal transduction pathway. The absence of recessive (loss-of-function) alleles of *ETR1* may indicate that this gene is required for plant viability; alternatively, it may indicate redundancy of ETR1 function. In keeping with this latter possibility, a second "strong" dominant ethylene-insensitive mutant, called *ein4*, has been identified and shows many of the characteristics of the *etr1* mutant (*26*). A third possibility for the absence of recessive alleles is that loss of ETR1 function may produce a phenotype that is not observed in the triple response screen, particularly if the *etr1* mutants are neomorphs.

A second well-characterized mutant, *ein2*, is also insensitive to high amounts of ethylene (Fig. 1) (*24, 26*). This recessive mutant is similar in phenotype to *etr1* and *ein4* in that strong alleles of *ein2* are pleiotropic, lacking all known ethylene responses (*24, 26*). Like *etr1* (*23*), *ein2* plants have larger rosette leaves (*24*) and larger cells (*31*) than wild-type plants, perhaps because of a failure to respond to a basal level of ethylene. These plants also show increased ethylene production relative to wild-type plants (*24*), which suggests that auto-inhibition of ethylene biosynthesis may be affected by the defect in ethylene perception (*23*).

Five additional ethylene-insensitive mutants have been characterized genetically (*26*). The *ain1*, *ein3*, *ein5*, *ein6*, and *ein7* mutants have a significantly less severe phenotype than *etr1*, *ein4*, or *ein2* (Table 1). A representative member of this class of insensitive mutants is the recessive mutant *ein3* (*13*). Consistent with the "weak" phenotype, the ethylene-regulated genes are induced by ethylene to higher levels in the *ein3* mutant than in alleles of *etr1* or *ein2* (*30*).

In another study, five ethylene-insensitive seedlings were isolated that were referred to as *eti* (*20*). These plants have been only partially characterized genetically, and so it is unclear whether they represent independent or previously unidentified loci. However, in support of Darwin's supposition (*18*), the ability of the *eti* mutant seedlings to emerge through compacted sand was found to be directly proportional to their ability to respond to ethylene and a triple response (*20*).

It is likely that additional ethylene-insensitivity loci remain to be detected because there is no evidence that the mutant screens are saturated (there are only single alleles for the *ein4*, *ein6*, and *ein7* mutations) and only a limited attempt has been made to recover mutants that are weak, lethal, or infertile. Genetic screens for second-site suppressor mutations may also provide a means to identify loci in the ethylene response pathway.

Table 1. Ethylene mutants of *Arabidopsis*.

Mutant	Seedling phenotype	Chromosome	Comments	Ref.
etr1	Insensitive (strong)	1, bottom	Dominant; reduced ethylene binding, putative "two-component" histidine kinase; *ein1* is an allele (*24*)	(*14, 23*)
ein2	Insensitive (strong)	5, top	Recessive; tolerant to virulent bacterial pathogens (*61*), gene cloned (*48*); *ckr1* is an allele (*83*)	(*24*)
ein3	Insensitive (weak)	3, top	Recessive; gene cloned (*48*)	(*13*)
ein4	Insensitive (strong)	3, top	Dominant; not allelic to *ein3*	(*26*)
ein5	Insensitive (weak)	1, middle	Recessive; possibly allelic to *ain1*	(*26*)
ein6	Insensitive (weak)	3, bottom	Recessive; increased sensitivity to Taxol (*84*)	(*26*)
ein7	Insensitive (weak)	1, middle	Semi-dominant; possibly allelic to *ein5*	(*26*)
ain1	Insensitive (weak)	1, middle	Recessive; ACC-insensitive	(*25*)
eti	Insensitive	?	Five isolates; incompletely characterized	(*20*)
eir1	Insensitive	5, bottom	Recessive; ethylene-insensitive root	(*26*)
eto1	Constitutive	3, bottom	Recessive; ethylene overproducer	(*24*)
eto2	Constitutive	5, bottom	Dominant; ethylene overproducer	(*13*)
eto3	Constitutive	3, bottom	Dominant; ethylene overproducer	(*13*)
ctr1	Constitutive	5, top	Recessive; putative serine-threonine kinase similar to the Raf (MAPKKK) family	(*13*)
hls1	Hookless	4, bottom	Recessive; ethylene-insensitive apical hook, gene cloned (*31*); *cop3* is an allele (*85*)	(*24*)

Cloning Genes That Act in the Pathway

A central component in the ethylene signaling pathway is the *CTR1* gene product; it acts downstream of *ETR1* and *EIN4* and is a negative regulator of *EIN2*, *EIN3*, *EIN5*, *EIN6*, *EIN7*, *EIR1*, and *HLS1*. The *CTR1* gene has been cloned by T-DNA mutagenesis (*13*). Conceptual translation of its mRNA revealed that it encodes a protein with the hallmark features of a serine-threonine protein kinase. CTR1 shows greatest amino acid similarity to the Raf family of protein kinases; the structure of CTR1 is similar to that of mitogen-activated protein kinase kinase kinase (MAPKKK). Several mutant alleles of CTR1 contained amino acid substitutions in residues that are invariant or nearly invariant in all known protein kinases (*32*), which suggests that kinase function is required for CTR1 activity. In a variety of multicellular eukaryotic and yeast cells (both budding and fission), MAPKK kinase, MAPK kinase, and MAP kinase (and related forms of these proteins) participate in phosphorylation cascades for a variety of developmental or stress signaling events (*33, 34*).

