CHAPTER

22

Ethylene: The Gaseous Hormone

During the nineteenth century, when coal gas was used for street illumination, it was observed that trees in the vicinity of streetlamps defoliated more extensively than other trees. Eventually it became apparent that coal gas and air pollutants affect plant growth and development, and ethylene was identified as the active component of coal gas (see **WEB TOPIC 22.1**).

In 1901, Dimitry Neljubov, a graduate student at the Botanical Institute of St. Petersburg in Russia, observed that dark-grown pea seedlings in the laboratory exhibited symptoms that were later termed the *triple response*: reduced stem elongation, increased lateral growth (swelling), and abnormal, horizontal growth (**FIGURE 22.1**). When the plants were allowed to grow in fresh air, they regained their normal morphology and rate of growth. Neljubov identified ethylene from coal gas, which was present in the laboratory air, as the molecule causing the response.

The first indication that ethylene is a natural product of plant tissues was published by H. H. Cousins in 1910. Cousins reported that "emanations" from oranges stored in a chamber caused the premature ripening of bananas when these gases were passed through a chamber containing the fruit. However, given that oranges synthesize relatively little ethylene compared to other fruits, such as apples, it is likely that the oranges used by Cousins were infected with the fungus *Penicillium*, which produces copious amounts of ethylene. In 1934, R. Gane and others identified ethylene chemically as a natural product of plant metabolism, and because of its dramatic effects on the plant it was classified as a hormone.

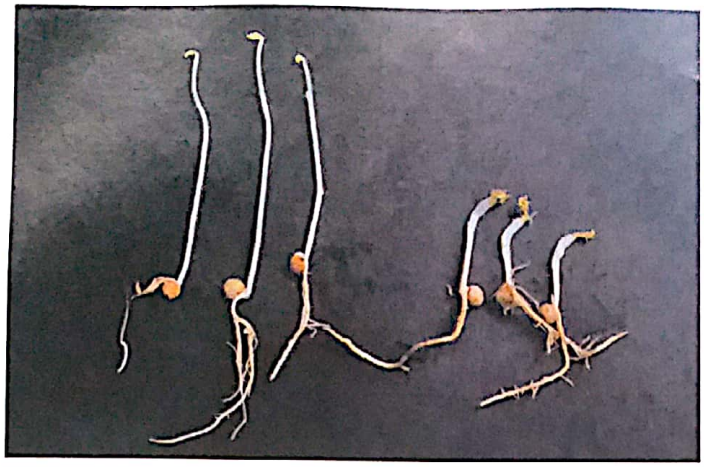


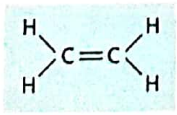
FIGURE 22.1 Triple response of etiolated pea seedlings. Six-day-old pea seedlings were grown in the presence of 10 ppm (parts per million) ethylene (right) or left untreated (left). The treated seedlings show radial swelling, inhibition of elongation of the epicotyl, and horizontal growth of the epicotyl (diagravitropism). (Courtesy of S. Gepstein.)

For 25 years ethylene was not recognized as an important plant hormone, mainly because many physiologists believed that the effects of ethylene were due to auxin, the first plant hormone to be discovered (see Chapter 19). Auxin was thought to be the main plant hormone, and ethylene was considered to play only an insignificant and indirect physiological role. Early work on ethylene was hampered by the lack of chemical techniques for its quantification. However, after gas chromatography was introduced in ethylene research in 1959, the importance of ethylene was rediscovered and its physiological significance as a plant growth regulator was recognized (Burg and Thimann 1959).

In this chapter we will describe the ethylene biosynthetic pathway and how ethylene acts at the cellular and molecular levels. At the end of the chapter we will outline some of the important effects of ethylene on plant growth and development.

Structure, Biosynthesis, and Measurement of Ethylene

Ethylene is the simplest olefin (its molecular weight is 28):



Ethylene

It is lighter than air under physiological conditions, and readily undergoes oxidation (see **WEB TOPIC 22.2**).

Ethylene can be produced by almost all parts of higher plants, although the rate of production depends on the type of tissue and the stage of development. It is usually measured by gas chromatography (see **WEB TOPIC 22.3**). Ethylene production increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as can physiological stresses such as flooding, disease, and temperature or drought stress. In additon, infection by various pathogens can also elevate ethylene biosyntheis.

The amino acid methionine is the precursor of ethylene, and 1-aminocyclopropane-1-carboxylic acid (ACC) serves as an intermediate in the conversion of methionine to ethylene. As we will see, the complete pathway is a cycle, taking its place among the many metabolic cycles that operate in plant cells.

Regulated biosynthesis determines the physiological activity of ethylene

In vivo experiments showed that plant tissues convert [^{14}C]methionine to [^{14}C]ethylene, and that the ethylene is derived from carbons 3 and 4 of methionine. The CH_3—S group of methionine is recycled via the *Yang cycle* (**FIGURE 22.2**). The immediate precursor of ethylene is **1-aminocyclopropane-1-carboxylic acid** (**ACC**). In general, when ACC is supplied exogenously to plant tissues, ethylene production increases substantially. This observation indicates that the synthesis of ACC is usually the limiting biosynthetic step in ethylene production in plant tissues.

ACC synthase (**ACS**) is the enzyme that catalyzes the conversion of AdoMet to ACC (see Figure 22.2). Its level is regulated by environmental and internal factors, such as wounding, drought stress, flooding, and auxin. ACC synthase is encoded by members of a divergent multigene family that are differentially regulated by various inducers of ethylene biosynthesis. In tomato, for example, there are at least ten ACC synthase genes, different subsets of which are induced by auxin, wounding, and/or fruit ripening. (For more details, see **WEB TOPIC 22.4**.)

ACC oxidase catalyzes the last step in ethylene biosynthesis: the conversion of ACC to ethylene (see Figure 22.2). In tissues that show high rates of ethylene production, such as ripening fruit, ACC oxidase activity can be the rate-limiting step in ethylene biosynthesis. Like ACC synthase, ACC oxidase is encoded by a multigene family, the members of which are differentially regulated (see **WEB TOPIC 22.5**). For example, in ripening tomato fruits and senescing petunia flowers, the mRNA levels of a subset of ACC oxidase genes are highly elevated.

CATABOLISM Researchers have studied the catabolism of ethylene by supplying $^{14}C_2H_4$ to plant tissues and tracing the radioactive compounds produced. Carbon dioxide, ethylene oxide, ethylene glycol, and the glucose conjugate

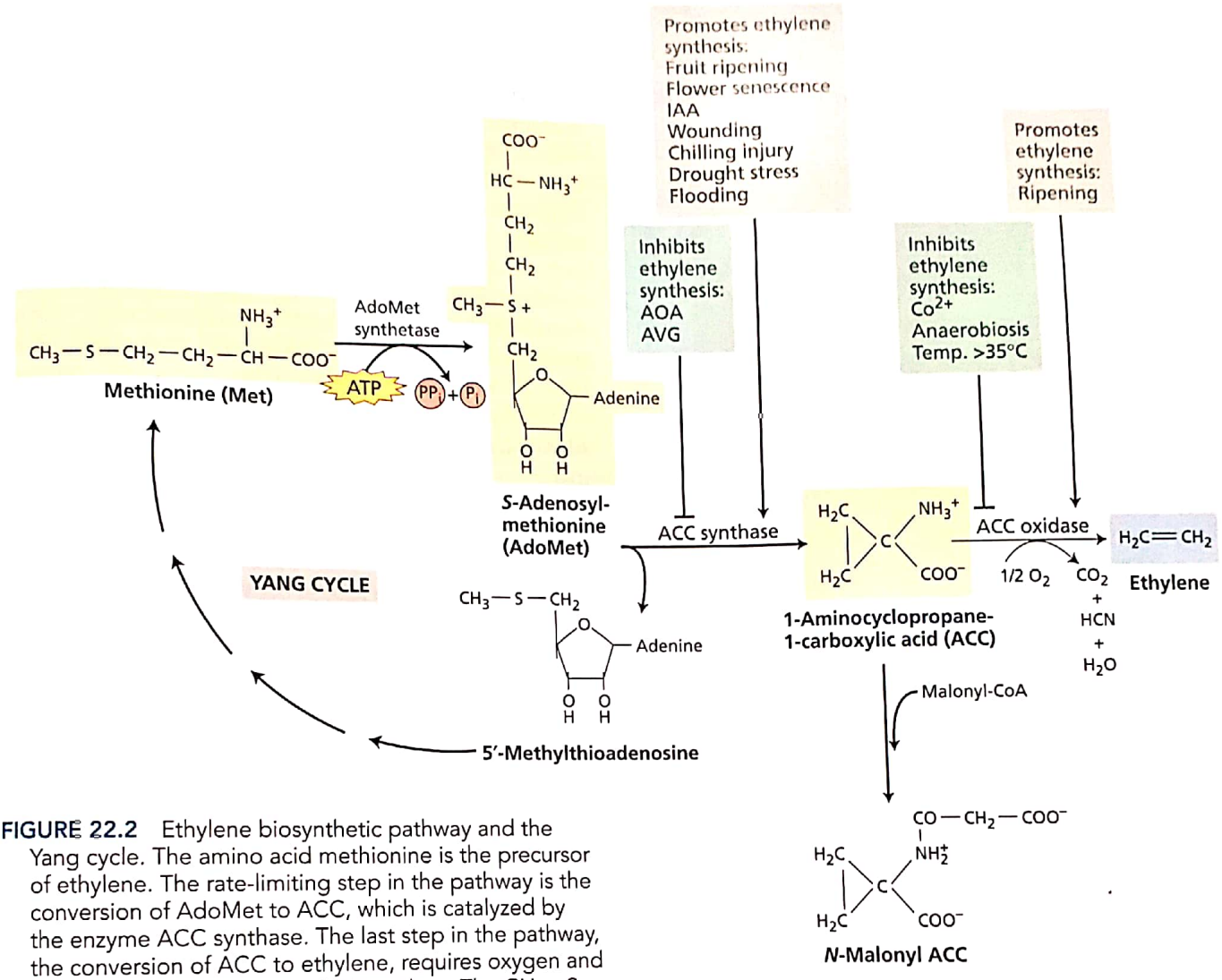


FIGURE 22.2 Ethylene biosynthetic pathway and the Yang cycle. The amino acid methionine is the precursor of ethylene. The rate-limiting step in the pathway is the conversion of AdoMet to ACC, which is catalyzed by the enzyme ACC synthase. The last step in the pathway, the conversion of ACC to ethylene, requires oxygen and is catalyzed by the enzyme ACC oxidase. The CH_3—S group of methionine is recycled via the Yang cycle and thus conserved for continued synthesis. Besides being converted to ethylene, ACC can be conjugated to *N*-malonyl ACC. AOA = aminooxyacetic acid; AVG = aminoethoxy-vinylglycine. (After McKeon et al. 1995.)

of ethylene glycol have been identified as metabolic breakdown products. However, because certain cyclic olefin compounds, such as 1,4-cyclohexadiene, have been shown to block ethylene breakdown without inhibiting ethylene action, ethylene catabolism does not appear to play a significant role in regulating the level of the hormone (Raskin and Beyer 1989).

CONJUGATION Not all the ACC found in the tissue is converted to ethylene. ACC can also be converted to a conjugated form, *N*-malonyl ACC (see Figure 22.2), which does not appear to break down and accumulates in the tissue, primarily in the vacuole. A second, minor, conjugated form of ACC, 1-(γ-L-glutamylamino) cyclopropane-1-carboxylic acid (GACC), has also been identified. The conjugation of ACC may play an important role in the control of ethylene biosynthesis, in a manner analogous to the conjugation of auxin and cytokinin.

ACC DEAMINASE A number of bacteria present in the soil express an enzyme called ACC deaminase that hydrolyzes ACC to ammonia and α-ketobutyrate (Glick 2005). These bacteria can promote plant growth by sequestering and cleaving ACC made and excreted by plants, thereby lowering the level of ethylene to which the plants are exposed. ACC deaminase has been expressed in transgenic plants to lower the level of ethylene produced. Recently, a gene encoding an ACC deaminase has been identified in Arabidopsis, suggesting that endogenous ACC deaminase plays a role in regulating ethylene biosynthesis.

