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.

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

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. Work on ethylene was also 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 discovery of the ethylene biosynthetic pathway and outline some of the important effects of ethylene on plant growth and development. At the end of the chapter we will consider how ethylene acts at the cellular and molecular levels.

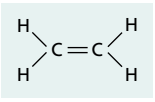
STRUCTURE, BIOSYNTHESIS, AND MEASUREMENT OF ETHYLENE

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. In general, meristematic regions and nodal regions are the most active in ethylene biosynthesis. However, ethylene production also 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, chilling, disease, and temperature or drought stress.

The amino acid methionine is the precursor of ethylene, and ACC (1-aminocyclopropane-1-carboxylic acid) 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.

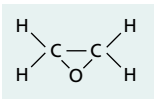
The Properties of Ethylene Are Deceptively Simple

Ethylene is the simplest known olefin (its molecular weight is 28), and it is lighter than air under physiological conditions:



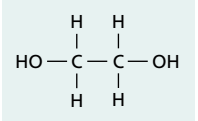
Ethylene

It is flammable and readily undergoes oxidation. Ethylene can be oxidized to ethylene oxide:



Ethylene oxide

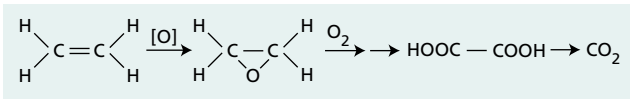
and ethylene oxide can be hydrolyzed to ethylene glycol:



Ethylene glycol

In most plant tissues, ethylene can be completely oxidized to CO_2, in the following reaction:

Complete oxidation of ethylene



Ethylene **Ethylene oxide** **Oxalic acid** **Carbon dioxide**

Ethylene is released easily from the tissue and diffuses in the gas phase through the intercellular spaces and outside the tissue. At an ethylene concentration of 1 μL L^{-1} in the gas phase at 25°C, the concentration of ethylene in water is 4.4×10^{-9} *M*. Because they are easier to measure, gas phase concentrations are normally given for ethylene.

Because ethylene gas is easily lost from the tissue 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 μL L^{-1}, markedly extending the storage life of the fruit.

Bacteria, Fungi, and Plant Organs Produce Ethylene

Even away from cities and industrial air pollutants, the environment is seldom free of ethylene because of its production by plants and microorganisms. The production of ethylene in plants is highest in senescing tissues and ripening fruits (>1.0 nL g-fresh-weight^{-1} h^{-1}), but all organs of higher plants can synthesize ethylene. Ethylene is biologically active at very low concentrations—less than 1 part per million (1 μL L^{-1}). The internal ethylene concentration in a ripe apple has been reported to be as high as 2500 μL L^{-1}.

Young developing leaves produce more ethylene than do fully expanded leaves. In bean (*Phaseolus vulgaris*), young leaves produce 0.4 nL g^{-1} h^{-1}, compared with 0.04 nL g^{-1} h^{-1} for older leaves. With few exceptions, nonsenescent tissues that are wounded or mechanically perturbed will temporarily increase their ethylene production severalfold within 30 minutes. Ethylene levels later return to normal.

Gymnosperms and lower plants, including ferns, mosses, liverworts, and certain cyanobacteria, all have shown the ability to produce ethylene. Ethylene production by fungi and bacteria contributes significantly to the ethylene content of soil. Certain strains of the common enteric bacterium *Escherichia coli* and of yeast (a fungus) produce large amounts of ethylene from methionine.

There is no evidence that healthy mammalian tissues produce ethylene, nor does ethylene appear to be a metabolic product of invertebrates. However, recently it was found that both a marine sponge and cultured mammalian

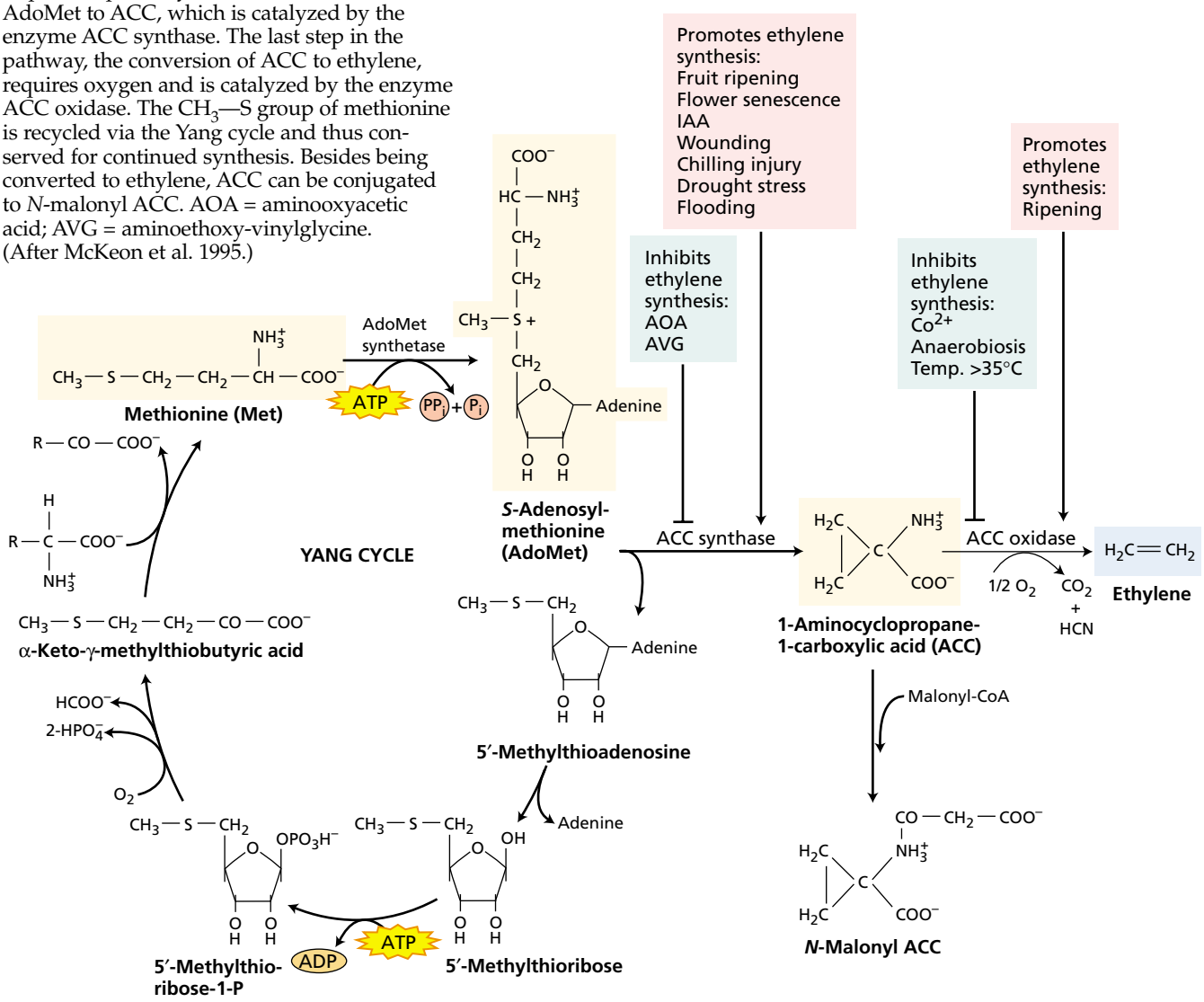


FIGURE 22.1 Ethylene biosynthetic pathway and the Yang cycle. The amino acid methionine is the precursor of ethylene. The rate-limiting step in the pathway is the conversion of AdoMet to ACC, which is catalyzed by the enzyme ACC synthase. The last step in the pathway, the conversion of ACC to ethylene, 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.)

cells can respond to ethylene, raising the possibility that this gaseous molecule acts as a signaling molecule in animal cells (Perovic et al. 2001).