If in fact CTR1 participates in such a scheme, then where are the mutations that correspond to the other components in the cascade (MAPK kinase and MAP kinase)? Given that there are at least 10 genes for MAPKs and several MAPKKs in *Arabidopsis* (*35–37*), the simple answer may be that

genetic redundancy prevents their detection by a classical mutagenesis approach. The lack of mutations corresponding to the MAPKKK in the yeast osmosensing pathway may underlie a similar situation (*38*, *39*). Alternatively, it is reasonable to conclude that since only five *ctr1* alleles are known, the screen for constitutive ethylene activation mutants (Ctr^-) is unlikely to have reached saturation.

The most significant (and intriguing) recent advance in our understanding of the ethylene signaling pathway has come as a result of positional cloning of the early-acting *Arabidopsis* ethylene response gene *ETR1* (*14*). Quite surprisingly, the predicted translation product of *ETR1* shows remarkable similarity to the bacterial two-component histidine kinases and an emerging family of eukaryotic putative histidine kinases (*40*–*42*). Four dominant *etr1* alleles were sequenced, and each of these mutations resulted from amino acid substitutions in either of three putative transmembrane domains. It is unclear from the sequence information alone whether *etr1* results from gain-of-function or dominant-negative mutations. The absence of recessive alleles of the locus implies that there may be similar genes with redundant function. In support of this hypothesis, several *ETR1*-homologous genes have been identified by low-stringency hybridization (*14*) and within the *Arabidopsis* expressed sequence tag (EST) collection (*37*). Ascertaining the nature of the *etr1* dominant effect will be crucial in determining its role in the ethylene signaling pathway.

Of particular interest is the resemblance between components of the ethylene and osmolarity response pathways in *Arabidopsis* and yeast, respectively. Both *ETR1* and *SLN1* gene products act early in ethylene- and osmotic-stress response pathways and encode putative "two-component" histidine kinases (*14*, *38*–*40*). SLN1 participates in the hyperosmolarity response pathway in budding yeast; it also inactivates the PBS2/HOG1 MAP kinase cascade, presumably under conditions of low osmolarity (*38*, *39*). Additionally, *SLN1* and *ETR1* mutations are suppressed by loss-of-function mutations in downstream genes that encode members of a MAP kinase cascade, CTR1 (MAPKKK) in the ethylene response pathway (*13*) and PBS2 (MAPKK) and HOG1 (MAPK) in the osmolarity response of yeast (*39*). Components of these stress response pathways are also conserved in mammals (*34*). HOG1- and PBS2-related kinases are activated in animal cells by conditions of high osmolarity, heat shock, chemical stress (arsenate), and lipopolysaccharide (LPS-endotoxins) (*43*–*47*).

Isolation of the *EIN2* and *EIN3* genes by positional cloning and insertional mutagenesis, respectively (*48*), should provide further insights into the ethylene response pathway. Analysis of the wild-type gene products may provide clues about their requirements for plant responses to ethylene (EIN3 and EIN2) or pathogen (EIN2) sensitivity. Perhaps genes that are related to *EIN2* and *EIN3* may participate in the stress response pathways of yeast (osmoregulation) and mammals (macrophage activation) in a similar fashion as they do in the response of plants to the stress hormone ethylene.

Order of Gene Action

Epistatic relationships between mutations in a biochemical or regulatory pathway can provide information about the order in which the protein products of these genes interact even without knowledge of their molecular identity. To define the order of gene action among the ethylene response genes, researchers have used epistasis analysis to build a framework model for the activities of these genes in the seedling stress-response pathway (*26*) (Fig. 2). The earliest steps in the pathway are defined by the *ETR1* and *EIN4* loci. The *etr1* and *ein4* mutants have strong Ein^- phenotypes, and all alleles of these mutations are dominant to the wild-type (*14*, *26*). Although their order of action is unknown, the *ETR1* and *EIN4* genes act in the same ethylene response pathway and before *CTR1* (*26*). The nature of action of the *ETR1* and *EIN4* gene products is not straightforward. If mutations in these genes result in a gain of function (that is, constitutive activation), then ethylene would act to negatively regulate the activities of the wild-type ETR1 and EIN4 proteins; these proteins would act as positive regulators of CTR1. Alternatively, if the products of *etr1* and *ein4* act in a dominant-negative fashion, then ethylene would act as a positive regulator of the wild-type gene products. In this case, ETR1 or EIN4 would act to inhibit the activity of CTR1. If, however, the *etr1* or *ein4* mutants are neomorphs, then it is possible that the wild-type gene products may not normally function in the ethylene signal transduction pathway.