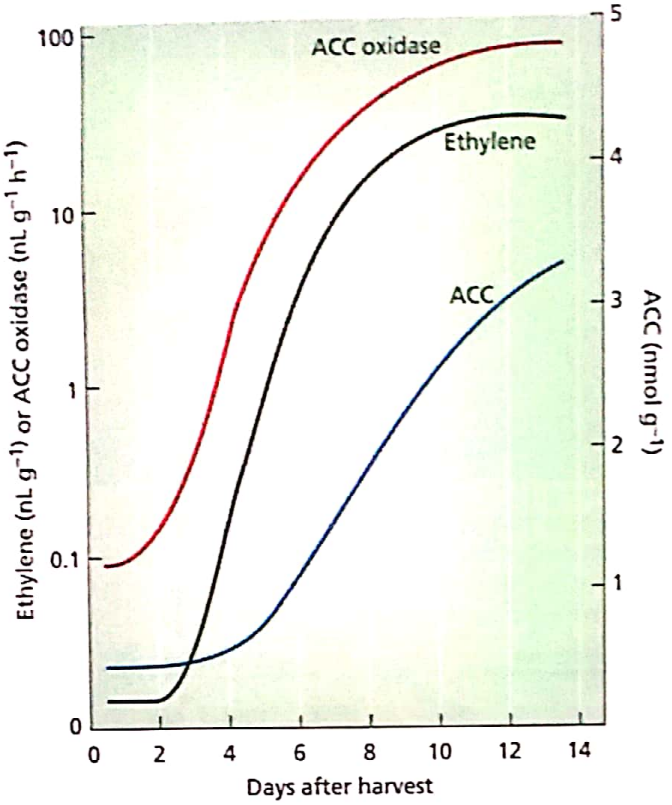


FIGURE 22.3 Changes in the ACC concentrations, ACC oxidase activity, and ethylene during ripening of Golden Delicious apples. The data are plotted as a function of days after harvest. Increases in ethylene and ACC concentrations and in ACC oxidase activity are closely correlated with ripening. (After Yang 1987.)

Ethylene biosynthesis is promoted by several factors

Ethylene biosynthesis is stimulated by several factors, including developmental state, environmental conditions, other plant hormones, and physical and chemical injury. Ethylene biosynthesis also varies in a circadian manner, peaking during the day and reaching a minimum at night.

FRUIT RIPENING As fruits mature, the rates of ACC and ethylene biosynthesis increase. Enzyme activities for both ACC oxidase (**FIGURE 22.3**) and ACC synthase increase, as do the mRNA levels for subsets of the genes encoding each enzyme. However, application of ACC to unripe fruits only slightly enhances ethylene production, indicating that an increase in the activity of ACC oxidase is the rate-limiting step in ripening (McKeon et al. 1995).

STRESS-INDUCED ETHYLENE PRODUCTION Ethylene biosynthesis is increased by stress conditions such as drought, flooding, chilling, exposure to ozone, and mechanical wounding. In all these cases ethylene is produced by the usual biosynthetic pathway, and the increased ethylene production has been shown to result at least in part from an increase in transcription of ACC synthase mRNA. This "stress ethylene" is involved in the onset of stress responses such as abscission, senescence, wound healing, and increased disease resistance (see Chapter 26).

CIRCADIAN REGULATION OF ETHYLENE PRODUCTION The circadian clock regulates the biosynthesis of ethylene in a number of plant species. There is generally a peak of ethylene evolution at midday, with a trough in the middle of the night. This regulation likely results from the transcriptional control of a subset of ACC synthase genes, which is mediated by the TOC1/CCA1 clock in Arabidopsis (Thain et al. 2004).

AUXIN-INDUCED ETHYLENE PRODUCTION In some instances, auxins and ethylene can cause similar plant responses, such as induction of flowering in pineapple and inhibition of stem elongation. These responses might be due to the ability of auxins to promote ethylene synthesis by enhancing ACC synthase activity. These observations suggest that some responses previously attributed to auxin (indole-3-acetic acid, or IAA) are in fact mediated by the ethylene produced in response to auxin.

Following application of exogenous IAA, the transcription of multiple ACC synthase genes is elevated and increased ethylene production is observed (Nakagawa et al. 1991; Liang et al. 1992; Tsuchisaka and Theologis 2004). Inhibitors of protein synthesis block IAA-induced ethylene synthesis, indicating that the synthesis of new ACC synthase protein caused by auxins brings about the marked increase in ethylene production.

Ethylene biosynthesis can be elevated through a stabilization of ACC synthase protein

In addition to auxin, brassinosteroids and cytokinins have been shown to elevate ethylene biosynthesis. As with auxin, these hormones act by increasing the activity of ACC synthase. However, in contrast to auxin, they act not by elevating ACC synthase transcription, but by increasing the stability of ACC synthase proteins (Chae and Kieber 2005).

For example, pathogen attack, cytokinin, and brassinosteroids all increase ethylene biosynthesis in part by stabilizing the ACC synthase protein so that it is broken down more slowly. The carboxy-terminal domain of ACC synthase plays a key role in this regulation (Vogel et al. 1998). This domain acts as a flag to target the protein for rapid degradation by the 26S proteasome (see Chapter 2). Phosphorylation of this domain by either a mitogen-activated protein (MAP) kinase (activated by pathogens) or a calcium-dependent kinase blocks its ability to target the protein for rapid turnover.

Various inhibitors can block ethylene biosynthesis

Inhibitors of hormone synthesis or action are valuable for the study of the biosynthetic pathways and physiological roles of hormones. The use of inhibitors is particularly helpful when it is difficult to distinguish between different hormones that have identical effects in plant tissue or when a hormone affects the synthesis or the action of another hormone.

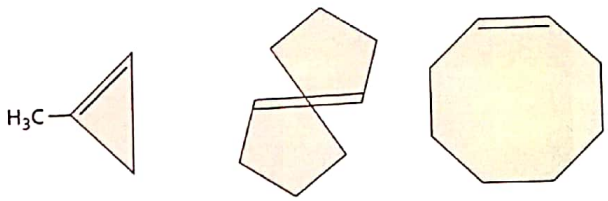
For example, ethylene mimics high concentrations of auxins by inhibiting stem growth and causing *epinasty* (a downward curvature of leaves). Use of specific inhibitors of ethylene biosynthesis and action made it possible to discriminate between the actions of auxin and ethylene. Studies using inhibitors showed that ethylene is the primary effector of epinasty and that auxin acts indirectly by causing a substantial increase in ethylene production.

INHIBITORS OF ETHYLENE SYNTHESIS **Aminoethoxyvinylglycine (AVG)** and **aminooxyacetic acid (AOA)** block the conversion of AdoMet to ACC (see Figure 22.2). AVG and AOA are known to inhibit enzymes that use the cofactor pyridoxal phosphate, including ACC synthase. α-Aminoisobutyric acid (AIBA) and cobalt ions (Co^{2+}) also inhibit the ethylene biosynthetic pathway, blocking the conversion of ACC to ethylene by ACC oxidase, the last step in ethylene biosynthesis.

INHIBITORS OF ETHYLENE ACTION Most of the effects of ethylene can be antagonized by specific ethylene inhibitors. Silver ions (Ag^+) applied as silver nitrate ($AgNO_3$) or as silver thiosulfate [$Ag(S_2O_3)_2^{3-}$] are potent inhibitors of ethylene action. Silver is very specific; the inhibition it causes cannot be induced by any other metal ion.

Carbon dioxide at high concentrations (in the range of 5 to 10%) also inhibits many effects of ethylene, such as the induction of fruit ripening, although CO_2 is less efficient than Ag^+. This effect of CO_2 has often been exploited in the storage of fruits, whose ripening is delayed at elevated CO_2 concentrations. The high concentrations of CO_2 required for inhibition make it unlikely that CO_2 acts as an ethylene antagonist under natural conditions. The volatile compound *trans*-cyclooctene, but not its isomer *cis*-cyclooctene, is a strong competitive inhibitor of ethylene binding (Sisler et al. 1990); *trans*-cyclooctene is thought to act by competing with ethylene for binding to the receptor. 1-Methylcyclopropene (MCP) binds almost irreversibly to the ethylene receptor (**FIGURE 22.4**), and effectively blocks multiple ethylene responses (Sisler and Serek 1997). This nearly odorless compound has been marketed under the trade name EthylBloc®, and is used to increase the shelf life of cut flowers and of some fruits, such as apples.

ETHYLENE ABSORPTION Because ethylene gas is easily lost from its tissue of origin and may affect other tissues or organs, ethylene-trapping systems are used during the storage of fruits, vegetables, and flowers. Potassium permanganate ($KMnO_4$) is an effective absorbent of ethylene and can reduce the concentration of ethylene in apple storage areas from 250 to 10 $\mu L\ L^{-1}$, markedly extending the storage life of the fruit.



1-Methylcyclopropene (MCP) *trans*-Cyclooctene *cis*-Cyclooctene

FIGURE 22.4 Two inhibitors that block ethylene binding to its receptor. The *cis* form of cyclooctene is not an effective inhibitor.

Ethylene Signal Transduction Pathways

Despite the broad range of ethylene's effects on development, the primary steps in ethylene action are likely similar in all cases: They all involve binding to a receptor, followed by activation of one or more signal transduction pathways (see Chapter 14) leading to the cellular response. Ultimately, ethylene exerts its effects primarily by altering the pattern of gene expression. Molecular genetic studies of selected mutants of *Arabidopsis thaliana* have contributed greatly to the elucidation of ethylene signaling components.

The triple-response morphology of etiolated Arabidopsis seedlings has been used as a screen to isolate mutants affected in their response to ethylene (**FIGURE 22.5**) (Guzman and Ecker 1990). Two classes of mutants have been identified by experiments in which mutagenized Arabidopsis seeds were grown on an agar medium in the presence or absence of ethylene for 3 days in the dark:

1. Mutants that fail to respond to exogenous ethylene (ethylene-resistant or ethylene-insensitive mutants)
2. Mutants that display the response even in the absence of ethylene (constitutive mutants)

Ethylene-insensitive mutants are identified as tall seedlings extending above the lawn of short, triple-responding seedlings when grown in the presence of ethylene (see Figure 22.5). Conversely, constitutive ethylene response mutants are identified as seedlings displaying the triple response in the absence of exogenous ethylene (see Figure 22.9).

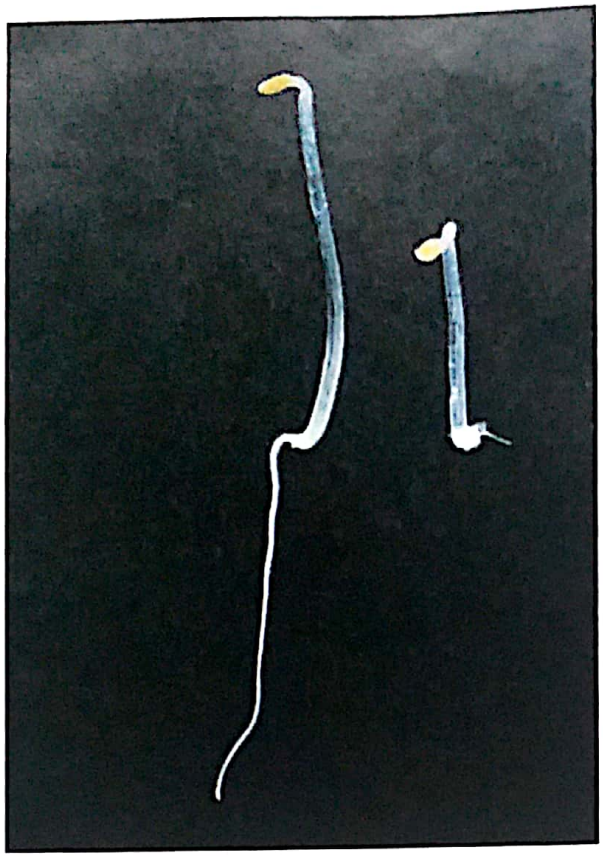


FIGURE 22.5 The triple response in Arabidopsis. Three-day-old etiolated seedlings grown in the presence (right) or absence (left) of 10 ppm ethylene. Note the shortened hypocotyl, reduced root elongation, and exaggeration of the curvature of the apical hook that results from the presence of ethylene. (Courtesy of J. Kieber.)