Regulated Biosynthesis Determines the Physiological Activity of Ethylene

In vivo experiments showed that plant tissues convert l-[^{14}C]methionine to [^{14}C]ethylene, and that the ethylene is derived from carbons 3 and 4 of methionine (Figure 22.1). The CH_3—S group of methionine is recycled via the Yang cycle. Without this recycling, the amount of reduced sulfur present would limit the available methionine and the synthesis of ethylene. *S*-adenosylmethionine (AdoMet), which is synthesized from methionine and ATP, is an intermediate in the ethylene biosynthetic pathway, and the immediate precursor of ethylene is **1-aminocyclopropane-1-carboxylic acid** (**ACC**) (see Figure 22.1).

The role of ACC became evident in experiments in which plants were treated with [^{14}C]methionine. Under anaerobic conditions, ethylene was not produced from the [^{14}C]methionine, and labeled ACC accumulated in the tissue. On exposure to oxygen, however, ethylene production surged. The labeled ACC was rapidly converted to ethylene in the presence of oxygen by various plant tissues, suggesting that ACC is the immediate precursor of ethylene in higher plants and that oxygen is required for the conversion.

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 biosynthetic step that limits ethylene production in plant tissues.

ACC synthase, the enzyme that catalyzes the conversion of AdoMet to ACC (see Figure 22.1), has been characterized in many types of tissues of various plants. ACC synthase is an unstable, cytosolic enzyme. Its level is regulated by environmental and internal factors, such as wounding, drought stress, flooding, and auxin. Because ACC synthase is present in such low amounts in plant tissues (0.0001% of the total protein of ripe tomato) and is very unstable, it is difficult to purify the enzyme for biochemical analysis (see **Web Topic 22.1**).

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 nine ACC synthase genes, different subsets of which are induced by auxin, wounding, and/or fruit ripening.

ACC oxidase catalyzes the last step in ethylene biosynthesis: the conversion of ACC to ethylene (see Figure 22.1). 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. The gene that encodes ACC oxidase has been cloned (see **Web Topic 22.2**). Like ACC synthase, ACC oxidase is encoded by a multigene family that is differentially regulated. For example, in ripening tomato fruits and senescing petunia flowers, the mRNA levels of a subset of ACC oxidase genes are highly elevated.

The deduced amino acid sequences of ACC oxidases revealed that these enzymes belong to the Fe^{2+}/ascorbate oxidase superfamily. This similarity suggested that ACC oxidase might require Fe^{2+} and ascorbate for activity—a requirement that has been confirmed by biochemical analysis of the protein. The low abundance of ACC oxidase and its requirement for cofactors presumably explain why the purification of this enzyme eluded researchers for so many years.

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 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 22.1), which does not appear to break down and accumulates in the tissue. A second 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.

Environmental Stresses and Auxins Promote Ethylene Biosynthesis

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 rate of ACC and ethylene biosynthesis increases. Enzyme activities for both ACC oxidase (Figure 22.2) 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).

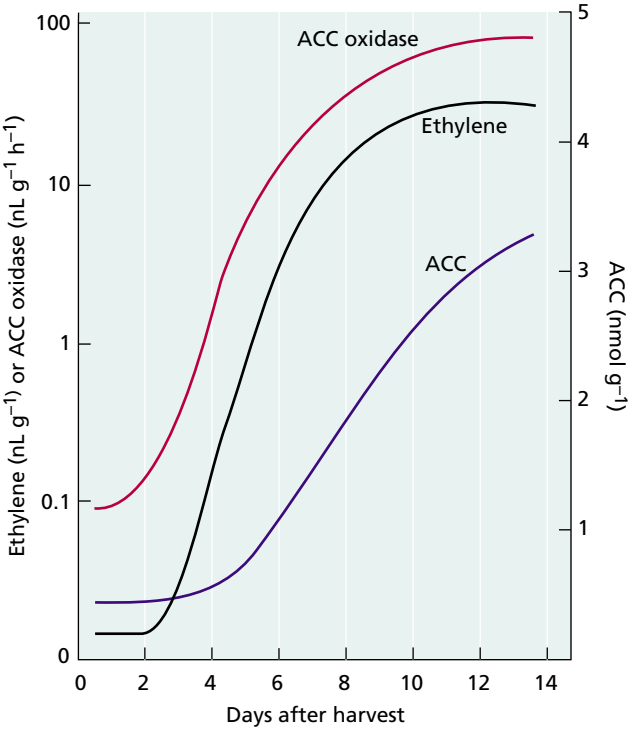


FIGURE 22.2 Changes in ethylene and ACC content and ACC oxidase activity during fruit ripening. Changes in the ACC oxidase activity and ethylene and ACC concentrations of Golden Delicious apples. The data are plotted as a function of days after harvest. Increases in ethylene and ACC concentrations and in ACC oxidase activity are closely correlated with ripening. (A from Hoffman and Yang 1980; B from Yang 1987.)

Stress-induced ethylene production. Ethylene biosynthesis is increased by stress conditions such as drought, flooding, chilling, exposure to ozone, or 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 25).

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.

Inhibitors of protein synthesis block both ACC and IAA-induced ethylene synthesis, indicating that the synthesis of new ACC synthase protein caused by auxins brings about the marked increase in ethylene production. Several ACC synthase genes have been identified whose transcription is elevated following application of exogenous IAA, suggesting that increased transcription is at least partly responsible for the increased ethylene production observed in response to auxin (Nakagawa et al. 1991; Liang et al. 1992).

Posttranscriptional regulation of ethylene production. Ethylene production can also be regulated posttranscriptionally. Cytokinins also promote ethylene biosynthesis in some plant tissues. For example, in etiolated *Arabidopsis* seedlings, application of exogenous cytokinins causes a rise in ethylene production, resulting in the triple-response phenotype (see Figure 22.5A).

Molecular genetic studies in *Arabidopsis* have shown that cytokinins elevate ethylene biosynthesis by increasing the stability and/or activity of one isoform of ACC synthase (Vogel et al. 1998). The carboxy-terminal domain of this ACC synthase isoform appears to be the target for this posttranscriptional regulation. Consistent with this, the carboxy-terminal domain of an ACC synthase isoform from tomato has been shown to be the target for a calcium-dependent phosphorylation (Tatsuki and Mori 2001).

Ethylene Production and Action Can Be Inhibited

Inhibitors of hormone synthesis or action are valuable for the study of the biosynthetic pathways and physiological roles of hormones. Inhibitors are 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.