The similarities between ETR1 and SLN1 suggest a putative function for the *EIN4* gene product. EIN4 may act after ETR1 and perform a function in ethylene signal transduction in *Arabidopsis* that is similar to SSK1 in the osmolarity-stress response in yeast, that of a response regulator or "second component." Alternatively, EIN4 may act before ETR1 in the ethylene signal transduction pathway. Indirect evidence suggests that the ethylene receptor

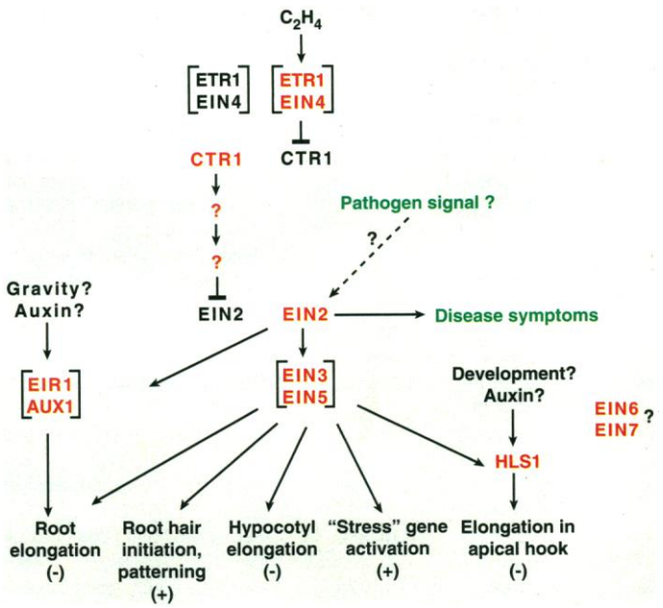

Fig. 2. A genetic pathway for ethylene action. A model of the ethylene signal transduction pathway is shown that is consistent with epistatic relationships of the various ethylene response mutants. The *etr1* and *ein4* mutations are assumed to act in a dominant-negative fashion; the wild-type gene products negatively regulate the activity of CTR1. *ctr1* mutations mask the phenotype of *etr1* and *ein4*; therefore, CTR1 is shown acting after ETR1 and EIN4. CTR1 negatively regulates the ethylene response pathway, possibly by inhibiting the activity of EIN2. These negative control points are indicated by a bar. Similarity between the ethylene response pathway and the mammalian and yeast stress response pathways suggests that additional proteins act after CTR1. These putative proteins are indicated by (?). *ein2*, *ein3*, *ein5*, *ein6*, *ein7*, *hls1*, *eir1*, and *aux1* are all epistatic to *ctr1* and likely act after CTR1. The effects of *eir1* and *aux1* mutations on root growth are distinct from that of the *ein3* and *ein5* mutations and, therefore, are shown to function in a separate pathway controlling root elongation. EIN2 is required for both EIN3/EIN5 and EIR1/AUX1 functions and is shown acting before these genes. EIN7 and EIN6 are shown outside of the genetic pathway because their interactions with the *EIR1/AUX1* genes have not been characterized. In addition to ethylene insensitivity, *eir1* and *aux1* mutants are defective in gravity or auxin responses (*26*). Brackets indicate uncertain gene order. Responses that are positively regulated by ethylene are indicated by (+), whereas negatively regulated responses are indicated by (−). The gene symbols in red correspond to the active state, whereas those in black correspond to the inactive state.

may contain a transition metal that is necessary to coordinate this olefin (*9*, *10*). The *ETR1* gene product does not appear to have features suggestive of a metal-binding domain (*14*); therefore, other proteins (possibly EIN4) that act before ETR1 may be required for ethylene binding. Another intriguing possibility is that ETR1 and EIN4 have "redundant" functions, which could account for the absence of recessive alleles of these loci.

EIN2, *EIN3*, *EIN5*, *EIN6*, and *EIN7* act after *CTR1* in the ethylene signal transduction pathway (Fig. 2). Mutations in the *CTR1* gene are recessive and likely represent a loss of function. This conclusion is supported by sequence analysis of *ctr1* alleles in which several are most likely null mutations (*13*). These results suggest that the wild-type *CTR1* gene product acts as a negative regulator of the ethylene response pathway and its loss of function (presumably kinase activity) results in plants that constitutively display ethylene responses. This same kind of reasoning leads to the conclusion that *EIN2*, *EIN3*, and the other downstream recessive Ein^- mutations act as positive regulators in this pathway, although nothing is known about their biochemical activities. The *ein3*, *ein5*, *ein6*, and *ein7* mutants have a significantly less severe Ein^- phenotype than *ein2* (*26*). It is possible that these mutants have a weak phenotype either because they are leaky mutations or because they affect only a portion of the *EIN2* functions. The sequence of one *ein3* mutant allele predicts that it gives rise to a truncated protein that should result in a severe reduction-of-function or a loss-of-function (*48*). Therefore, the weak phenotype of *ein3* mutants cannot be attributed to a simple model of reduced activity, but must be explained by the function of this gene in the ethylene response pathway. Thus, the *EIN3* locus affects only a subset of the functions of *EIN2*. The molecular identities of the *EIN5*, *EIN6*, and *EIN7* loci remain to be determined.