Ethylene receptors are related to bacterial two-component system histidine kinases

The first ethylene-insensitive mutant isolated was *etr1* (*e*thylene-response*1*) (**FIGURE 22.6**). The *etr1* mutant was identified in a screen for mutations that block the response of Arabidopsis seedlings to ethylene. The amino acid sequence of the carboxy-terminal half of ETR1 is similar to bacterial two-component histidine kinases—receptors used by bacteria to perceive environmental cues such as chemosensory stimuli, phosphate availability, and osmolarity.

FIGURE 22.6 Screen for the *etr1* mutant of Arabidopsis. Seedlings were grown for 3 days in the dark in ethylene. Note that all but one of the seedlings exhibit the triple response: exaggeration in curvature of the apical hook, inhibition and radial swelling of the hypocotyl, and horizontal growth. The *etr1* mutant is completely insensitive to the hormone and grows like an untreated seedling. (Photograph by K. Stepnitz of the MSU/DOE Plant Research Laboratory.)

As previously discussed in Chapter 14, bacterial two-component systems consist of a sensor histidine kinase and a response regulator. ETR1 was the first example of a eukaryotic histidine kinase, but others have since been found in yeast, mammals, and plants. Phytochrome (see Chapter 17) and the cytokinin receptor (see Chapter 21) also share sequence similarity to bacterial two-component histidine kinases. The similarity to bacterial receptors and the ethylene insensitivity of the *etr1* mutants suggested that ETR1 might be an ethylene receptor. Binding studies confirmed this hypothesis (see **WEB TOPIC 22.6**).

The Arabidopsis genome encodes four additional proteins similar to ETR1 that also function as ethylene receptors: ETR2, ERS1 (*ETHYLENE-RESPONSE SENSOR 1*), ERS2, and EIN4 (**FIGURE 22.7**). Like ETR1, these receptors have been shown to bind ethylene, and missense mutations in the genes that encode these proteins, analogous to the original *etr1* mutation, prevent ethylene binding to the receptor while allowing the receptor to function normally as a regulator of the ethylene response pathway in the absence of ethylene.

All of these five receptor proteins share at least two domains:

1. The amino-terminal domain spans the membrane at least three times and contains the ethylene-binding site. Ethylene can readily access this site because of its hydrophobicity.

2. The carboxy-terminal half of the ethylene receptors contains a domain homologous to histidine kinase catalytic domains.

A subset of the ethylene receptors also have a carboxy-terminal domain that is similar to bacterial two-component receiver domains. In other two-component systems, binding of ligand regulates the activity of the histidine kinase domain, which autophosphorylates a conserved histidine residue. The phosphate is then transferred to an aspartic acid residue located within the fused receiver domain (see Figure 14.4). Histidine kinase activity has been demonstrated for the ethylene receptor ETR1. However, genetic studies have shown that, in contrast to bacterial two-component systems, the histidine kinase activity of ETR1 does *not* play a primary role in signaling by this ethylene receptor (Wang et al. 2003). Instead, the kinase activity of ETR1 appears to have more subtle effects on ethylene signaling that may modulate the ethylene response (Qu and Schaller 2004; Binder et al. 2004a). Several other ethylene receptors are missing amino acids critical for histidine kinase activity ("Subfamily 2" in Figure 22.7), making it unlikely that they possess histidine kinase activity.

The five Arabidopsis ethylene receptors have been shown to interact with each other in the plant, forming large multisubunit complexes (Gao et al. 2008). Furthermore, binding of ethylene appears to induce degradation of the receptors via the 26S proteasome (Kevany et al. 2007).

Unlike most receptors, which are associated with the plasma membrane, ETR1 and the other four ethylene receptors in Arabidopsis are located on the endoplasmic reticulum. However, ETR1 may also be localized to the Golgi apparatus, at least in roots. In either case, an intracellular location for the ethylene receptor is consistent with the hydrophobic nature of ethylene, which enables it to pass freely through the plasma membrane into the cell. In this respect ethylene is similar to the hydrophobic signaling molecules of animals, such as steroids and the gas nitric oxide, which also bind to intracellular receptors.

High-affinity binding of ethylene to its receptor requires a copper cofactor

Even before the identification of its receptor, scientists had predicted that ethylene would bind to its receptor via a transition metal cofactor, most likely copper or zinc. This prediction was based on the high affinity of olefins, such as ethylene, for these transition metals. Recent genetic and biochemical studies have borne out these predictions.

Analysis of the ETR1 ethylene receptor expressed in yeast demonstrated that a copper ion was coordinated to the protein and that this copper was necessary for high-affinity ethylene binding (Rodriguez et al. 1999). Silver ion could substitute for copper to yield high-affinity binding, which indicates that silver blocks the action of ethylene not by interfering with ethylene binding, but by preventing the changes in the protein that normally occur when ethylene binds to the receptor.

Evidence that copper binding is required for ethylene receptor function in vivo came from identification of the **RAN1** (*RESPONSIVE-TO-ANTAGONIST1*) gene in Arabidopsis (Hirayama et al. 1999). Strong *ran1* mutations block the formation of functional ethylene receptors (Woeste and Kieber 2000). Cloning of *RAN1* revealed that it encodes a protein similar to a yeast protein required for the transfer of a copper ion to an iron transport protein. In an analogous manner, RAN1 is likely to be involved in the addition of a copper ion cofactor necessary for the function of the ethylene receptors.

Unbound ethylene receptors are negative regulators of the response pathway

In Arabidopsis, tomato, and probably most other plant species, the ethylene receptors are encoded by multigene families. Targeted disruption (complete inactivation) of the five Arabidopsis ethylene receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) has revealed that they are functionally redundant (Hua and Meyerowitz 1998). That is, disruption of any single gene encoding one of these proteins has no effect, but a plant with disruptions in multiple receptor

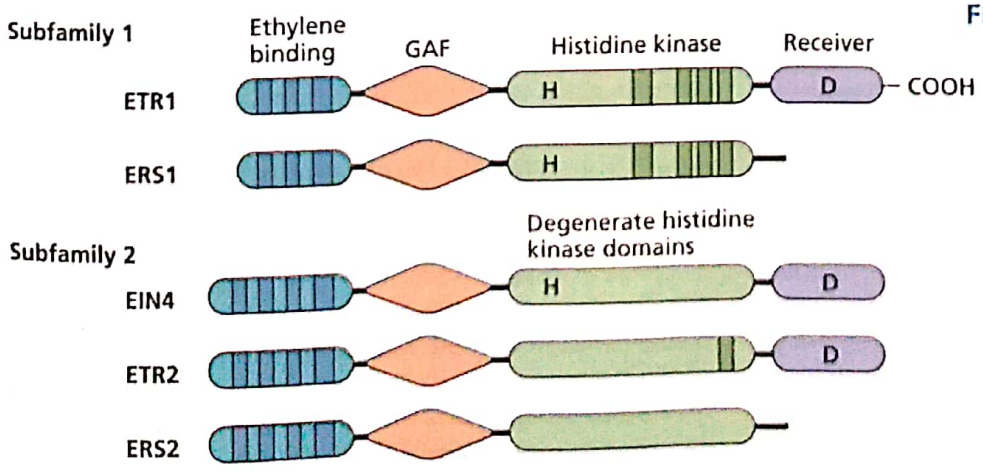


FIGURE 22.7 Schematic diagram of five ethylene receptor proteins and their functional domains. The GAF domain is a conserved cGMP-binding domain, found in a diverse group of proteins, that generally acts as small molecule–binding regulatory domains. H and D are histidine and aspartate residues that participate in phosphorylation. Note that EIN4, ETR2, and ERS2 have degenerate histidine kinase domains, meaning that they are missing critical, highly conserved amino acids that are required for histidine kinase catalytic activity.

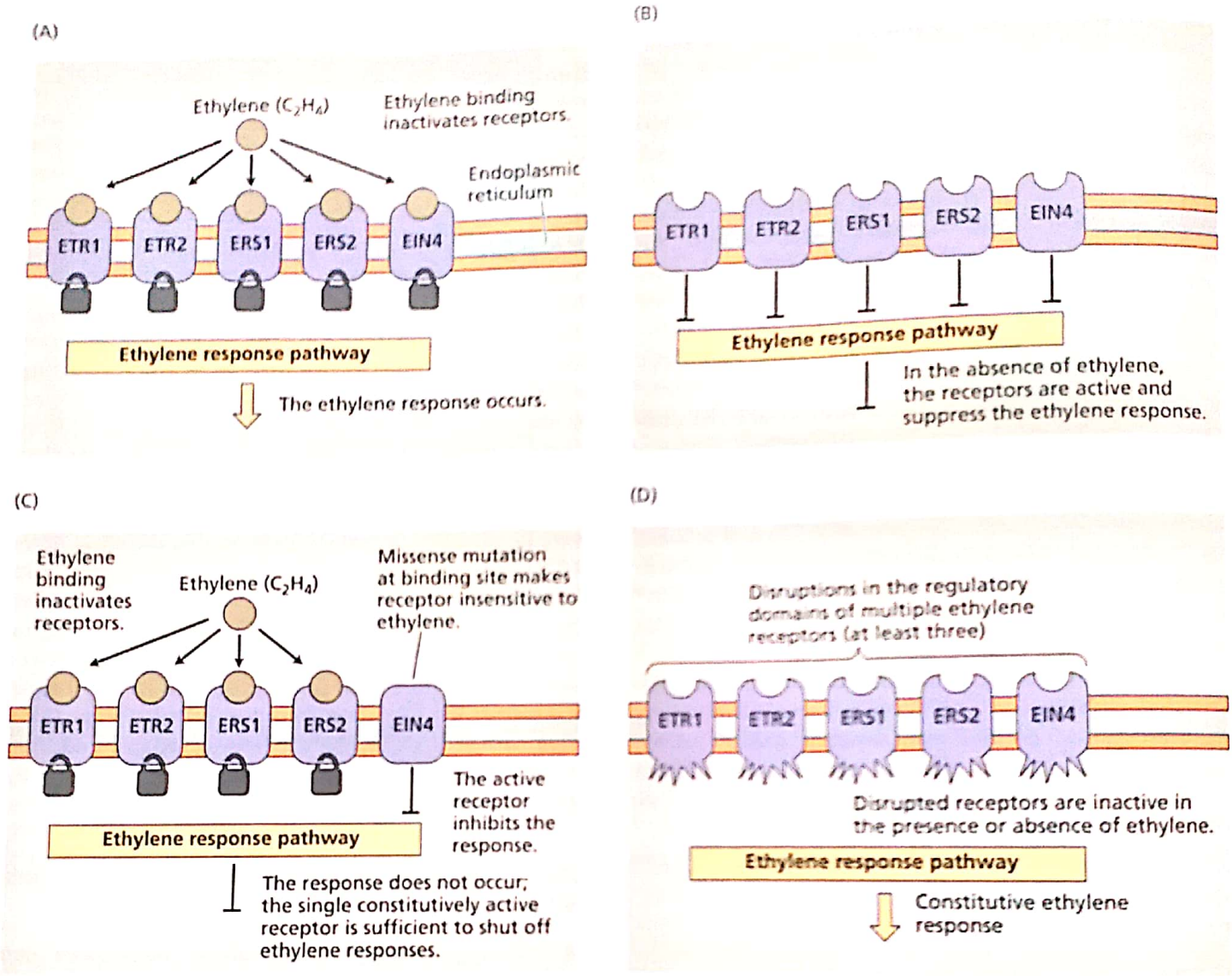


FIGURE 22.8 Model for ethylene receptor action based on the phenotype of receptor mutants. (A) In the wild type, ethylene binding inactivates the receptors, allowing the response to occur. (B) In the absence of ethylene the receptors act as negative regulators of the response pathway. (C) A missense mutation that interferes with ethylene binding to its receptor, but leaves the regulatory site active, results in an ethylene-insensitive phenotype. (D) Disruption mutations in the regulatory sites result in a constitutive ethylene response.

genes exhibits a constitutive ethylene response phenotype (FIGURE 22.8D).

The observation that ethylene responses, such as the triple response, become constitutive when the receptors are disrupted indicates that the receptors are normally "on" (i.e., in the active state) in the *absence* of ethylene, and that the function of the receptor *minus* its ligand (ethylene), is to *shut off* the signaling pathway that leads to the response (FIGURE 22.8B). Binding of ethylene "turns off" (inactivates) the receptors, thus allowing the response pathway to proceed (FIGURE 22.8A).