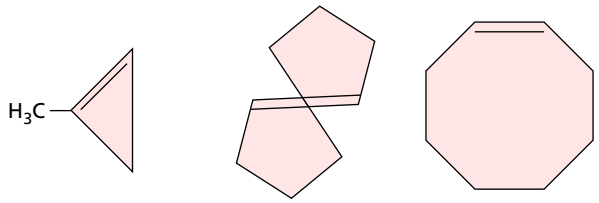
For example, ethylene mimics high concentrations of auxins by inhibiting stem growth and causing epinasty (a downward curvature of leaves). Use of specific inhibitors of ethylene biosynthesis and action made it possible to discriminate between the actions of auxin and ethylene. Studies using inhibitors 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. **Aminoethoxy-vinylglycine** (**AVG**) and **aminooxyacetic acid** (**AOA**) block the conversion of AdoMet to ACC (see Figure 22.1). AVG and AOA are known to inhibit enzymes that use the cofactor pyridoxal phosphate. The cobalt ion (Co^{2+}) is also an inhibitor of 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. A novel inhibitor, **1-methylcyclopropene** (**MCP**), was recently found that binds almost irreversibly to the ethylene receptor (Figure 22.3) (Sisler and Serek 1997). MCP shows tremendous promise in commercial applications.



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

FIGURE 22.3 Inhibitors that block ethylene binding to its receptor. Only the *trans* form of cyclooctene is active.

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**. As little as 5 parts per billion (ppb) (5 pL per liter)[1] of ethylene can be detected, and the analysis time is only 1 to 5 minutes.

Usually the ethylene produced by a plant tissue is allowed to accumulate in a sealed vial, and a sample is withdrawn with a syringe. The sample is injected into a gas chromatograph column in which the different gases are separated and detected by a flame ionization detector. Quantification of ethylene by this method is very accurate. Recently a novel method to measure ethylene was developed that uses a laser-driven photoacoustic detector that can detect as little as 50 parts per trillion (50 ppt = 0.05 pL L^{-1}) ethylene (Voesenek et al. 1997).

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.

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.

[1] pL = picoliter = 10^{-12} L.

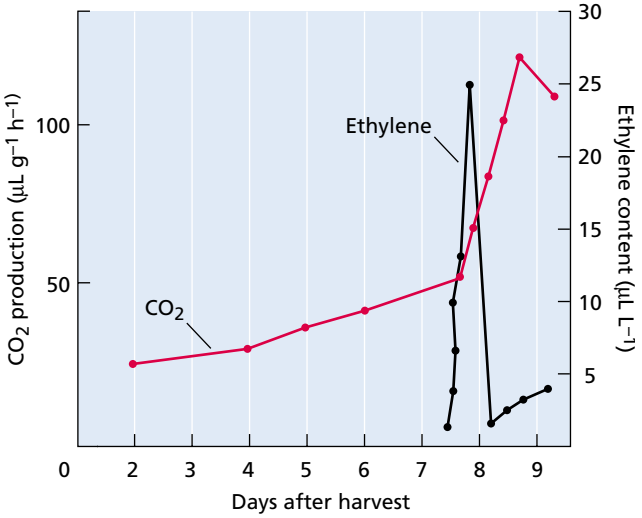


FIGURE 22.4 Ethylene production and respiration. In banana, ripening is characterized by a climacteric rise in respiration rate, as evidenced by the increased CO_2 production. A climacteric rise in ethylene production precedes the increase in CO_2 production, suggesting that ethylene is the hormone that triggers the ripening process. (From Burg and Burg 1965.)

All fruits that ripen in response to ethylene exhibit a characteristic respiratory rise before the ripening phase called a **climacteric**.[2] Such fruits also show a spike of ethylene production immediately before the respiratory rise (Figure 22.4). Inasmuch as treatment with ethylene induces the fruit to produce additional ethylene, its action can be described as **autocatalytic**. 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.

When unripe climacteric fruits are treated with ethylene, the onset of the climacteric rise is hastened. When nonclimacteric fruits are treated in the same way, the magnitude of the respiratory rise 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 biosynthe-

[2] 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.

TABLE 22.1
Climacteric and nonclimacteric fruits

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

sis (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 22.3**). Elimination of ethylene biosynthesis in these transgenic tomatoes completely blocked fruit ripening, and ripening was restored by application of exogenous ethylene (Oeller et al. 1991).

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). These experiments provided unequivocal proof of the role of ethylene in fruit ripening, and they opened the door to the manipulation of fruit ripening through biotechnology.

In tomatoes several genes have been identified that are highly regulated during ripening (Gray et al. 1994). During tomato fruit ripening, the fruit softens as the result of cell wall hydrolysis and 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 tomato fruits from 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 lycopene and aroma biosynthesis, respiratory metabolism, and ACC synthase.
2. *A developmental, ethylene-independent pathway* includes genes encoding 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 22.5B). Ethylene 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. There is no known physiological function for the response.

In tomato and other dicots, flooding (waterlogging) or anaerobic conditions around the roots enhances 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 after flooding of tomato roots for 1 to 2 days (Figure 22.6) (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. The elevated production of ethylene appears to be caused by the accumulation of ACC in the roots under anaerobic conditions, since the conversion of ACC to ethylene requires oxygen (see Figure 22.1). The ACC accumulated in the anaerobic roots is then transported to shoots via the transpiration stream, where it is readily converted to ethylene.

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 region below the hook. These effects of ethylene are common to growing shoots of most dicots, forming part of the **triple response**. In *Arabidopsis*, the triple response consists of inhibition and swelling of the hypocotyl, inhibition of root elongation, and exaggeration of the apical hook (Figure 22.7).

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 in turn is 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.