Ethylene Response Mutants in Tomato

Identification of the *Arabidopsis* ethylene-insensitive mutants has been based on a phenotype displayed by etiolated seedlings; however, these mutations also affect all known adult plant responses to ethylene. Such results suggest that the ethylene signal perception or transduction pathways in seedlings and adult plants must share common components. This may also be true for other plants, including those such as tomato that undergo an ethylene-mediated climacteric (fruit ripening). A number of tomato mutants that are affected in the ripening process are known (*1*). The *Never-ripe* (*Nr*), *ripening inhibitor* (*rin*), and *nonripening* (*nor*) mutants are delayed in fruit ripening (*49*), whereas the *epi* mutant shows features of a plant with a constitutive ethylene response (Ctr^-) (*50*). The partially dominant *Nr* mutation shows pleiotropic effects on plant development; *Nr* has a profound effect on both seedling and adult ethylene responses (*49*) (Fig. 3). Not only is fruit ripening blocked, but also flower and petal abscission and epinasty are affected in *Nr*. This mutant also fails to display the seedling triple response to ethylene (Ein^-), although *rin* and *nor* seedlings show a normal response (*49*). These results strongly suggest that *Nr* may generally affect ethylene perception, but that *rin* and *nor* may affect ethylene sensitivity specifically during fruit ripening. In addition, the *Nr* locus has a profound effect on ethylene-inducible gene expression (*51*). *ETR1*-homologous genes have been isolated from tomato, and genetic mapping experiments indicate that one of these genes is very tightly linked to the *Nr* mutation (*51*). To the surprise of many post-harvest physiologists but to the delight of most plant geneticists, signaling components required for the seemingly simple triple response of the commercially useless weed *Arabidopsis* are, in all likelihood, quite similar to those required for fruit ripening in economically important crop plants.

The ease with which millions of plants can be examined by the triple response assay should provide a rapid means to iso-

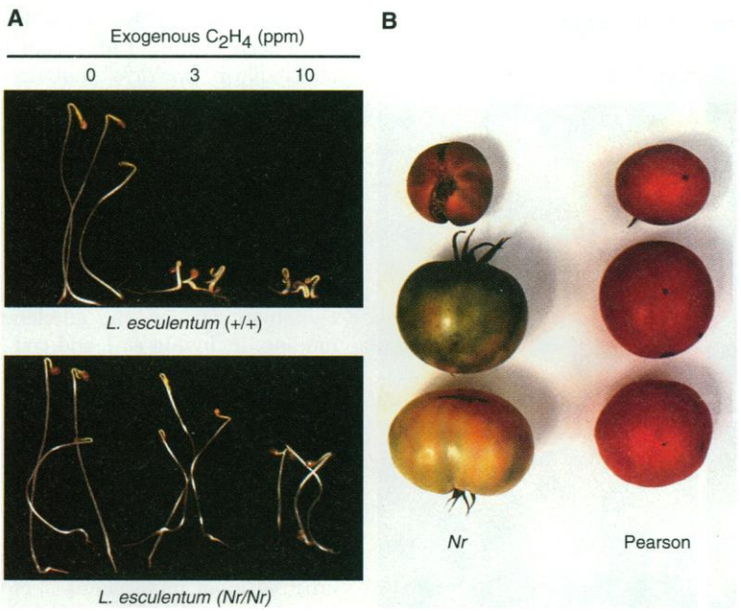

Fig. 3. Phenotypes of the ethylene-insensitive tomato mutant *Never-ripe*. (**A**) *Never-ripe* inhibits the triple response of tomato seedlings. Normal (Pearson) and *Nr* mutant seedlings were germinated and grown in the dark for 12 days in the presence of various concentrations of ethylene (0, 3, or 10 μl of ethylene per liter of air). (**B**) The effects of the *Never-ripe* mutation on tomato fruit ripening. Tomato fruits from wild-type plants (Pearson) show normal ripening, whereas fruits of the *Nr* mutant are inhibited in ripening.

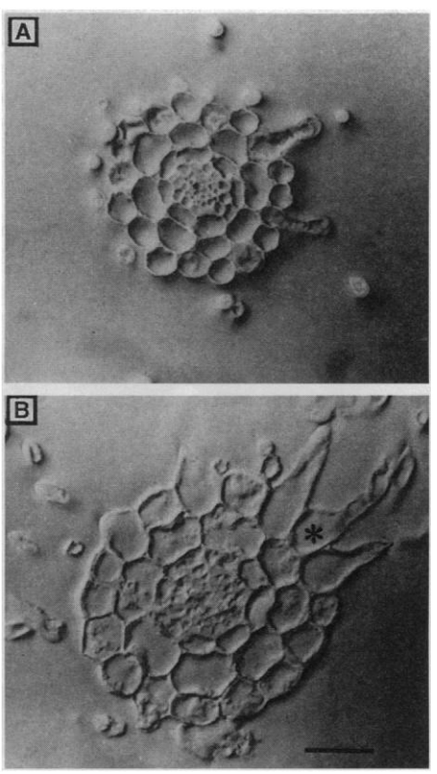

Fig. 4. Effect of ethylene on cell fate patterning in the root epidermis. (**A**) Cross sections showing the location of hair cells in wild-type *Arabidopsis* root; hairs are normally positioned only over the anticlinal walls of cortical cells. (**B**) In *ctr1* roots, the presence of three adjacent hair-cell files is evident, with the middle cell being an ectopic position (indicated by an asterisk). [Reproduced from (*53*) with permission from the Company of Biologists]

late new alleles of *Nr* and to identify new genes that control tomato fruit ripening. In principle, the assay can be used to identify ethylene response mutations for any plant species in which it is possible to generate large quantities of mutagenized seeds. New plant varieties, such as those whose fruits or flowers show limited response to ethylene, may be developed.