As discussed in Chapter 14, this somewhat counterintuitive model for ethylene receptors as negative regulators of a signaling pathway is unlike the mechanism of most animal receptors, which, after binding their ligands, serve as positive regulators of their respective signal transduction pathways. That is, animal receptors typically *activate* previously inactive signaling pathways, bringing about the response. Ethylene receptors, on the other hand, actively *repress* the hormone response in the absence of the hormone.

In contrast to the disrupted receptors, receptors with missense mutations at the ethylene binding site (as occurs in the original *etr1* mutant) are unable to bind ethylene, but are still active as negative regulators of the ethylene response pathway. Such missense mutations result in a plant that expresses a subset of receptors that can no longer be turned off by ethylene, and thus confer a *dominant ethylene-insensitive phenotype* (FIGURE 22.8C). Even though the normal receptors can all be turned off by ethylene, the mutant receptors continue to signal the cell to suppress

ethylene responses whether ethylene is present or not. In tomato, the *never-ripe* mutation, which, as the name suggests, sets fruit that fails to ripen, is such a dominant ethylene-insensitive mutation in a tomato ethylene receptor.

A consequence of this negative signaling is that a decrease in the level of ethylene receptors actually makes a tissue more sensitive to ethylene. Thus, the level of functional ethylene receptors is an important mechanism by which plants regulate their sensitivity to this hormone. For example, in tomato fruit, two of the ethylene receptors are rapidly degraded by the 26S proteasome in response to ethylene, resulting in an *increase* in ethylene sensitivity (Kevany et al. 2007). This is important in coordinating the timing of ripening throughout the large tomato fruit.

A serine/threonine protein kinase is also involved in ethylene signaling

The recessive ***ctr1*** (constitutive *t*riple *r*esponse *1* = triple response in the absence of ethylene) mutation was identified in screens for mutations that constitutively activate ethylene responses (FIGURE 22.9). The fact that the recessive mutation caused an *activation* of the ethylene response suggests that the wild-type protein, like the ethylene receptors, acts as a *negative regulator* of the response pathway (Kieber et al. 1993).

CTR1 appears to be related to Raf, a MAPKKK (*mito*gen-*a*ctivated *p*rotein *k*inase *k*inase *k*inase) type of serine/threonine protein kinase that is involved in the transduction of various external regulatory signals and developmental signaling pathways in organisms ranging from yeast to humans (see Chapter 14). In animal cells, the final product in the MAP kinase cascade is a phosphorylated transcription factor that regulates gene expression in the nucleus. There is some evidence that a MAP kinase cascade acts downstream of CTR1 in ethylene signaling (Yoo et al. 2008), but as yet there is no consensus on this point.

Various lines of evidence indicate that the CTR1 protein directly interacts with the ethylene receptors, forming part of a protein complex involved in perceiving ethylene. Genetic analysis has shown that the interaction of CTR1 with the ethylene receptors is necessary for its function, as mutations in CTR1 that block this interaction, but otherwise do not affect the protein, cause CTR1 to be inactive in the plant (Huang et al. 2003). The precise mechanism by which CTR1 is regulated by ETR1 and the other ethylene receptors is still not known.

EIN2 encodes a transmembrane protein

The ***ein2*** (ethylene-*in*sensitive 2) mutation blocks all ethylene responses in both seedling and adult Arabidopsis plants. The *EIN2* gene encodes a protein containing 12 membrane-spanning domains that is most similar to the N-RAMP (*n*atural *r*esistance–*a*ssociated *m*acrophage *p*ro-

FIGURE 22.9 Screen for Arabidopsis mutants that constitutively display the triple response. Seedlings were grown for 3 days in the dark in air (no ethylene). A single *ctr1* mutant seedling is evident among the taller, wild-type seedlings. (Courtesy of J. Kieber.)

tein) family of cation transporters in animals (Alonso et al. 1999), suggesting that it may act as a channel or pore. To date, however, researchers have failed to demonstrate a transport activity for this protein, and the intracellular location of the protein is not known. The EIN2 protein is rapidly degraded by the 26S proteasome, and ethylene inhibits the degradation of EIN2 (Qiao et al. 2009). Because EIN2 alters the sensitivity to ethylene, the degradation of EIN2 provides a further mechanism for regulating the sensitivity of plant cells to ethylene.

Ethylene Regulation of Gene Expression

One of the primary effects of ethylene signaling is an alteration in the expression of various target genes. Ethylene affects the mRNA transcript levels of numerous genes, including those that encode cellulase and genes related to ripening and ethylene biosynthesis. Regulatory sequences called **ethylene response elements**, or **ERES**, have been identified among the ethylene-regulated genes.

Specific transcription factors are involved in ethylene-regulated gene expression

Key components mediating ethylene's effects on gene expression are the EIN3 family of transcription factors (Chao et al. 1997). There are at least four *EIN3*-like genes in Arabidopsis, and homologs have been identified in tomato and tobacco. In response to an ethylene signal, homodimers of EIN3 or closely related proteins bind to the promot-

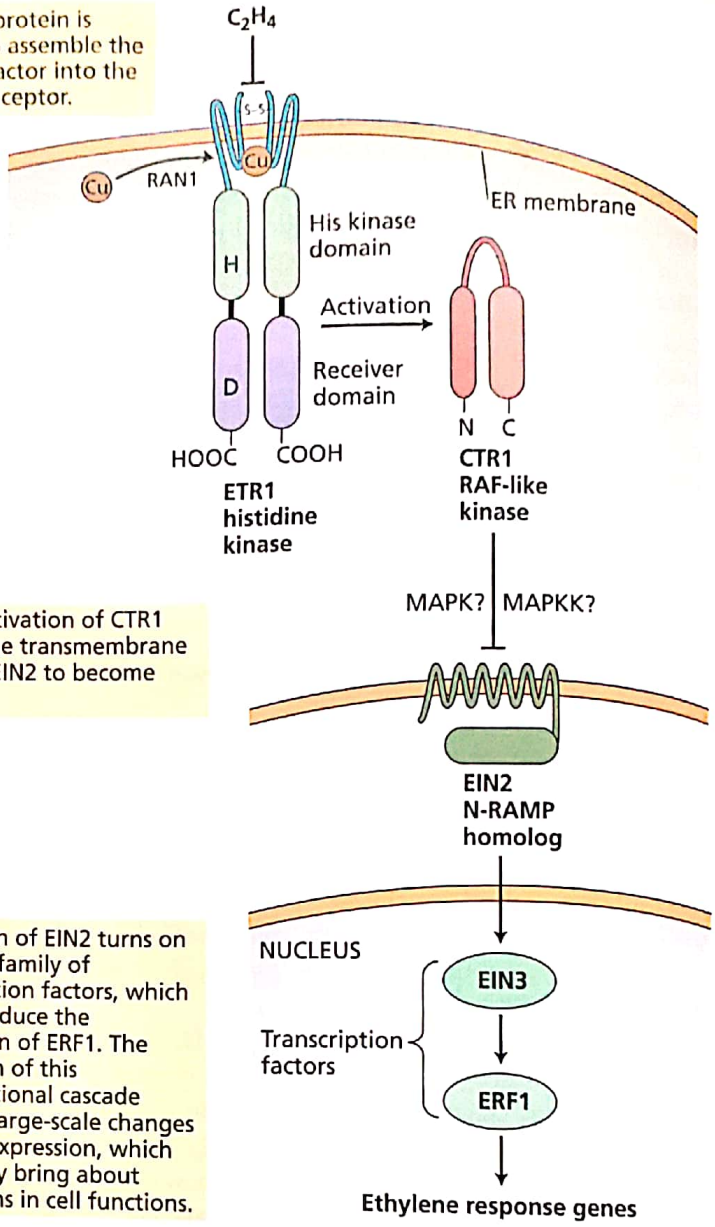


FIGURE 22.10 Model of ethylene signaling in Arabidopsis. Ethylene binds to the ETR1 receptor, which is an integral membrane protein of the endoplasmic reticulum membrane. Multiple isoforms of ethylene receptors may be present in a cell; only ETR1 is shown for simplicity. The receptor is a dimer, held together by disulfide bonds. Ethylene binds within the transmembrane domain, through a copper cofactor, which is assembled into the ethylene receptors by the RAN1 protein.

ers of genes that are rapidly induced by ethylene, including *ERF1* (*E*THYLENE *R*ESPONSE *F*ACTOR *1*), to activate their transcription (Solano et al. 1998).

ERF1 encodes a protein that belongs to the **ERE-binding protein (EREBP)** family of transcription factors, which were first identified in tobacco as proteins that bind to ERE sequences (Ohme-Takagi and Shinshi 1995). Several EREBPs are rapidly up-regulated in response to ethylene. The *EREBP* genes exist in Arabidopsis as a very large gene family, but only a few of the genes are inducible by ethylene.

The regulation of EIN3 protein stability plays an important role in ethylene signaling, as well as in regulating the ethylene biosynthetic pathway. Two redundant F-box proteins, EBF1 and EBF2 (*EIN3-b*inding *F*-box 1 and 2), promote ubiquitination and thus targeting of EIN3 for degradation by the 26S proteasome (see Chapter 14). Ethylene inhibits this EBF1/EBF2-dependent degradation of EIN3, possibly through phosphorylation of EIN3 by a MAP kinase (Yoo et al. 2008), resulting in the accumulation of EIN3 and the subsequent expression of ethylene-regulated genes. Thus, ethylene acts, at least in part, by regulating the level of the EIN3 and *EIN3-l*ike (EIL) proteins.

Genetic epistasis reveals the order of the ethylene signaling components

The order of action of the genes *ETR1*, *EIN2*, *EIN3*, and *CTR1* has been determined by the analysis of how the mutations interact with each other (i.e., their epistatic order). Two mutants with opposite phenotypes are crossed, and a line harboring both mutations (the double mutant) is identified in the F_2 generation. In the case of the ethylene response mutants, researchers constructed a line doubly mutant for *ctr1* (a constitutive ethylene response mutant) and one of the ethylene-insensitive mutations.

The phenotype displayed by the double mutant reveals which of the mutations is epistatic to the other (Avery and Wasserman 1992). For example, if an *etr1 ctr1* double mutant displays a *ctr1* mutant phenotype, the *ctr1* mutation is said to be epistatic to *etr1*. From this it can be inferred that CTR1 acts downstream of ETR1. Similar genetic studies were used to determine the order of action of *ETR1*, *EIN2*, and *EIN3* relative to *CTR1*.

The ETR1 protein has been shown to interact physically with the predicted downstream protein, CTR1, suggesting

that the ethylene receptors may directly regulate the kinase activity of CTR1 (Clark et al. 1998). The model in FIGURE 22.10 summarizes these and other data. Genes similar to several of these Arabidopsis signaling genes have been found in other species (see WEB TOPIC 22.7).

This model is still incomplete and there are likely additional components of this pathway that have yet to be uncovered. In addition, we are only beginning to understand the biochemical properties of these proteins and how they interact. Further research will be needed to provide a more complete picture of the molecular basis for the perception and transduction of the ethylene signal.

Developmental and Physiological Effects of Ethylene

As we have seen, ethylene was discovered in connection with its effects on seedling growth and fruit ripening. It has since been shown to regulate a wide range of responses in plants, including seed germination, cell expansion, cell differentiation, flowering, senescence, and abscission. In this section we will consider the phenotypic effects of ethylene in more detail.

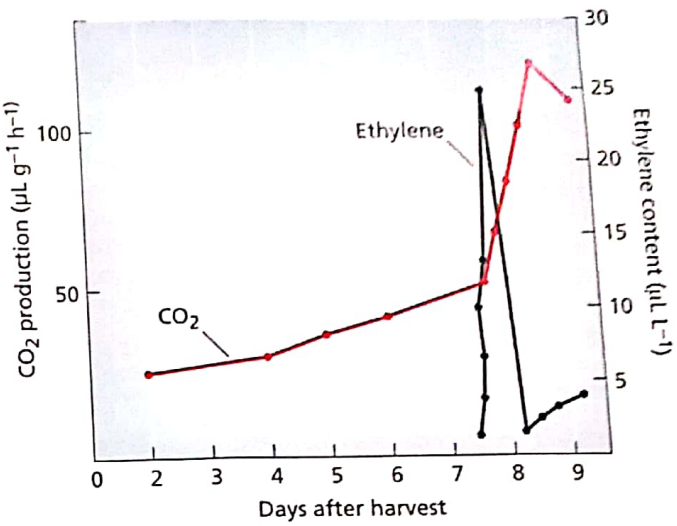


FIGURE 22.11 Ethylene production and respiration. In banana, ripening is characterized by a climacteric rise in respiration rate, as evidenced by the increased CO_2 production. A climacteric rise in ethylene production precedes the increase in CO_2 production, suggesting that ethylene is the hormone that triggers the ripening process. (After Burg and Burg 1965.)