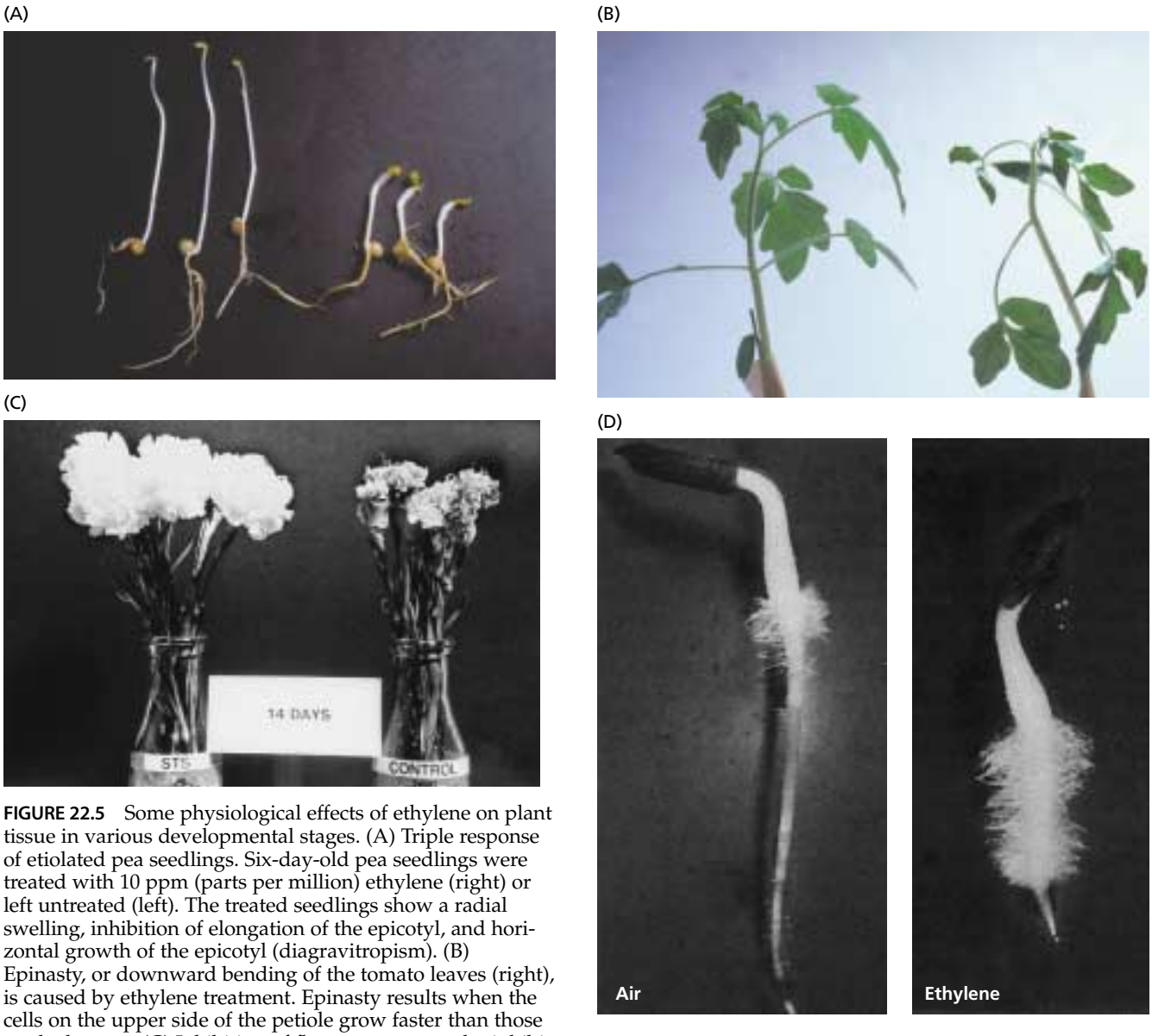


FIGURE 22.5 Some physiological effects of ethylene on plant tissue in various developmental stages. (A) Triple response of etiolated pea seedlings. Six-day-old pea seedlings were treated with 10 ppm (parts per million) ethylene (right) or left untreated (left). The treated seedlings show a radial swelling, inhibition of elongation of the epicotyl, and horizontal growth of the epicotyl (diagravitropism). (B) 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. (C) 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 results in a marked inhibition of floral senescence. (D) 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. (A and B courtesy of S. Gepstein; C from Reid 1995, courtesy of M. Reid; D from Abeles et al. 1992, courtesy of F. Abeles.)

During the seedling triple response to ethylene, 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

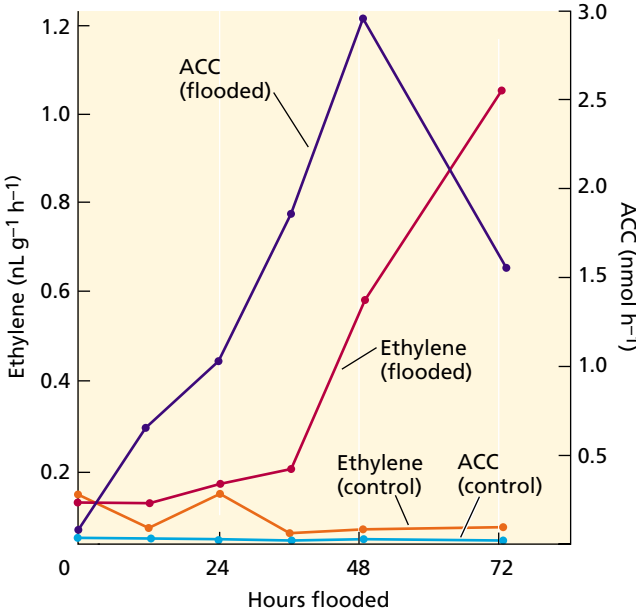


FIGURE 22.6 Changes in the amounts of ACC in the xylem sap and ethylene production in the petiole following flooding of tomato plants. ACC is synthesized in roots, but it is converted to ethylene very slowly under anaerobic conditions of flooding. ACC is transported via the xylem to the shoot, where it is converted to ethylene. The gaseous ethylene cannot be transported, so it usually affects the tissue near the site of its production. The ethylene precursor ACC is transportable and can produce ethylene far from the site of ACC synthesis. (From Bradford and Yang 1980.)

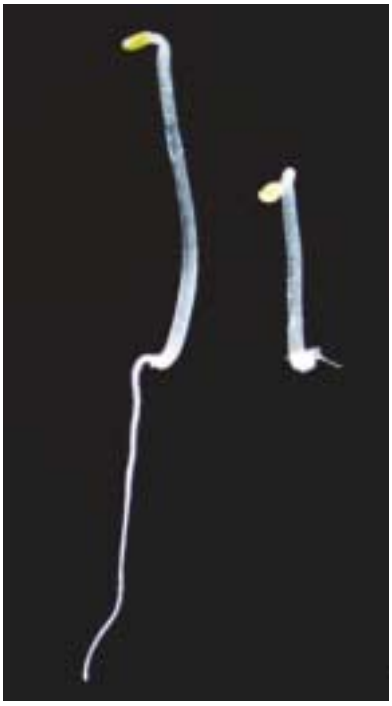
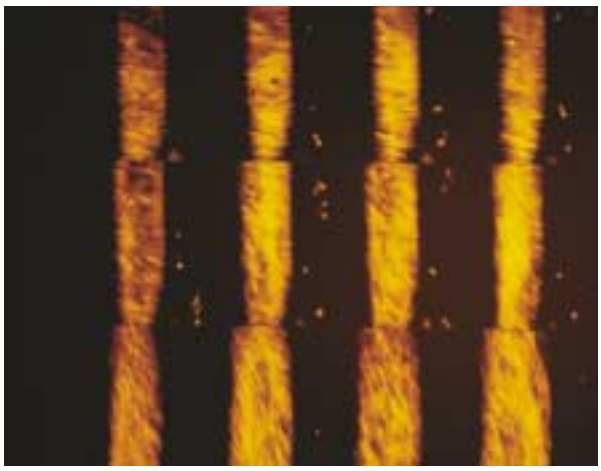


FIGURE 22.7 The triple response in *Arabidopsis*. Three-day-old etiolated seedlings grown in the presence (right) or absence (left) of 10 ppm ethylene. Note the shortened hypocotyl, reduced root elongation and exaggeration of the curvature of the apical hook that results from the presence of ethylene.

aligned microtubules appear in particular locations (Figure 22.8). Neighboring microtubules then adopt the new alignment, so at one stage different alignments coexist before they adopt a uniformly longitudinal orientation (Yuan et al., 1994). Although the reorientations observed in this study were spontaneous rather than induced by ethylene, it is presumed that ethylene-induced microtubule reorientation operates by a similar mechanism.