Specification of Cell Fate in Patterning of the Root

In addition to its dramatic effect on cell elongation in the root, ethylene causes a proliferation of root hairs. So called "stress-dependent" root hair production may aid the plant in absorption of nutrients or in stabilization of the seedling in the soil. Ethylene's ability to promote root hair differentiation in a variety of plant species has been known for many years (*52*); however, only recently has its critical role in regulating the spatial organization of the root epidermis been demonstrated (*53*). The *Arabidopsis* root epidermis is highly patterned and contains two cell types: hair cells (derived from trichoblasts) and non-hair cells (derived from atricoblasts). Because root epidermal cells are precisely and predictably arranged (*53, 54*), the *Arabidopsis* root provides an excellent model system to study pattern formation and cell differentiation in plants (*55*). From the early studies of Bunning (*56*), the existence of a diffusible regulator of root hair cell fate was proposed. Surgical manipulation of epidermal cells indicated that a short-range signal may play a role in the cellular interactions that control the decision to differentiate into a hair cell. Examination of *ctr1* has provided new insight into the process of cell communication and pattern formation in the root epidermis (*53*). Ectopic hairs (hair production on "non-hair" cells) are present in the *ctr1* root epidermis, which suggests that ethylene may be the diffusible signal (Fig. 4). Moreover, root hair cell formation is dramatically reduced in ethylene-insensitive mutants and by treatment with inhibitors of ethylene biosynthesis or action (*57*). Lastly, the identification of hairless mutants in *Arabidopsis* root that can be complemented for hair growth by the addition of exogenous ethylene confirms the essential role of ethylene in specifying root epidermal cell fate (*57*). A model for the action of ethylene and the *CTR1* gene product in specifying cell patterns in the *Arabidopsis* root has been devised (*53*).

Influences on Differential Cell Elongation Processes

Ethylene controls many plant responses that require differential cell elongation, such as epinasty and apical hook formation (*1*). The apex of the hypocotyl in most dicot seedlings contains a region that forms a hook-like structure (Fig. 5). The shape of this apical hook is defined by differential cell elongation, and the hook itself can be considered as a "standing wave" of plant growth; cells produced at the apical meristem appear to flow through the hook as they elongate (*58*). At different points in time in its passage through the hook, each cell must be capable of accelerating and decelerating its rate of elongation. A complex pattern of coordinated cell elongation is needed to establish and maintain this structure where cell passage through the apical hook occurs over a relatively short time (hours).

Ethylene regulates the development and maintenance of the apical hook in etiolated dicot seedlings (*21*). One can speculate that a gradient of ethylene responsiveness of cells in the hypocotyl may result in exaggeration of the curvature of the hook in the presence of ethylene. Cells in the hook region may perceive or respond to ethylene (or some downstream effector) in a differential manner; those on the inside of the apical hook are more inhibited in elongation than those on the outside (*58*). Application of exogenous ethylene results in exaggeration in hook curvature (Fig. 5), whereas chemicals that reduce or block perception of ethylene or mutants that block ethylene response significantly reduce hook formation (*24*).

Establishment of the apical hook likely involves an intricate balance of ethylene with a second plant hormone, auxin, to coordinate the rates of cell elongation among cells throughout the hook structure (*31, 59*). As in the root gravitropic response, inhibitors of auxin transport disrupt the formation of the apical hook (*31, 59*). Similarly, germination of seedlings on medium containing high concentrations of auxin also prevents apical hook development; hookless seedlings are produced (*31*). Auxin may be asymmetrically distributed in the apical hook (*60*), and treatment with high concentrations of IAA or auxin transport inhibitors presumably swamps or disrupts the hormone gradient, respectively.

Mutations that affect differential cell elongation in the hypocotyl hook region have been identified in screens for "hook-affected" or "hookless" mutants (*24*) (Fig. 5). Such mutations may identify genes that control the hypothetical "gradient" of ethylene or auxin as well as those that regulate auxin transport in the hook. One mutation in particular, *hookless1* (*hls1*), completely abolishes apical hook development in *Arabidopsis* (*24*). The apical hookless phenotype of *hls1* is retained in all double-mutant combinations with the ethylene response mutants including *eto1* and *ctr1* (*26*), and thus *hls1* likely acts downstream of these genes. Interestingly, certain alleles of this locus, such as *hls1-2*, can be partially compensated in hook formation by the addition of ethylene gas (Fig. 5), thereby suggesting that expression of *HLS1* mRNA (or its protein product) may be regulated by ethylene. The *HLS1* gene has been cloned by an insertional mutagenesis approach (*31*). Its primary sequence may yield clues as to the role of ethylene in apical hook development and, more generally, may contribute to our understanding of differential cell growth processes in plants.