Ethylene promotes the ripening of some fruits

In everyday usage, the term *fruit ripening* refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins.

From the perspective of the plant, fruit ripening means that the seeds are ready for dispersal. For seeds whose dispersal depends on animal ingestion, *ripeness* and *edibility* are synonymous. Brightly colored anthocyanins and carotenoids often accumulate in the epidermis of such fruits, enhancing their visibility. However, for seeds that rely on mechanical or other means for dispersal, *fruit ripening* may mean drying followed by splitting.

Because of their importance in agriculture, the vast majority of studies on fruit ripening have focused on edible fruits. Ethylene has long been recognized as the hormone that accelerates the ripening of edible fruits. Exposure of such fruits to ethylene hastens the processes associated with ripening, and a dramatic increase in ethylene production accompanies the initiation of ripening. However, surveys of a wide range of fruits have shown that not all of them respond to ethylene.

Fruits that respond to ethylene exhibit a climacteric

All fruits that ripen in response to ethylene exhibit a characteristic respiratory rise called a **climacteric** before the ripening phase.* Such fruits also show a spike of ethylene production immediately before the respiratory rise (FIGURE 22.11). Apples, bananas, avocados, and tomatoes are examples of climacteric fruits.

In contrast, fruits such as citrus fruits and grapes do not exhibit the respiration and ethylene production rise and are called **nonclimacteric** fruits. Other examples of climacteric and nonclimacteric fruits are given in TABLE 22.1.

In climacteric fruits, treatment with ethylene induces the fruit to produce additional ethylene, a response that can be described as **autocatalytic**. In climacteric plants, two systems of ethylene production operate:

- **System 1**, which acts in vegetative tissue, and in which ethylene inhibits its own biosynthesis
- **System 2**, which occurs in ripening climacteric fruit and in the senescing petals in some species, and in which ethylene stimulates its own biosynthesis—that is, it is autocatalytic

The positive feedback loop for ethylene biosynthesis in System 2 integrates ripening of the entire fruit once it has commenced. When unripe climacteric fruits are treated with ethylene, the onset of the climacteric rise is hastened.

*The term climacteric can be used either as a noun, as in "most fruits exhibit a climacteric during ripening" or as an adjective, as in "a climacteric rise in respiration." The term nonclimacteric, however, is used only as an adjective, as in "nonclimacteric fruit."

TABLE 22.1
Climacteric and nonclimacteric fruits

Climacteric		Nonclimacteric
Apple	Olive	Bell pepper
Avocado	Peach	Cherry
Banana	Pear	Citrus
Cantaloupe	Persimmon	Grape
Cherimoya	Plum	Pineapple
Fig	Tomato	Snap bean
Mango		Strawberry
		Watermelon

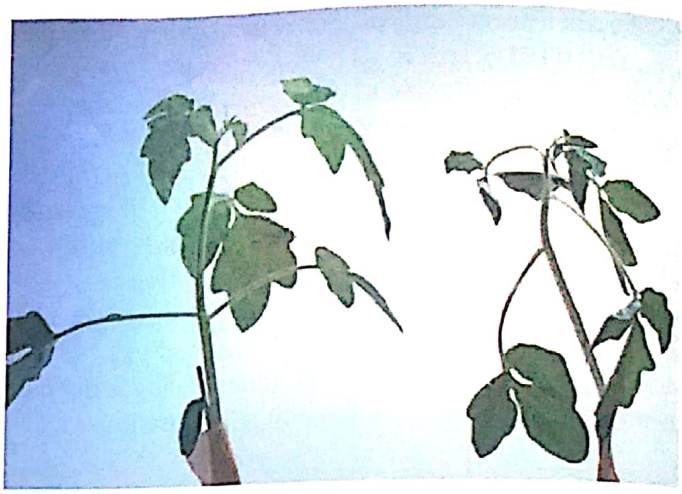


FIGURE 22.12 Leaf epinasty in tomato. Epinasty, or downward bending of the tomato leaves (right), is caused by ethylene treatment. Epinasty results when the cells on the upper side of the petiole grow faster than those on the bottom. (Courtesy of S. Gepstein.)

In contrast, when nonclimacteric fruits are treated with ethylene, the respiration rate increases as a function of the ethylene concentration, but the treatment does not trigger production of endogenous ethylene and does not accelerate ripening. Elucidation of the role of ethylene in the ripening of climacteric fruits has resulted in many practical applications aimed at either uniform ripening or the delay of ripening.

Although the effects of exogenous ethylene on fruit ripening are straightforward and clear, establishing a causal relation between the level of endogenous ethylene and fruit ripening is more difficult. Inhibitors of ethylene biosynthesis (such as AVG) or of ethylene action (such as CO_2, MCP, or Ag^+) have been shown to delay or even prevent ripening. However, the definitive demonstration that ethylene is required for fruit ripening was provided by experiments in which ethylene biosynthesis was blocked by expression of an antisense version of either ACC synthase or ACC oxidase in transgenic tomatoes (see **WEB TOPIC 22.8**). Elimination of ethylene biosynthesis in these transgenic tomatoes completely blocked fruit ripening, and application of exogenous ethylene restored ripening (Oeller et al. 1991).

The receptors of never-ripe *mutants of tomato fail to bind ethylene*

Further demonstration of the requirement for ethylene in fruit ripening came from the analysis of the *never-ripe* mutation in tomato. As the name implies, this mutation completely blocks the ripening of tomato fruit. Molecular analysis revealed that *never-ripe* was due to a mutation in an ethylene receptor that rendered it unable to bind ethylene (Lanahan et al. 1994). This analysis, together with the demonstration that inhibiting ethylene biosynthesis via antisense technology blocked ripening, provided unequivocal proof of the role of ethylene in fruit ripening, and opened the door to the manipulation of fruit ripening through biotechnology.

In tomatoes many genes that are highly regulated during ripening have been identified using tomato complementary DNA (cDNA) microarrays.* During tomato fruit ripening, the fruit softens as the result of cell wall hydrolysis, and it changes from green to red as a consequence of chlorophyll loss and the synthesis of the carotenoid pigment lycopene. At the same time, aroma and flavor components are produced.

Analysis of mRNA from fruits of wild-type and transgenic tomato plants genetically engineered to lack ethylene has revealed that gene expression during ripening is regulated by at least two independent pathways:

1. *An ethylene-dependent pathway* includes genes involved in the biosynthesis of lycopene, volatile aromatic compounds, ACC synthase, and the enzymes of respiratory metabolism.
2. *A developmental, ethylene-independent pathway* includes genes that encode ACC oxidase and chlorophyllase.

Thus, not all of the processes associated with ripening in tomato are ethylene dependent.

Leaf epinasty results when ACC from the root is transported to the shoot

The downward curvature of leaves that occurs when the upper (adaxial) side of the petiole grows faster than the lower (abaxial) side is termed *epinasty* (**FIGURE 22.12**). Eth-

*cDNA microarrays (also known as biochips, DNA chips, or gene arrays) are small chips on which cDNAs or oligonucleotides, each representing a given gene, have been immobilized. When probed with the appropriately tagged cDNAs, DNA chips allow one to measure the expression of thousands of genes in a tissue simultaneously. See Chapter 2 for a more detailed description of this technique.

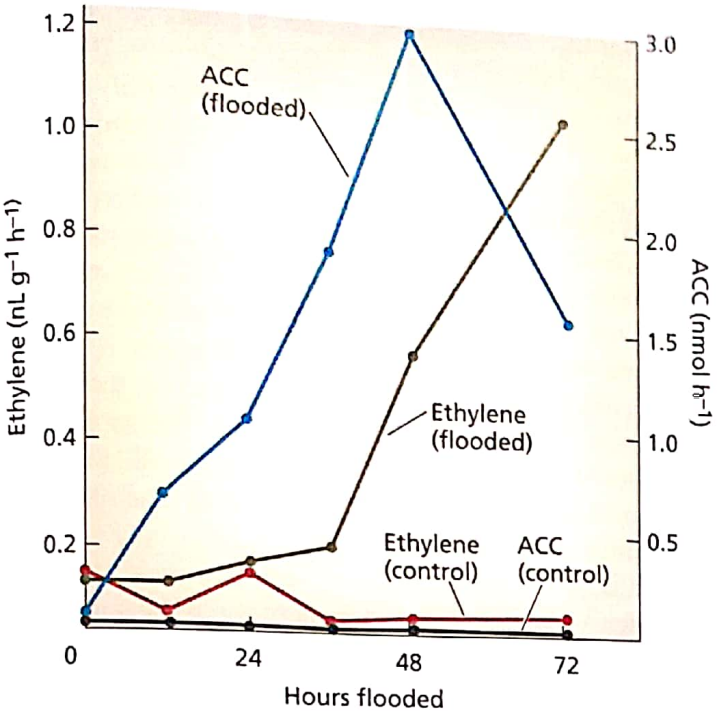


FIGURE 22.13 Changes in the amounts of ACC in the xylem sap and ethylene production in the petiole following flooding of tomato plants. ACC is synthesized in roots, but it is converted to ethylene very slowly under the anaerobic conditions of flooding. ACC is transported via the xylem to the shoot, where it is converted to ethylene. Gaseous ethylene cannot be transported, so it usually affects the tissue nearest the site of its production. In contrast, the ethylene precursor ACC is transportable and can produce ethylene far from the site of ACC synthesis. (After Bradford and Yang 1980.)

ylene and high concentrations of auxin induce epinasty, and it has now been established that auxin acts indirectly by inducing ethylene production. As will be discussed later in the chapter, a variety of stress conditions, such as salt stress or pathogen infection, increase ethylene production and also induce epinasty.

In tomato and other dicots, flooding (waterlogging) or anaerobic conditions around the roots enhance the synthesis of ethylene in the shoot, leading to the epinastic response. Because these environmental stresses are sensed by the roots and the response is displayed by the shoots, a signal from the roots must be transported to the shoots. This signal is ACC, the immediate precursor of ethylene. ACC levels were found to be significantly higher in the xylem sap of tomato plants after the roots had been flooded for 1 to 2 days (**FIGURE 22.13**) (Bradford and Yang 1980).

Because water fills the air spaces in waterlogged soil, and O_2 diffuses slowly through water, the concentration of oxygen around flooded roots decreases dramatically. ACC accumulates in the anaerobic roots, and is then transported to shoots via the transpiration stream, where it is readily converted to ethylene in the presence of oxygen (see Figure 22.2).

Ethylene induces lateral cell expansion

At concentrations above 0.1 $\mu L\ L^{-1}$, ethylene changes the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the hypocotyl or the epicotyl. In dicots, this swelling is part of the **triple response**, which, in Arabidopsis, consists of inhibition of hypocotyl elongation combined with hypocotyl swelling, inhibition of root elongation, and exaggeration of the curvature of the apical hook (see Figure 22.5).

As discussed in Chapter 15, the directionality of plant cell expansion is determined by the orientation of the cellulose microfibrils in the cell wall. Transverse microfibrils reinforce the cell wall in the lateral direction, so that turgor pressure is channeled into cell elongation. The orientation of the microfibrils is in turn determined by the orientation of the cortical array of microtubules in the cortical (peripheral) cytoplasm. In typical elongating plant cells, the cortical microtubules are arranged transversely, giving rise to transversely arranged cellulose microfibrils.

During the seedling triple response to ethylene, in the hypocotyl, the transverse pattern of microtubule alignment is disrupted, and the microtubules switch over to a longitudinal orientation. This 90° shift in microtubule orientation leads to a parallel shift in cellulose microfibril deposition. The newly deposited wall is reinforced in the longitudinal direction rather than the transverse direction, which promotes lateral expansion instead of elongation.