Transverse microtubules

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 Figure 22.7). 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

FIGURE 22.8 Reorientation of microtubules from transverse to vertical in pea stem epidermis 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.)

increases, equalizing the growth rates on both sides. The kinematic aspects of hook growth (i.e., maintenance of the hook shape over time) were discussed in Chapter 16.

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* and treatment of wild-type seedlings with NPA (*1*-N-naphthylphthalamic acid), an inhibitor of polar auxin transport, both block the formation of the apical hook in *Arabidopsis*. 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).

A gene required for formation of the apical hook, *HOOKLESS1* (so called because mutations in this gene result in seedlings lacking an apical hook), was identified in *Arabidopsis* (Lehman et al. 1996). Disruption of this gene severely alters the pattern of expression of auxin-responsive genes. When the gene is overexpressed in *Arabidopsis*, it causes constitutive hook formation even in the light. *HOOKLESS1* encodes a putative *N*-acetyltransferase that is hypothesized to regulate—by an unknown mechanism—differential auxin distribution in the apical hook induced by ethylene.

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 the cereal deepwater rice (see Chapter 20).

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.

As we saw in Chapter 20, in deepwater rice it has been shown that ethylene stimulates internode elongation by increasing the amount of, and the sensitivity to, gibberellin in the cells of the intercalary meristem. The increased sensitivity to GA (gibberellic acid) 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.

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. Ethylene has also been shown to act as a positive regulator of root hair formation in several species (see Figure 22.5D). This relationship 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, extra hairs form in abnormal locations in the epidermis; that is, cells not overlying a cortical cell junction differentiate into hair cells (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 in response to ethylene. These observations suggest that ethylene acts as a positive regulator in the differentiation of root hairs.

Ethylene Induces Flowering in the Pineapple Family

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially in pineapple for synchronization of 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 (see Chapter 24). The promotion of female flower formation in cucumber is one example of this effect.

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. Several lines of physiological evidence 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 (see Figure 22.5C); 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 senescence.

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

Senescence in ethylene mutants. Direct evidence for the involvement of ethylene in the regulation of leaf senescence has come from molecular genetic studies on *Arabidopsis*. As will be discussed later in the chapter, several mutants affecting the response to ethylene have been identified. The specific bioassay employed was the triple-response assay in which ethylene significantly inhibits seedling hypocotyl elongation and promotes lateral expansion.

Ethylene-insensitive mutants, such as *etr1* (*e*thylene-*r*esistant *1*) and *ein2* (*e*thylene-*i*nsensitive *2*), were identified by their failure to respond to ethylene (as will be described later in the chapter). The *etr1* mutant is unable to perceive the ethylene signal because of a mutation in the gene that codes for the ethylene receptor protein; the *ein2* mutant is blocked at a later step in the signal transduction pathway.

Consistent with a role for ethylene in leaf senescence, both *etr1* and *ein2* 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; Grbič 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.

Use of genetic engineering to probe senescence. Another very useful genetic approach that offers direct evidence for the function of specific gene(s) is based on transgenic plants. Through genetic engineering technology, the roles of both ethylene and cytokinins in the regulation of leaf senescence have been confirmed.

One way to suppress the expression of a gene is to transform the plant with antisense DNA, which consists of the gene of interest in the reverse orientation with respect to the promoter. When the antisense gene is transcribed, the resulting antisense mRNA is complementary to the sense mRNA and will hybridize to it. Because double-stranded RNA is rapidly degraded in the cell, the effect of the antisense gene is to deplete the cell of the sense mRNA.

Transgenic plants expressing antisense versions of genes that encode enzymes involved in the ethylene biosynthetic pathway, such as ACC synthase and ACC oxidase, can synthesize ethylene only at very low levels. Consistent with a role for ethylene in senescence, such antisense mutants have been shown to exhibit delayed leaf senescence, as well as fruit ripening, in tomato (see **Web Topic 22.1**).

The Role of Ethylene in Defense Responses Is Complex

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 allowed the role of ethylene in the response to various pathogens to be assessed. The emerging picture is that the involvement of ethylene in pathogenesis is complex and depends on the particular host–pathogen interaction. For example, blocking the ethylene response does not affect the resistance response to *Pseudomonas* bacteria in *Arabidopsis* or to tobacco mosaic virus in tobacco. 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 jasmonic acid (see Chapter 13), is required for the activation of several plant defense genes. In addition, ethylene-insensitive tobacco and *Arabidopsis* mutants become susceptible to several necrotrophic (cell-killing) soil fungal pathogens that are normally not plant pathogens. Thus, ethylene appears to be involved in the resistance response to some pathogens, but not others.

Ethylene Biosynthesis in the Abscission Zone Is Regulated by Auxin

The shedding of leaves, fruits, flowers, and other plant organs is termed **abscission** (see **Web Topic 22.4**). 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 22.9).

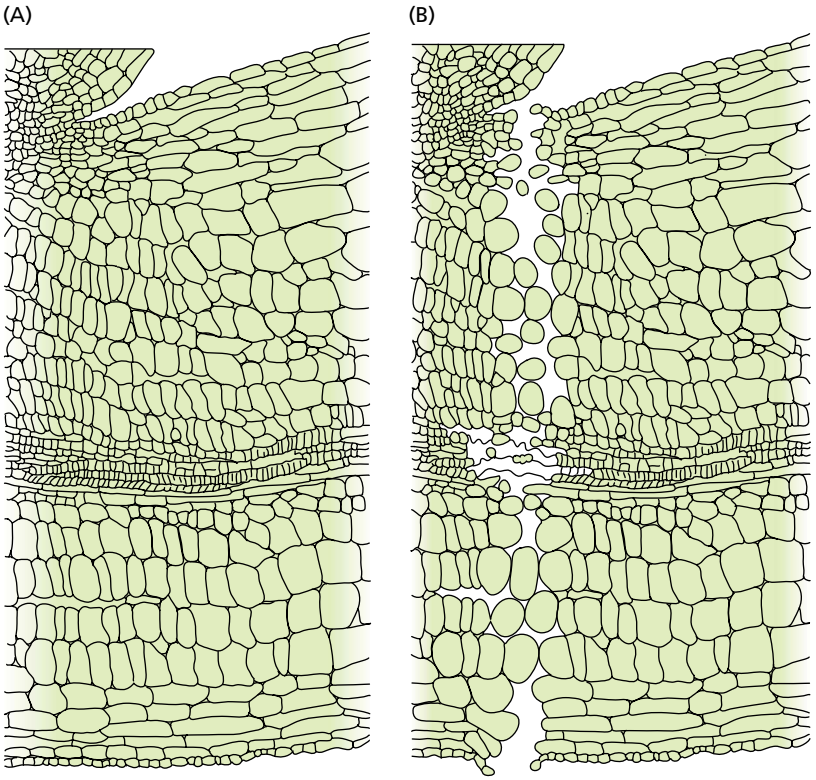


FIGURE 22.9 During the formation of the abscission layer, in this case that of jewelweed (*Impatiens*), two or three rows of cells in the abscission zone (A) undergo cell wall breakdown because of an increase in cell wall–hydrolyzing enzymes (B). The resulting protoplasts round up and increase in volume, pushing apart the xylem tracheary cells, and facilitating the separation of the leaf from the stem. (From Sexton et al. 1984.)