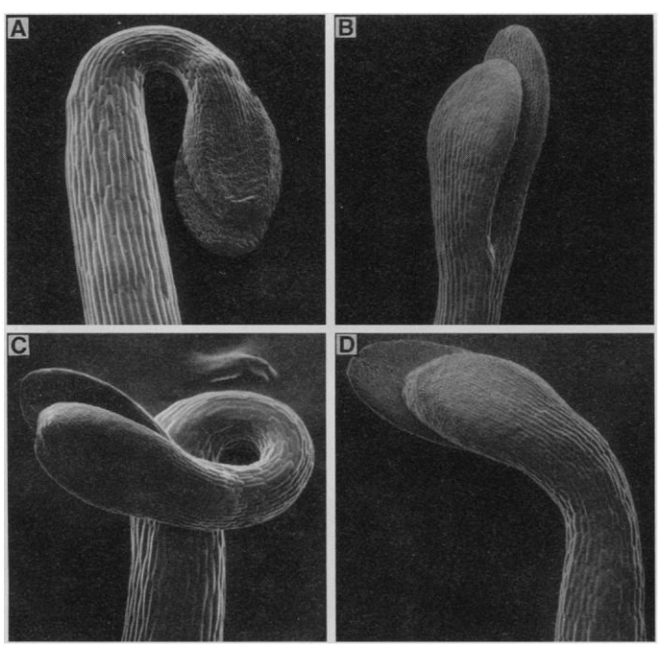

Fig. 5. Effect of ethylene on differential growth in the hypocotyl hook. Scanning electron microscope images of the apical hook region of wild-type *Arabidopsis* and *hookless1* mutant seedlings. (**A** and **C**) Wild-type, (**B**) *hls1-1*, and (**D**) *hls1-2* seedlings were germinated and grown in the dark for 3 days (A) in the absence or (B to D) presence of 100 μM 1-aminocyclopropane-1-carboxylic acid (ACC). ACC, an ethylene precursor, is readily taken up by the seedlings and converted to ethylene.

Ethylene in Plant Disease and Defense Gene Regulation

Ethylene has been implicated in the response of plants to pathogen attack (*1*). Its biosynthesis is promoted by many stresses, including wounding and pathogen infection, and correlates with the induction of mRNAs for a diverse array of pathogen-related (PR) genes. Through use of the *Arabidopsis* ethylene-insensitive mutants, the role of ethylene in these processes has been clarified (*30, 61*). Plants can respond to pathogen infection by inducing broad-spectrum resistance, a phenomenon known as systemic acquired resistance (SAR) (*62*). Inducers of SAR include pathogens, salicylic acid, and ethephon (an ethylene-releasing compound), and it has been suggested that ethylene may act as a signal involved in salicylic acid–mediated SAR in tobacco (*63*). The role of ethylene in SAR has been evaluated with *Arabidopsis* mutants that are insensitive to ethylene (*30*). Examination of the pattern of gene expression in these mutants has revealed that chemical breakdown products of ethephon (hydrochloric and phosphonic acids) and not ethylene are responsible for the induction of SAR gene expression, although ethylene potentiates the effect of salicylic acid on PR gene induction. In light of these results, all previous experiments in which ethephon has been used as an ethylene source must be reevaluated.

Similarly, it does not appear that ethylene plays a major role in resistance to plant disease (*61*). Plants, challenged with a pathogen to which they are resistant, display the hypersensitive response (HR), which manifests as patches of localized cell death at the sites of infection (*64*). The HR is mediated by recognition of the pathogen by plant resistance gene products and effectively isolates the infection, thereby preventing further damage to the plant (*65*). The ethylene-insensitive mutants *etr1*, *ein2*, and *ein3* display a normal HR response when challenged with an avirulent strain of *Pseudomonas syringae*, suggesting that ethylene responsiveness is not critical for this process. Interestingly, evidence linking one gene involved in ethylene signal transduction to disease symptom formation has been obtained (*61*). Upon infection with several strains of virulent bacteria, wild-type, *etr1*, and *ein3* plants showed typical disease symptoms, including chlorosis and the presence of water-soaked legions. However, these symptoms were significantly abated in the *ein2* mutant, even though pathogen growth on *ein2* plants was identical to growth on wild-type plants. The tolerance of *ein2* plants was not limited to *P. syringae*, as these plants also showed reduced symptoms when infected with a virulent strain of *Xanthomonas campestris*. The differing reactions of *etr1*, *ein3*, and *ein2* mutants indicate that the ethylene response pathway may branch; *EIN2* may play a role in plants mediating both ethylene sensitivity and pathogen-induced damage (Fig. 2). Alternatively, the difference in symptoms observed between the *ein2* and the other ethylene-insensitive mutants may be due to "leakiness" of the *etr1* and *ein3* alleles used in these studies. Recent isolation of the *EIN2* gene by positional cloning may clarify its role in ethylene sensitivity and tolerance to virulent pathogens (*48*).

Many of ethylene's effects on plant growth and development are likely to be mediated by changes in the expression pattern of target genes. Complex patterns of ethylene-induced gene expression have been described for tomato fruit ripening (*4, 8*), and there is evidence that ethylene acts posttranscriptionally as well (*4*). Ethylene can elevate the steady-state level of mRNA for genes related to plant defense against pathogens, including β-1,3-glucanase, basic chitinase, pathogen-related protein PR1, chalcone synthase, and hydroxyproline-rich glycoproteins (*66–73*). The application of exogenous ethylene fails to induce ethylene-regulated defense genes in *etr1*, *ein2*, and *ein3* (*29, 30, 48*), and in *ctr1* these genes are expressed constitutively at a high level (*13*).