How do microtubules shift from one orientation to another? To study this phenomenon, pea (*Pisum sativum*) epidermal cells were injected with the microtubule protein tubulin, to which a fluorescent dye was covalently attached. The fluorescent "tag" did not interfere with the assembly of microtubules. This procedure allowed researchers to monitor the assembly of microtubules in living cells using a confocal laser scanning microscope, which can focus in many planes throughout the cell.

It was found that microtubules do not reorient from the transverse to the longitudinal direction by complete depolymerization of the transverse microtubules followed by repolymerization of a new longitudinal array of microtubules. Instead, increasing numbers of nontransversely aligned microtubules appear in particular locations (**FIGURE 22.14**). Neighboring microtubules then adopt the new alignment, so at one stage different alignments coexist before all the microtubules adopt a uniformly longitudinal orientation (Yuan et al. 1994). Although the reorientations observed in this study were in response to wounding rather than induced by ethylene, it is presumed that

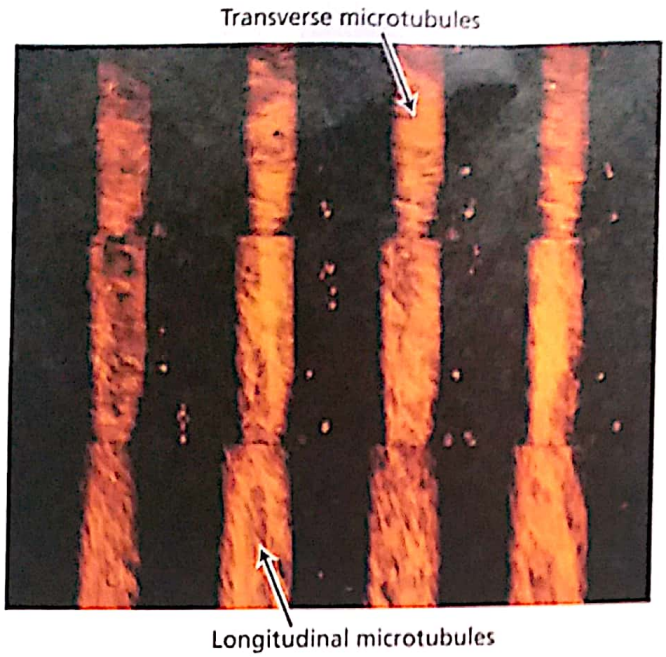


FIGURE 22.14 Reorientation of microtubules from transverse to vertical in pea stem epidermal cells in response to wounding. A living epidermal cell was microinjected with rhodamine-conjugated tubulin, which incorporates into the plant microtubules. A time series of approximately 6-minute intervals shows the cortical microtubules undergoing reorientation from net transverse to oblique/longitudinal. The reorientation seems to involve the appearance of patches of new, "discordant" microtubules in the new direction, concomitant with the disappearance of microtubules from the previous alignment. (From Yuan et al. 1994, photo courtesy of C. Lloyd.)

ethylene-induced microtubule reorientation operates by a similar mechanism.

There are two distinct phases to growth inhibition by ethylene

As noted above, growth of seedlings in the presence of ethylene inhibits the elongation of hypocotyls in dark-grown seedlings. Careful kinetic analysis of this response indicates that it occurs in two distinct phases (**FIGURE 22.15A**) (Binder et al. 2004a).

- The first, rapid phase of inhibition occurs within 15 minutes of exposure to ethylene and lasts approximately 30 minutes.
- A second deceleration of growth then ensues, with the hypocotyl growth reaching a new steady-state level of growth that is slower than that of untreated seedlings.

These two phases are growth are mechanistically distinct: The first phase is more sensitive to ethylene as compared to the second phase; EIN2 is required for both phases, but the EIN3/EIL1 transcription factors are only required for the second phase (**FIGURE 22.15B**) (Binder et al. 2004b).

Following removal from ethylene, seedlings fully recover to untreated growth rates within 90 minutes (Binder et al. 2004a). How can we reconcile this observation with the fact that the half-life of ethylene bound to its receptors is 11 hours? Recall that the turnover of some ethylene receptors is stimulated by binding to ethylene. Thus, when ethylene is removed from seedlings, the bound receptors are rapidly degraded and are replaced by newly synthesized receptors that are not bound to ethylene. Remember that the newly synthesized unbound ethylene receptors act as *negative regulators* of the ethylene response (see Figure 22.8). Thus, these unbound receptors shut off the ethylene response (i.e., inhibited hypocotyl elongation) relatively rapidly, even though some receptors remain that are still bound to ethylene. The histidine kinase activity of the subfamily 1 receptors appears to play an important role during this recovery phase following removal of ethylene.

The hooks of dark-grown seedlings are maintained by ethylene production

Etiolated dicot seedlings are usually characterized by a pronounced hook located just behind the shoot apex (see Figures 22.1 and 22.5). This hook shape facilitates penetration of the seedling through the soil, protecting the tender apical meristem.

Like epinasty, hook formation and maintenance result from ethylene-induced asymmetric growth. The closed shape of the hook is a consequence of the more rapid elongation of the outer side of the stem compared with the inner side. When the hook is exposed to white light it opens, because the elongation rate of the inner side increases, equalizing the growth rates on both sides (see Appendix 2).

Red light induces hook opening, and far-red light reverses the effect of red, indicating that phytochrome is the photoreceptor involved in this process (see Chapter 17). A close interaction between phytochrome and ethylene controls hook opening. As long as ethylene is produced by the hook tissue in the dark, elongation of the cells on the inner side is inhibited. Red light inhibits ethylene formation, promoting growth on the inner side, thereby causing the hook to open.

The auxin-insensitive mutation *axr1* does not develop an apical hook; and treatment of wild-type Arabidopsis seedlings with NPA (*N*-1-naphthylphthalamic acid), an inhibitor of polar auxin transport, blocks apical hook formation. These and other results indicate a role for auxin in maintaining hook structure. The more rapid growth rate of the outer tissues relative to the inner tissues could reflect an ethylene-dependent auxin gradient, analogous to the lateral auxin gradient that develops during phototropic curvature (see Chapter 19 and **WEB TOPIC 22.9**).

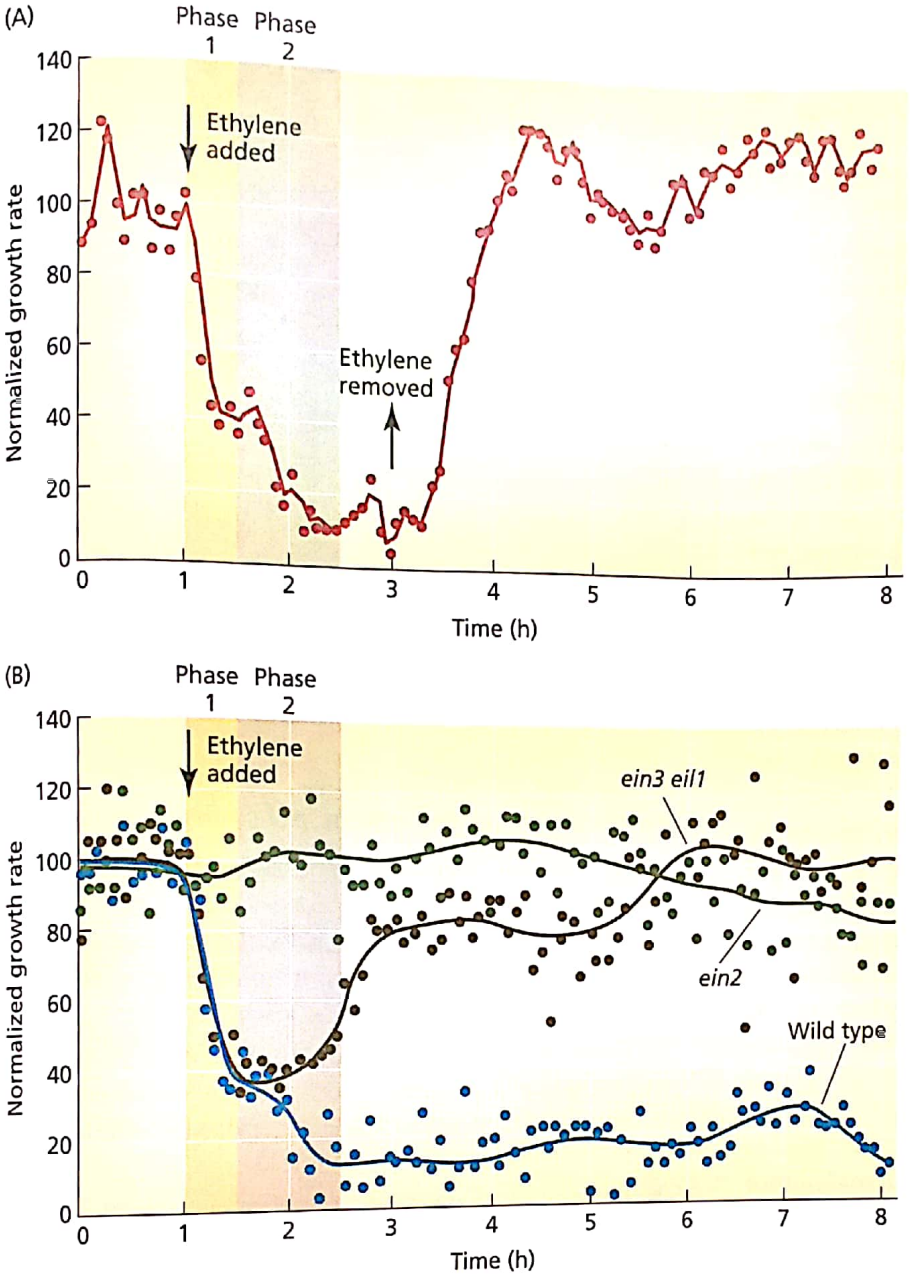


FIGURE 22.15 Kinetics of the effects of ethylene addition and removal on hypocotyl elongation in dark-grown Arabidopsis seedlings. (A) Growth rate of etiolated wild-type Arabidopsis after exposure to ethylene and subsequent removal of ethylene at the times indicated by the arrows. Note that the reduction in the growth rate following exposure to ethylene occurs in two distinct phases. (B) Growth rate of etiolated wild-type, *ein2*, and *ein3 eil1* Arabidopsis seedlings following exposure to ethylene at the time indicated by the arrow. Note that the phase 1 response of the *ein3 eil1* seedlings is identical to that of the wild type, but the phase 2 response is absent. (After Binder et al. 2004a, b.)

Ethylene breaks seed and bud dormancy in some species

Seeds that fail to germinate under normal conditions (water, oxygen, temperature suitable for growth) are said to be dormant (see Chapter 23). Ethylene has the ability to break dormancy and initiate germination in certain seeds, such as cereals. In addition to its effect on dormancy, ethylene increases the rate of seed germination of several species. In peanuts (*Arachis hypogaea*), ethylene production and seed germination are closely correlated. Ethylene can also break bud dormancy, and ethylene treatment is sometimes used to promote bud sprouting in potato and other tubers.

Ethylene promotes the elongation growth of submerged aquatic species

Although usually thought of as an inhibitor of stem elongation, ethylene is able to promote stem and petiole elongation in various submerged or partially submerged aquatic plants. These include the dicots *Ranunculus sceleratus*, *Nymphoides peltata*, and *Callitriche platycarpa*, and the fern *Regnellidium diphyllum*. Another agriculturally important example is deep-water rice (*Oryza sativa*), a cereal.

In these species, submergence induces rapid internode or petiole elongation, which allows the leaves or upper parts of the shoot to remain above water. Treatment with ethylene mimics the effects of submergence.

Growth is stimulated in the submerged plants because ethylene builds up in the tissues. In the absence of O_2, ethylene synthesis is diminished, but the loss of ethylene by diffusion is retarded under water. Sufficient oxygen for growth and ethylene synthesis in the underwater parts is usually provided by aerenchyma tissue (see Chapter 26).

Ethylene stimulates internode elongation in deep-water rice by increasing the amount of, and the sensitivity to, gibberellin in the cells of the intercalary meristem. The increased sensitivity to gibberellic acid (GA) in these cells in response to ethylene is brought about by a decrease in the level of abscisic acid (ABA), a potent antagonist of GA.