FIGURE 22.10 Effect of ethylene on abscission in birch (*Betaul 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, ETR1-1. 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 3 days with 50 ppm of ethylene.

The ability of ethylene gas to cause defoliation in birch trees is shown in Figure 22.10. The wild-type tree on the left has lost all its leaves. The tree on the right has been transformed with a gene for the *Arabidopsis* ethylene receptor ETR1-1 carrying a dominant mutation (discussed in the next section). This tree is unable to respond to ethylene and 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.

A model of the hormonal control of leaf abscission describes the process in three distinct sequential phases (Figure 22.11) (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 abscis-

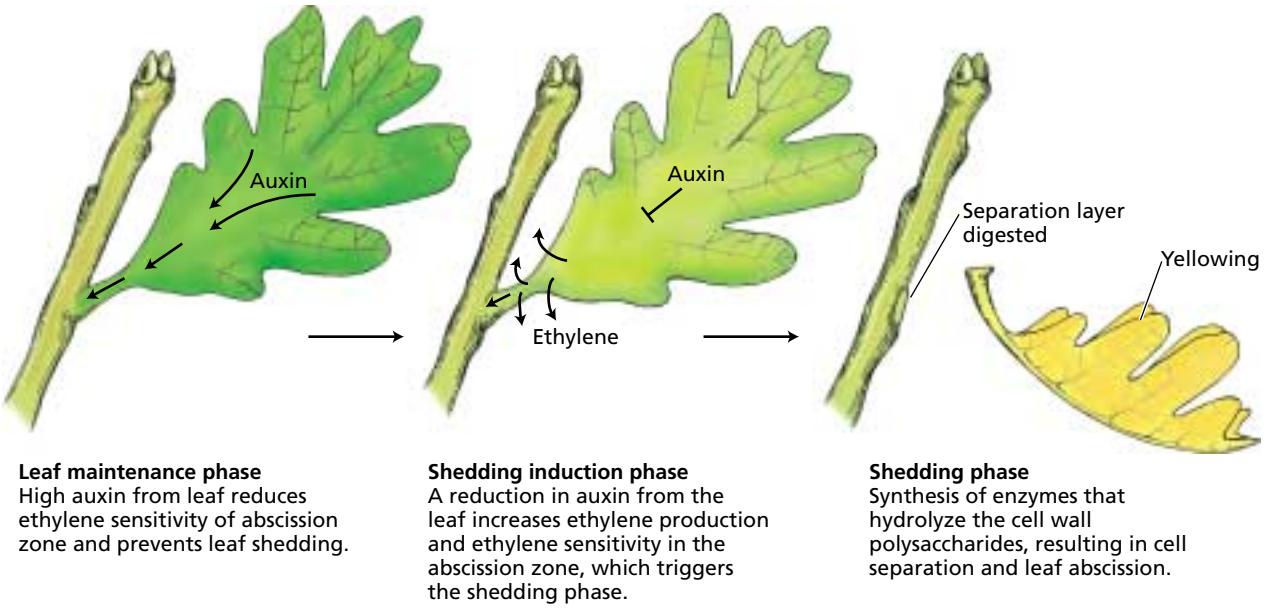


FIGURE 22.11 Schematic view of the roles of auxin and ethylene during leaf abscission. In the shedding induction phase, the level of auxin decreases, and the level of ethylene increases. These changes in the hormonal balance increase the sensitivity of the target cells to ethylene. (After Morgan 1984.)

sion 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 via secretory vesicles derived from the Golgi. The action of these enzymes leads 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.

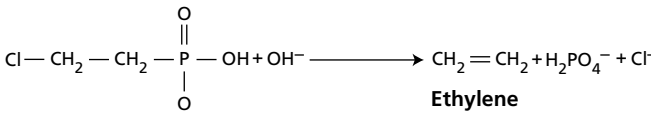
Ethephon is sprayed in aqueous solution and is readily absorbed and transported within the plant. It releases ethylene slowly by a chemical reaction, allowing the hormone to exert its effects:

Ethephon hastens fruit ripening of apple and tomato and degreening of citrus, 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 that inhibits 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.

Specific inhibitors of ethylene biosynthesis and action are also useful in postharvest preservation. Silver (Ag^+) is

used extensively to increase the longevity of cut carnations and several other flowers. The potential inhibitor AVG retards fruit ripening and flower fading, but its commercial use has not yet been approved by regulatory agencies. The strong,



2-Chloroethylphosphonic acid (ethephon)

offensive odor of *trans*-cyclooctene precludes its use in agriculture. Currently, 1-methylcyclopropene (MCP) is being developed for use in a variety of postharvest applications.

The near future may see a variety of agriculturally important species that have been genetically modified to manipulate the biosynthesis of ethylene or its perception. The inhibition of ripening in tomato by expression of an antisense version of ACC synthase and ACC oxidase has already been mentioned. Another example of this technology is in petunia, in which ethylene biosynthesis has been blocked by transformation of an antisense version of ACC oxidase. Senescence and petal wilting of cut flowers are delayed for weeks in these transgenic plants.

CELLULAR AND MOLECULAR MODES OF ETHYLENE ACTION

Despite the broad range of ethylene's effects on development, the primary steps in ethylene action are assumed to be similar in all cases: They all involve binding to a receptor, followed by activation of one or more signal transduction pathways (see Chapter 14 on the web site) leading to the cellular response. Ultimately, ethylene exerts its effects primarily by altering the pattern of gene expression. In recent years, remarkable progress has been made in our understanding of ethylene perception, as the result of molecular genetic studies of *Arabidopsis thaliana*.

One key to the elucidation of ethylene signaling components has been the use of the triple-response morphology of etiolated *Arabidopsis* seedlings to isolate mutants affected in their response to ethylene (see Figure 22.7) (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. Conversely, constitutive ethylene response mutants are identified as seedlings displaying the triple response in the absence of exogenous ethylene.

Ethylene Receptors Are Related to Bacterial Two-Component System Histidine Kinases

The first ethylene-insensitive mutant isolated was *etr1* (*et*hylene-*r*esistant *1*) (Figure 22.12). 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 various environmental cues, such as chemo-sensory stimuli, phosphate availability, and osmolarity.

Bacterial two-component systems consist of a sensor histidine kinase and a response regulator, which often acts as a transcription factor (see Chapter 14 on the web site). ETR1 was the first example of a eukaryotic histidine kinase,

FIGURE 22.12 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 are exhibiting 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.)

but others have since been found in yeast, mammals, and plants. Both 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. Consistent with this hypothesis, *ETR1* expression in yeast conferred the ability to bind radiolabeled ethylene with an affinity that closely parallels the dose-response curve of *Arabidopsis* seedlings to ethylene (see **Web Topic 22.5**).

The *Arabidopsis* genome encodes four additional proteins similar to ETR1 that also function as ethylene receptors: ETR2, ERS1 (*E*TR1-*r*elated *s*equence *1*), ERS2, and EIN4 (Figure 22.13). 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 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 middle portion of the ethylene receptors contains a histidine kinase catalytic domain.