DNA sequence elements that confer ethylene responsiveness to a minimal promoter have been identified for a number of ethylene-responsive PR genes, including basic chitinase from bean and *Nicotiana* genes for a basic-type PR protein and a β-1,3-glucanase protein (*67–73*). Within the promoters of each of these genes, one or more GCCGCC sequence motifs were recognized. Mutational analysis of a 47–base pair (bp) ethylene-responsive element (ERE), containing two 11-bp GCC boxes, indicates that these elements are necessary and sufficient for transcriptional control by ethylene (*74*). Four ERE-binding proteins (EREBPs) were identified in tobacco that interact directly with the GCC box in the ERE (*74*). Although the amino acid sequences of these EREBPs are quite distinct, they share a region of 59 amino acids which, for EREBP2, has been determined to be necessary and sufficient for DNA binding. This protein domain was found to share a high degree of amino acid sequence similarity with several previously identified but uncharacterized proteins from a variety of plants, including several cadmium-inducible genes from *Arabidopsis* (*74*). Additional searches have revealed that this domain is similar to the AP2 domains (AP2-R1 and AP2-R2) found in the *Arabidopsis* floral homeotic protein APETALA2 (AP2) (Fig. 6). The AP2-R1 and AP2-R2 domains share 53% amino acid identity and may have an amphipathic, α-helical character (*75*). Mutations within the AP2 domain in three *ap2* mutant alleles suggest their requirement for AP2 function (*75*), one of which is to negatively regulate a second floral homeotic gene, *AGAMOUS* (*AG*) (*76*). Although AP2 has not been reported to bind DNA or to localize to the nucleus, it has features that suggest it is a nuclear protein (*75*). Sequence similarity between the AP2 domain and the EREBP DNA-binding domain suggests that AP2 many have a similar activity toward its genetic targets (such as AG); *AP2* may directly regulate transcription from the AG promoter.

The steady-state mRNA levels of all four EREBPs are dramatically increased by ethylene (*74*). These results explain the requirement of protein synthesis for the transcriptional induction of ERE-containing plant defense–responsive genes such as β-1,3-glucanase and basic chitinase. Thus, EREBP2 (and possibly EREBP-1, -3, and -4) may be targets for proteins that act downstream of constitutive ethylene response pathway genes such as the nuclear-localized EIN3 protein (*48*).

A Biochemical Model for the Ethylene Response Pathway

The first step in ethylene perception is presumably the binding of this gas to a receptor molecule. Given the similarity of the ethylene and osmotic response pathways, it is

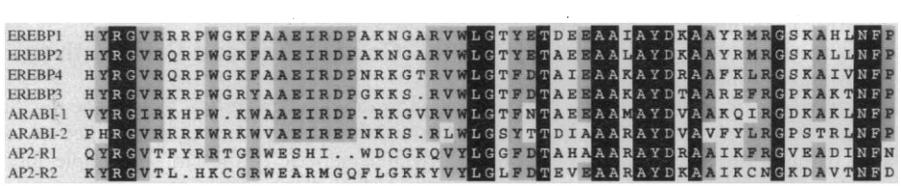

Fig. 6. Amino acid sequence similarity of the ethylene-responsive element binding proteins (EREBPs) and the floral homeotic protein APETALA2 (AP2). The conserved amino acid sequences in the DNA-binding domains of four tobacco EREBPs (*74*) are aligned along with the AP2 domains R1 and R2 of the *Arabidopsis* APETALA2 protein (*75*) and the predicted translation products of two *Arabidopsis* expressed sequence tags (ESTs): ARABI-1 (GenBank T04320) and ARABI-2 (GenBank Z27045). Regions of amino acid identity are shown in reverse shading and regions of similarity in light shading. 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.

tempting to speculate that perception of ethylene may occur at the plasma membrane and end in alterations in gene expression in the nucleus. However, ethylene has a much higher solubility in lipids than water and at high concentrations can even act as an anesthetic in animals. Thus, a plasma membrane receptor is not a prerequisite for ethylene's entry into cells. Ethylene-binding components that fit the pharmacological criteria for authentic receptors have been identified in several plant species, including *Arabidopsis* (*10*); they show high affinity and saturable binding with a dissociation constant (K_d) that is consistent with physiologically active concentrations of ethylene. Two classes of binding proteins are present in a variety of plant species, one with a relatively low rate constant of association and dissociation and one with a high rate. Inhibitors of ethylene action such as transcyclooctene can inhibit binding of ethylene to these proteins; however, it is unclear if any of these binding proteins are authentic ethylene receptors. Biochemical characterization of ethylene receptors, in the absence of a robust biochemical assay for receptor function, presents a major challenge to the field.

It is possible that one of the genetically identified genes discussed above encodes an ethylene receptor. The best candidate receptor is ETR1, which acts early in the signaling pathway and, in mutant form, demonstrates pleiotropic effects on ethylene physiology (*14*, *23*). The *ETR1* gene product shows strong similarity to bacterial two-component histidine kinase "sensors," and *etr1* plants showed an 80% reduction in the amount of ethylene that they can bind in a competitive binding assay (*24*). The significance of this result is somewhat diminished by the finding that at least one allele of *etr1* produces significantly increased amounts of ethylene (*23*), which will have profound consequences in a competitive ethylene-binding assay.

CTR1 shows significant similarity to Raf-1 (*13*). Control of Raf-1 is exerted by phosphorylation and protein-protein interactions in its NH_2-terminus by numerous upstream regulators, in particular Ras (*77–81*). It is unlikely that CTR1 interacts with the same ligands as Raf because an authentic homolog of Ras has not been discovered in plants. However, like Raf, the kinase activity of CTR1 may be regulated through phosphorylation by a number of upstream activators and repressors or possibly by direct interaction with a putative response regulator (SSK1-like protein), as may be the case in the yeast osmosensing pathway (*39*). Similarly, ethylene application induces very rapid and transient protein phosphorylation in tobacco leaves (*63*). Furthermore, this effect was abolished in the presence of protein kinase inhibitors. Conversely, treatment of excised tobacco leaves with inhibitors of type 1 and 2A protein phosphatases caused increased phosphorylation and accumulation of pathogenesis-related proteins. Earlier pharmacological studies revealed that calcium is required for the ethylene-mediated pathogenesis response in tobacco, as exemplified by the induction of the chitinase gene (*82*). Taken together, the results of biochemical and pharmacological studies in tobacco support the conclusion that several ethylene-evoked responses in plants are propagated through phosphorylation of intermediates and suggest that, at least in tobacco, some of these processes may require calcium.