Two genes encoding transcription factors in the ethylene response factor family, SNORKEL1 and SNORKEL2, have recently been identified in deep-water rice that mediate this response (Hattori et al. 2009). In flooding conditions, ethylene accumulates and induces the expression of SNORKEL1 and SNORKEL2, which then triggers the dramatic internode elongation.

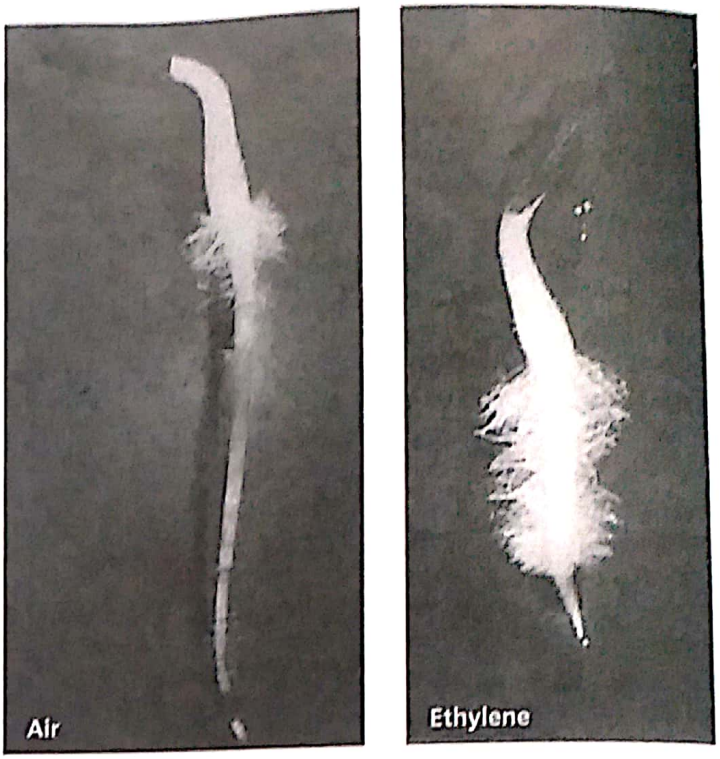


FIGURE 22.16 Promotion of root hair formation by ethylene in lettuce seedlings. Two-day-old seedlings were treated with air (left) or 10 ppm ethylene (right) for 24 hours before the photo was taken. Note the profusion of root hairs on the ethylene-treated seedling. (From Abeles et al. 1992, courtesy of F. Abeles.)

Ethylene induces the formation of roots and root hairs

Ethylene is capable of inducing adventitious root formation in leaves, stems, flower stems, and even other roots. Vegetative stem cuttings from tomato and petunia make many adventitious roots in response to applied auxin, but in ethylene-insensitive mutants auxin has little or no effect, indicating that the promotive effect of auxin on adventitious rooting is mediated by ethylene (Clark et al. 1999). Ethylene also plays a role in the morphogenesis of crown gall tissue (see **WEB ESSAY 22.1**), and is a negative regulator of root nodule formation in legumes (see **WEB TOPIC 22.10**).

Ethylene has also been shown to act as a positive regulator of root hair formation in several species (**FIGURE 22.16**). This regulation has been best studied in Arabidopsis, in which root hairs normally are located in the epidermal cells that overlie a junction between the underlying cortical cells (Dolan et al. 1994). In ethylene-treated roots, cells not overlying a cortical cell junction differentiate into hair cells, and produce root hairs in abnormal locations (Tanimoto et al. 1995). Seedlings grown in the presence of ethylene inhibitors (such as Ag^+), as well as ethylene-insensitive mutants, display a reduction in root hair formation. These observations suggest that ethylene acts as a positive regulator in the differentiation of root hairs.

Ethylene regulates flowering and sex determination in some species

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially for synchronization of pineapple fruit set. Flowering of other species, such as mango, is also initiated by ethylene. On plants that have separate male and female flowers (monoecious species), ethylene may change the sex of developing flowers. The promotion of female flower formation in cucumber is one example of this effect. Recently, a gene responsible for andromonoecy (plants carrying both male and bisexual flowers) in melons was identified as encoding an ACC synthase (Boualem et al. 2008). A mutation that reduces the activity of this ACC synthase gene results in the formation of the bisexual flowers in these andromonoecious lines.

Ethylene enhances the rate of leaf senescence

As described in Chapter 16, senescence is a genetically programmed developmental process that affects all tissues of the plant. Research has provided several lines of physiological evidence that support roles for ethylene and cytokinins in the control of leaf senescence:

- Exogenous applications of ethylene or ACC (the precursor of ethylene) accelerate leaf senescence, and treatment with exogenous cytokinins delays leaf senescence (see Chapter 21).
- Enhanced ethylene production is associated with chlorophyll loss and color fading, which are characteristic features of leaf and flower senescence; an inverse correlation has been found between cytokinin levels in leaves and the onset of senescence.
- Inhibitors of ethylene synthesis (e.g., AVG or Co^{2+}) and action (e.g., Ag^+ or CO_2) retard leaf and flower senescence (**FIGURE 22.17**).

Taken together, these physiological studies suggest that senescence is regulated by the balance of ethylene and cytokinin. In addition, abscisic acid has been implicated in the control of leaf senescence. The role of ABA in senescence will be discussed in Chapter 23.

Direct evidence for the involvement of ethylene in the regulation of leaf senescence has come from molecular genetic studies on Arabidopsis. As discussed above, ethylene-insensitive mutants, such as ***etr1*** (*e*thylene-*r*esistant *1*) and ***ein2*** (*e*thylene-*in*sensitive *2*), were identified by their failure to respond to ethylene. Consistent with a role for ethylene in leaf senescence, both *etr1* and *ein2* plants

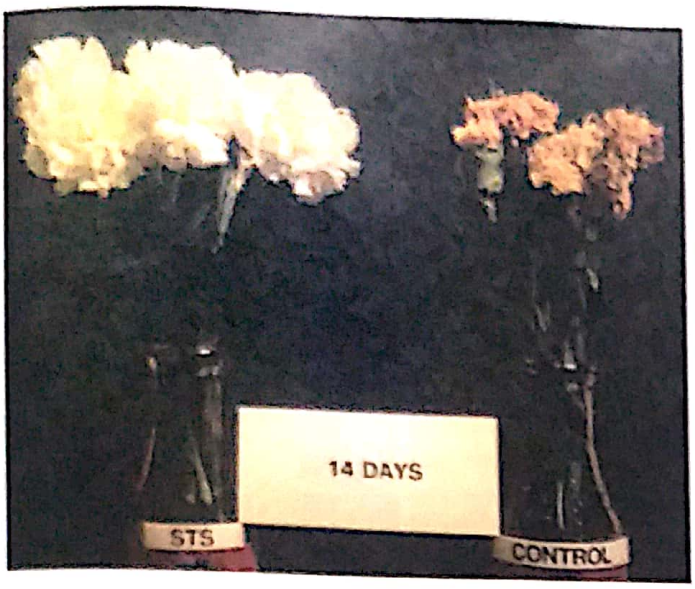


FIGURE 22.17 Inhibition of flower senescence by inhibition of ethylene action. Carnation flowers were held in deionized water for 14 days with (left) or without (right) silver thiosulfate (STS), a potent inhibitor of ethylene action. Blocking of ethylene action results in a marked inhibition of floral senescence. (From Reid 1995, courtesy of M. Reid.)

were found to be affected not only during the early stages of germination, but throughout the life cycle, including senescence (Zacarias and Reid 1990; Hensel et al. 1993; Grbi and Bleecker 1995). The ethylene mutants retained their chlorophyll and other chloroplast components for a longer period of time compared to the wild type. However, because the total life spans of these mutants were increased by only 30% over that of the wild type, ethylene appears to increase the *rate* of senescence, rather than acting as a developmental switch that initiates the senescence process (see WEB TOPIC 22.11).

Ethylene mediates some defense responses

Pathogen infection and disease will occur only if the interactions between host and pathogen are genetically compatible. However, ethylene production generally increases in response to pathogen attack in both compatible (i.e., pathogenic) and noncompatible (nonpathogenic) interactions.

The discovery of ethylene-insensitive mutants has facilitated the assessment of the role of ethylene in the response to various pathogens. The involvement of ethylene in pathogenesis is complex and depends on the particular host–pathogen interaction. For example, blocking ethylene responsiveness does not affect the resistance responses of Arabidopsis to *Pseudomonas* bacteria or of tobacco to tobacco mosaic virus. In compatible interactions of these pathogens and hosts, however, elimination of ethylene responsiveness prevents the development of disease symptoms, even though the growth of the pathogen appears to be unaffected.

On the other hand, ethylene, in combination with the plant hormone jasmonic acid (see Chapter 13), is required for the activation of several plant defense genes. In addition, ethylene-insensitive tobacco and Arabidopsis mutants are susceptible to several necrotrophic (growing on dead host tissue) soil fungi that are normally not pathogenic. Thus ethylene, in combination with jasmonic acid, plays an important role in plant defense against necrotrophic pathogens. On the other hand, ethylene does not appear to play a major role in the response of plants to biotrophic (growing on living tissue) pathogens.

Ethylene acts on the abscission layer

The shedding of leaves, fruits, flowers, and other plant organs is termed **abscission** (see WEB TOPIC 22.12). Abscission takes place in specific layers of cells called **abscission layers**, which become morphologically and biochemically differentiated during organ development. Weakening of the cell walls at the abscission layer depends on cell wall–degrading enzymes such as cellulase and polygalacturonase (FIGURE 22.18).

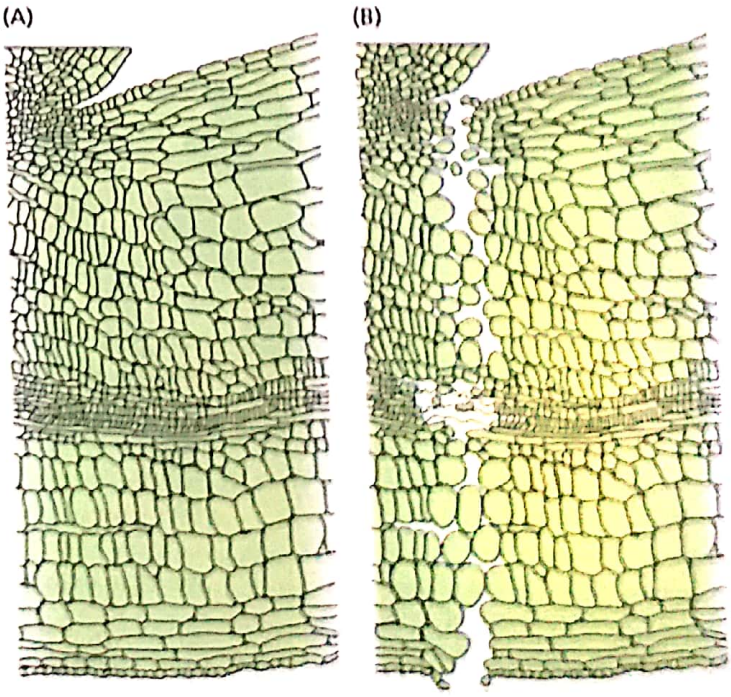


FIGURE 22.18 Formation of the abscission layer of jewelweed (*Impatiens*). (A) During leaf abscission, two or three rows of cells in the abscission zone undergo cell wall breakdown because of an increase in cell wall–hydrolyzing enzymes. (B) The protoplasts, released from the restraint of their cell walls, expand and push apart the xylem tracheary cells, facilitating the separation of the leaf from the stem. (After Sexton et al. 1984.)

FIGURE 22.19 Effect of ethylene on abscission in birch (*Betula pendula*). The plant on the left is the wild type; the plant on the right was transformed with a mutated version of the Arabidopsis ethylene receptor *etr1*. The expression of this gene was under the transcriptional control of its own promoter. One of the characteristics of these mutant trees is that they do not drop their leaves when fumigated for 3 days with 50 ppm ethylene. (From Vahala et al. 2003.)

The ability of ethylene gas to cause defoliation in birch trees is shown in **FIGURE 22.19**. Both trees have been subjected to 3 days of fumigation with 50 ppm ethylene. The wild-type tree on the left has lost most of its leaves. The tree on the right has been transformed with a gene for the Arabidopsis ethylene receptor, ETR1, which carries the dominant *etr1* mutation (discussed earlier). This tree is unable to respond to ethylene and therefore does not shed its leaves after ethylene treatment.