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.Although histidine kinase activity has been demonstrated for one of the ethylene receptors—ETR1—several others are missing critical amino acids, making it unlikely that they possess histidine kinase activity. Thus the biochemical mechanism of these ethylene receptors is not known.

Recent studies indicate that ETR1 is located on the *endoplasmic reticulum*, rather than on the plasma membrane as originally assumed. Such 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 prior to 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* 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 cofactor 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.

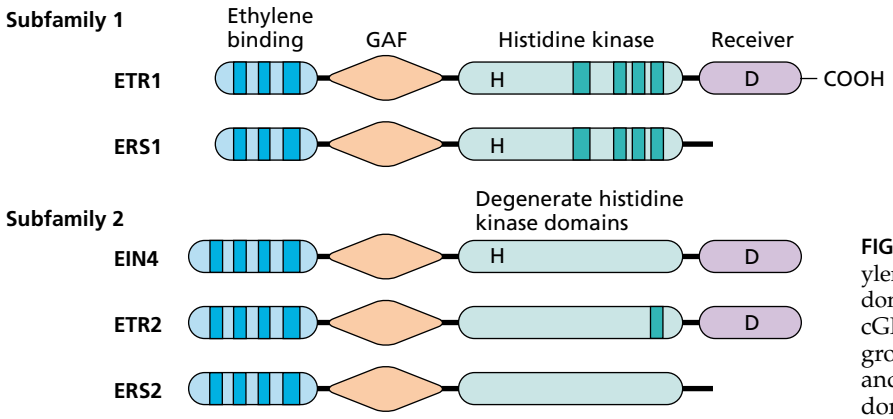


FIGURE 22.13 Schematic diagram of five ethylene receptor proteins and their functional domains. The GAF domain is a conserved cGMP-binding domain found in a diverse group of proteins. Note that EIN4, ETR2, and ERS2 have degenerate histidine kinase domains.

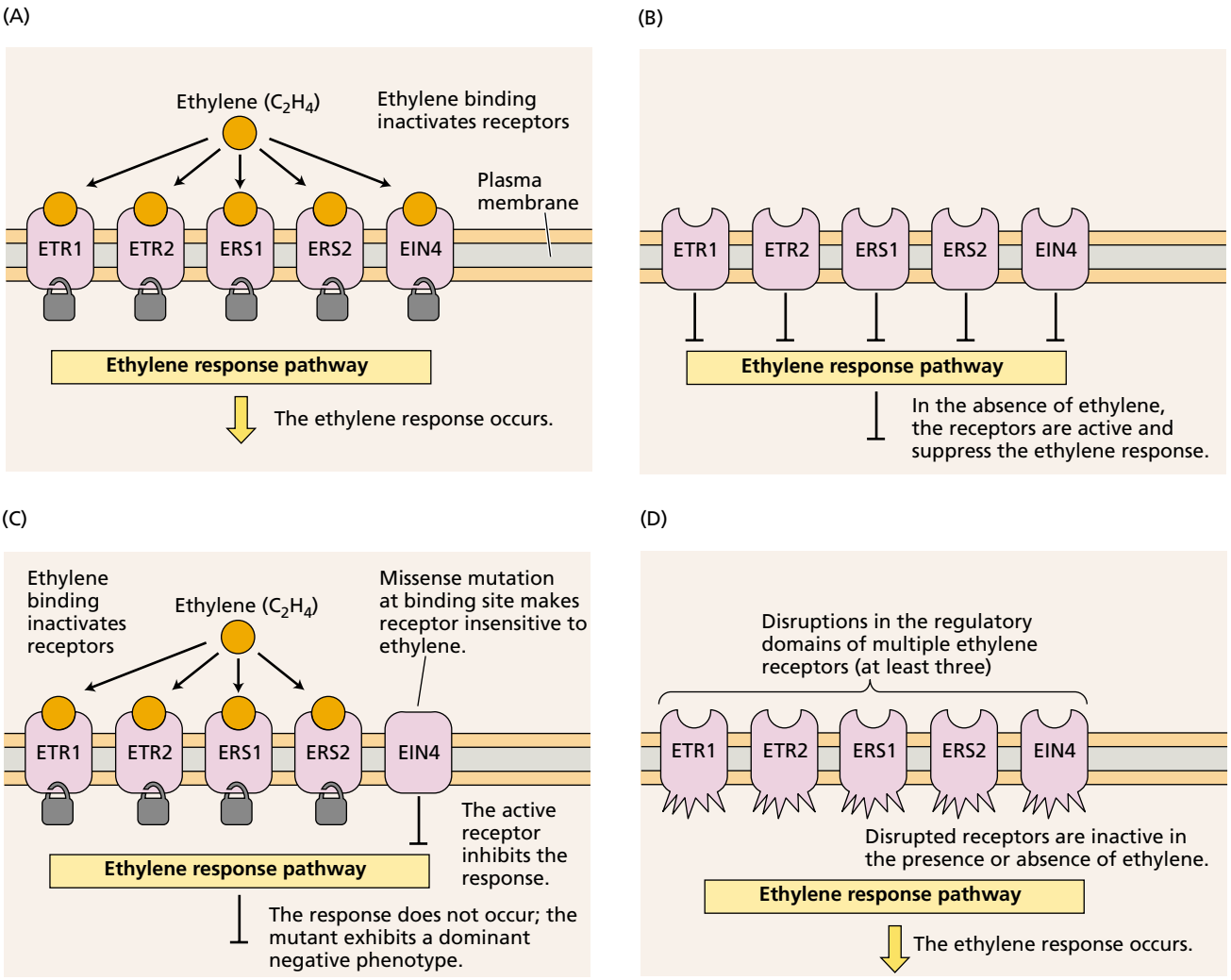


FIGURE 22.14 Model for ethylene receptor action based on the phenotype of receptor mutants. (A) In the wild type, ethylene binding inactivates the receptors, allowing the response to occur. (B) In the absence of ethylene the receptors act as negative regulators of the response pathway. (C) A missense mutation that interferes with ethylene binding to its receptor, but leaves the regulatory site active, results in a dominant negative phenotype. (D) Disruption mutations in the regulatory sites result in a constitutive ethylene response.

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 all five receptor genes exhibits a constitutive ethylene response phenotype (Figure 22.14D).

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 22.14B). Binding of ethylene turns off the receptors, thus allowing the response pathway to proceed (Figure 22.14A).

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.

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 22.14C). 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.

A Serine/Threonine Protein Kinase Is Also Involved in Ethylene Signaling

The recessive *ctr1* (*c*onstitutive *t*riple *r*esponse *1* = triple response in the absence of ethylene) mutation was identified in screens for mutations that constitutively activated ethylene responses (Figure 22.15). The fact that the mutation caused an *activation* of the ethylene response suggests that the wild-type protein also acts as a *negative regulator* of the response pathway (Kieber et al. 1993), similar to the ethylene receptors.

CTR1 appears to be related to RAF-1, a MAPKKK serine/threonine protein kinase (*m*itogen-*a*ctivated *p*rotein *k*inase *k*inase *k*inase) 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 on the web site). In animal cells, the final product in the MAP kinase cascade is a phosphorylated transcription factor that regulates gene expression in the nucleus.

EIN2 Encodes a Transmembrane Protein

The *ein2* (*e*thylene-*i*nsensitive *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*rotein) 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.