These results are consistent with a model in which ETR1 and CTR1 act in a multistep signal transduction pathway through phosphorylation of proteins in a cascade, possibly including the *EIN2* and *EIN3* gene products. In the absence of ethylene, the kinase of CTR1 is predicted to be active and it may subsequently phosphorylate (and inactivate) genetically downstream targets (possibly the *EIN2* gene product). When ethylene is present, it may bind to and activate a receptor (possibly the *ETR1* gene product). This ethylene-receptor complex may then inactivate CTR1, either directly or indirectly (through an interaction with EIN4) and possibly through phosphorylation. The *EIN2* gene product may then activate a number of terminal ethylene-regulated genes, including those that control cell elongation and stress-gene activation, possibly by acting on genes like *EIN3* and the EREBPs. This model is consistent with what is known about the genetic interactions of the ethylene response mutations and the biochemical activities inferred from their predicted amino acid sequences. However, due to the lack of direct biochemical experimentation, it must be considered a highly speculative one. Cloning of additional genes in this pathway and biochemical analysis of their protein products should rapidly increase our understanding of the molecular basis for ethylene signal transduction.

Conclusions and Perspectives

Hormones play a central role in the regulation of plant growth and development. Molecular genetic studies in the simple mustard *Arabidopsis* are beginning to unravel the biochemical processes that control ethylene biosynthesis, perception, and signal transduction. The use of *Arabidopsis* mutants has made it possible to better understand the ethylene-mediated signal transduction pathway that leads to tomato fruit ripening. Several components of the pathway have now been identified by cloning of the *Arabidopsis* mutant genes, and results emerging from these early studies are beginning to paint a tantalizing picture of an evolutionarily conserved signaling system. Genetic engineering of *ETR1*, *CTR1*, and (when cloned) other ethylene response genes will provide agriculture with new tools to prevent or modify ethylene responses in a variety of plants.

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Phytochromes: Photosensory Perception and Signal Transduction

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The phytochrome family of photoreceptors monitors the light environment and dictates patterns of gene expression that enable the plant to optimize growth and development in accordance with prevailing conditions. The enduring challenge is to define the biochemical mechanism of phytochrome action and to dissect the signaling circuitry by which the photoreceptor molecules relay sensory information to the genes they regulate. Evidence indicates that individual phytochromes have specialized photosensory functions. The amino-terminal domain of the molecule determines this photosensory specificity, whereas a short segment in the carboxyl-terminal domain is critical for signal transfer to downstream components. Heterotrimeric GTP–binding proteins, calcium-calmodulin, cyclic guanosine 5′-phosphate, and the COP-DET-FUS class of master regulators are implicated as signaling intermediates in phototransduction.

Light is a critical environmental factor for plants. It provides not only the radiant energy for photosynthesis, but also the informational signals that plants use to adapt and optimize growth and development in response to the ambient conditions (*1*). Perception, interpretation, and transduction of these light signals is accomplished with the use of regulatory photoreceptors: the phytochromes [responsive to red (R) and far-red (FR) light], the blue-light (B) receptors, the ultraviolet A (UV-A) receptor or receptors, and the UV-B receptor or receptors (*2*). This article focuses on recent developments regarding the phytochromes (*2–5*).

Phytochromes are cytosolically localized dimers composed of two ~125-kD polypeptides, each carrying a covalently linked tetrapyrrole chromophore in the NH_2-terminal domain and dimerization determinants in the COOH-terminal domain. The photosensory function of the molecule is based on its capacity for reversible interconversion between the R-absorbing Pr form and the FR-absorbing Pfr form upon sequential absorption of R and FR light. Photosignal perception by the receptor activates signaling pathways leading to the changes in gene expression that underlie the physiological and developmental responses to light (*2, 3*). These responses occur throughout the life of the plant and range from seed germination, seedling deetiolation, and shade avoidance to flowering (*1*). The molecular nature of the primary transduction processes by which the photoreceptors relay their sensory information to the cell is unknown. However, various analytical approaches have converged in recent years to provide insights into possible mechanisms.

Phytochrome genes encode a small family of photoreceptors (*6*). In *Arabidopsis*, the apoprotein is encoded by five genes, designated *PHYA*, *-B*, *-C*, *-D*, and *-E* (*7, 8*). Sequences related to these genes have been found in species ranging from algae to angiosperms (*6, 9, 10*). Evidence indicates that the phytochrome variants have distinct photosensory functions, but their regulatory mechanisms of action remain unclear. In this discussion, the distinction is made between the photosensory function of the molecule, defined as perception and interpretation of the incoming light signal, and the regulatory function, defined as induction of changes in downstream transduction components by the activated photoreceptor molecule. Investigations of the mechanism of action and the downstream signaling pathways focus on three broad areas: the photoreceptor molecule itself,

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