Ethylene appears to be the primary regulator of the abscission process, with auxin acting as a suppressor of the ethylene effect (see Chapter 19). However, supraoptimal auxin concentrations stimulate ethylene production, which has led to the use of auxin analogs as defoliants. For example, 2,4,5-T, the active ingredient in Agent Orange, was widely used as a defoliant during the Vietnam War. Its action is based on its ability to increase ethylene biosynthesis, thereby stimulating leaf abscission.

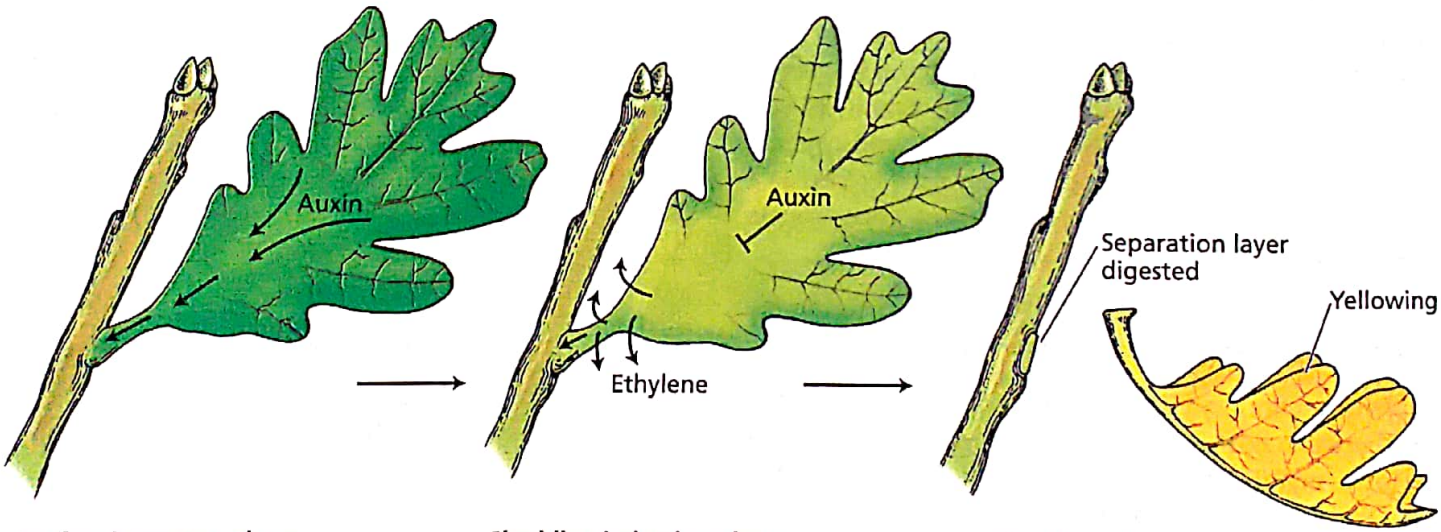


FIGURE 22.20 Schematic view of the roles of auxin and ethylene during leaf abscission. In the shedding induction phase, the level of auxin decreases, and the level of ethylene increases. These changes in the hormonal balance increase the sensitivity of the target cells to ethylene. (After Morgan 1984.)

A model of the hormonal control of leaf abscission describes the process in three distinct sequential phases (**FIGURE 22.20**) (Reid 1995):

1. *Leaf maintenance phase.* Prior to the perception of any signal (internal or external) that initiates the abscission process, the leaf remains healthy and fully functional in the plant. A gradient of auxin from the blade to the stem maintains the abscission zone in a nonsensitive state.
2. *Shedding induction phase.* A reduction or reversal in the auxin gradient from the leaf, normally associated with leaf senescence, causes the abscission zone to become sensitive to ethylene. Treatments that enhance leaf senescence may promote abscission by interfering with auxin synthesis and/or transport in the leaf.
3. *Shedding phase.* The sensitized cells of the abscission zone respond to low concentrations of endogenous ethylene by synthesizing and secreting cellulase and other cell wall–degrading enzymes, resulting in shedding.

During the early phase of leaf maintenance, auxin from the leaf prevents abscission by maintaining the cells of the abscission zone in an ethylene-insensitive state. It has long been known that removal of the leaf blade (the site of auxin production) promotes petiole abscission. Application of exogenous auxin to petioles from which the leaf blade has been removed delays the abscission process. However, application of auxin to the proximal side of the abscission zone (i.e., the side closest to the stem) actually accelerates the abscission process. These results indicate that it is not the absolute amount of auxin at the abscission zone, but rather the auxin *gradient*, that controls the ethylene sensitivity of these cells.

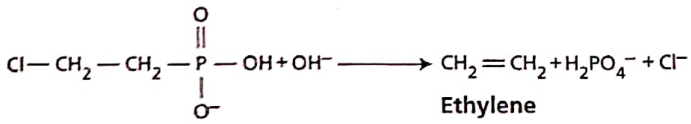
In the shedding induction phase, the amount of auxin from the leaf decreases and the ethylene level rises. Ethylene appears to decrease the activity of auxin both by reducing its synthesis and transport and by increasing its destruction. The reduction in the concentration of free auxin increases the response of specific target cells to ethylene. The shedding phase is characterized by the induction of genes encoding specific hydrolytic enzymes of cell wall polysaccharides and proteins.

The target cells, located in the abscission zone, synthesize cellulase and other polysaccharide-degrading enzymes, and secrete them into the cell wall. The activities of these enzymes lead to cell wall loosening, cell separation, and abscission.

Ethylene has important commercial uses

Because ethylene regulates so many physiological processes in plant development, it is one of the most widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice. Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be overcome if an ethylene-releasing compound is used. The most widely used such compound is Ethephon, or 2-chloroethylphosphonic acid, which was discovered in the 1960s and is known by various trade names, such as Ethrel.

Ethephon is sprayed in aqueous solution and is readily absorbed and transported within the plant. It releases ethylene slowly by a chemical reaction, allowing the hormone to exert its effects:



2-Chloroethylphosphonic acid (Ethephon)

Ethephon hastens fruit ripening of apple and tomato and degreening of citrus fruits, synchronizes flowering and fruit set in pineapple, and accelerates abscission of flowers and fruits. It can be used to induce fruit thinning or fruit drop in cotton, cherry, and walnut. It is also used to promote female sex expression in cucumber, to prevent self-pollination and increase yield, and to inhibit terminal growth of some plants in order to promote lateral growth and compact flowering stems.

Storage facilities developed to inhibit ethylene production and promote preservation of fruits have a controlled atmosphere of low O_2 concentration and low temperature for the inhibition of ethylene biosynthesis. A relatively high concentration of CO_2 (3 to 5%) prevents ethylene's action as a ripening promoter. Low pressure (vacuum) is used to remove ethylene and oxygen from the storage chambers, reducing the rate of ripening and preventing overripening. The ethylene binding inhibitor Ethylbloc® is increasingly being used to extend the shelf life of various climacteric fruits.

It is estimated that from 15 to 35% of the cut flowers harvested in the U.S. are lost because of postharvest spoilage. Specific inhibitors of ethylene biosynthesis and action have proven useful in postharvest preservation (see **WEB TOPIC 22.13**).

SUMMARY

Ethylene regulates fruit ripening and processes associated with leaf and flower senescence and abscission, root hair development and nodulation, and seedling growth and hook opening, and does so at least in part by altering gene expression.

Structure, Biosynthesis, and Measurement of Ethylene

- Ethylene gas induces the triple response in dicots (**Figures 22.1, 22.5**).
- The ethylene precursor is methionine, which is converted sequentially to *S*-adenosylmethionine, ACC, and ethylene (**Figure 22.2**). Ethylene acts near its site of synthesis. The immediate precursor of ethylene, ACC can be transported and thus can produce ethylene at a site distant from its synthesis.
- Ethylene biosynthesis is stimulated by several factors, including developmental state, environmental conditions, other plant hormones, and physical and chemical stimuli (**Figure 22.3**).
- The biosynthesis and perception of ethylene can be antagonized by inhibitors, some of which have commercial applications (**Figure 22.4**).

Ethylene Signal Transduction Pathways

- The triple-response morphology of etiolated Arabidopsis seedlings has aided identification of functionally redundant ethylene receptor genes and other signaling elements (**Figures 22.5–22.7**).
- Ethylene receptors are located on the endoplasmic reticulum, and also on the Golgi apparatus.
- Ethylene binds to its receptor via a copper cofactor.
- Unbound receptors actively shut off the signaling pathway that leads to the response; binding of ethylene inactivates the receptors, allowing the response pathway to proceed (**Figures 22.8, 22.9**).
- ETR1 activates CTR1, a protein kinase that shuts off ethylene responses.

Ethylene Regulation of Gene Expression

- Ethylene affects the transcription of numerous genes via specific transcription factors.
- Analysis of epistatic interactions revealed the sequence of action for the genes *ETR1*, *EIN2*, *EIN3*, and *CTR1* (**Figure 22.10**).

Developmental and Physiological Effects of Ethylene

- Ethylene is involved in seedling growth and fruit ripening (**Figure 22.11, Table 22.1**), epinasty (**Figures 22.12, 22.13**), and seed germination.
- The hormone influences cell expansion and the orientation of the cellulose microfibrils in the cell wall (**Figure 22.14**).
- There are two mechanistically distinct phases to ethylene inhibition of hypocotyl elongation (**Figure 22.15**).
- Ethylene stimulates rapid internode or petiole elongation when some species are submerged. The hormone regulates flowering, sex determination, and defense responses in some species.
- Ethylene stimulates root hair formation (**Figure 22.16**).
- Ethylene is active in leaf and flower senescence and in leaf abscission (**Figures 22.17–22.20**).

WEB MATERIAL

Web Topics

22.1 Ethylene in the Environment Arises Biotically and Abiotically

Ethylene in the environment arises from a variety of sources, including pollution, photochemical reactions in the atmosphere, and production by microbes, algae, and plants.

22.2 Ethylene Readily Undergoes Oxidation

Ethylene can be oxidized to ethylene oxide, which can then be hydrolyzed to ethylene glycol.

22.3 Ethylene Can Be Measured by Gas Chromatography

Historically, bioassays based on the seedling triple response were used to measure ethylene levels, but they have been replaced by gas chromatography.

22.4 Cloning of the Gene That Encodes ACC Synthase

A brief description of the cloning of the gene for ACC synthase using antibodies raised against the partially purified protein.

WEB MATERIAL continued

Web Topics

22.5 Cloning of the Gene That Encodes ACC Oxidase
The ACC oxidase gene was cloned by a circuitous route using antisense DNA.

22.6 Ethylene Binding to ETR1 and Seedling Response to Ethylene
Ethylene binding to its receptor ETR1 was first demonstrated by expressing the gene in yeast.

22.7 Conservation of Ethylene Signaling Components in Other Plant Species
The evidence suggests that ethylene signaling is similar in all plant species.

22.8 ACC Synthase Gene Expression and Biotechnology
A discussion of the use of the ACC synthase gene in biotechnology.

22.9 The *hookless* Mutation Alters the Pattern of Auxin Gene Expression
The *hookless* mutation of Arabidopsis confirms the interaction between auxin and ethylene in hook formation.

22.10 Ethylene Inhibits the Formation of Nitrogen-Fixing Root Nodules in Legumes
Hyper-nodulating mutants are blocked in the ethylene signal transduction pathway.

22.11 Ethylene Biosynthesis Can Be Blocked with Anti-Sense DNA
Mutants expressing anti-sense DNA coding for ethylene biosynthesis enzymes have delayed leaf senescence and fruit ripening.

22.12 Abscission and the Dawn of Agriculture
A short essay on the domestication of modern cereals based on artificial selection for nonshattering rachises.

22.13 Specific Inhibitors of Ethylene Biosynthesis Are Used Commercially to Preserve Cut Flowers
Some inhibitors of ethylene biosynthesis are suitable for commercial use in flower preservation.

Web Essay

22.1 Tumor-Induced Ethylene Controls Crown Gall Morphogenesis
Agrobacterium tumefaciens-induced galls produce very high ethylene concentrations, which reduce vessel diameter in the host stem adjacent to the tumor and enlarge the gall surface giving priority in water supply to the growing tumor over the host shoot.

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