Interestingly, mutations in the *EIN2* gene have also been identified in genetic screens for resistance to other hormones, such as jasmonic acid and ABA, suggesting that EIN2 may be a common intermediate in the signal transduction pathways of various hormones and other chemical signals.

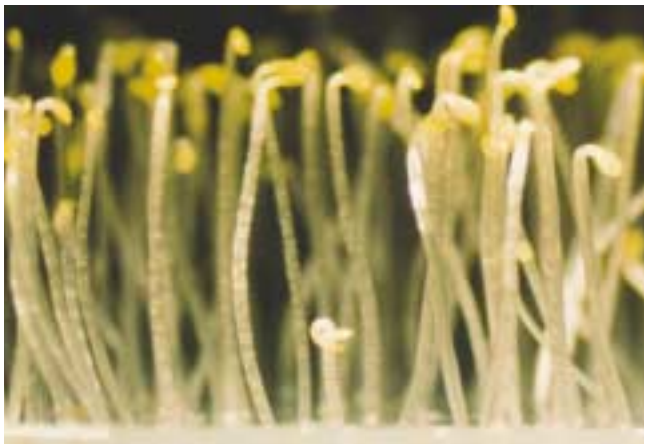


FIGURE 22.15 Screen for *Arabidopsis* mutants that constitutively display the triple response. Seedlings were grown for 3 days in the dark in air. A single *ctr1* mutant seedling is evident among the taller, wild-type seedlings. (Courtesy of J. Kieber.)

Ethylene Regulates 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 the genes that encode cellulase, as well as ripening-related genes and ethylene biosynthesis genes. Regulatory sequences called **ethylene response elements**, or **EREs**, have been identified from the ethylene-regulated genes.

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 both tomato and tobacco. In response to an ethylene signal, homodimers of EIN3 or its paralogs (closely related proteins), bind to the promoter of a gene called *ERF1* (*e*thylene *r*esponse *f*actor *1*) and activate its 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.

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 that the double mutant displays reveals which of the mutations is epistatic to the other. 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 (Avery and Wasserman 1992). In this way, the order of action of *ETR1*, *EIN2*, and *EIN3* were determined 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 22.16 summarizes these and other data. Genes that are similar to several of these *Arabidopsis* signaling genes have been found in other species (see **Web Topic 22.6**).

This model is still incomplete because other ethylene response mutations have been identified that act in this pathway. In addition, we are only beginning to understand the biochemical properties of these proteins and how they interact. However, we are beginning to glimpse the outline of the molecular basis for the perception and transduction of this hormonal signal.

SUMMARY

Ethylene is formed in most organs of higher plants. Senescing tissues and ripening fruits produce more ethylene than do young or mature tissues. The precursor of ethylene in vivo is the amino acid methionine, which is converted to AdoMet (*S*-adenosylmethionine), ACC (1-aminocyclopropane-1-carboxylic acid), and ethylene. The rate-limiting step of this pathway is the conversion of AdoMet to ACC, which is catalyzed by ACC synthase. ACC synthase is encoded by members of a multigene family that are differentially regulated in various plant tissues and in response to various inducers of ethylene biosynthesis.

Ethylene biosynthesis is triggered by various developmental processes, by auxins, and by environmental stresses. In all these cases the level of activity and of mRNA of ACC synthase increases. The physiological effects of ethylene can

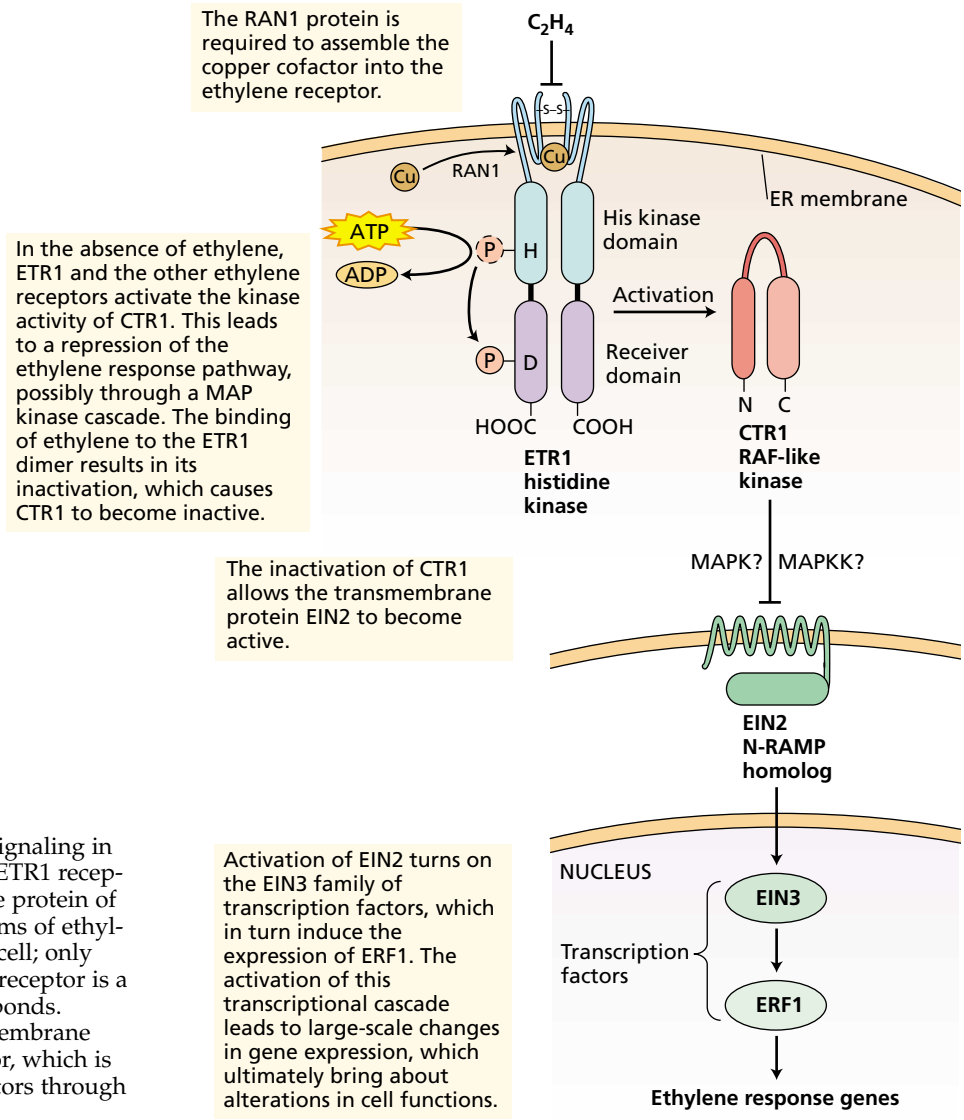


FIGURE 22.16 Model of ethylene signaling in *Arabidopsis*. Ethylene binds to the ETR1 receptor, which is an integral membrane protein of the ER membrane. Multiple isoforms of ethylene receptors may be present in a cell; only ETR1 is shown for simplicity. The receptor is a dimer, held together by disulfide bonds. Ethylene binds within the trans-membrane domain, through a copper co-factor, which is assembled into the ethylene receptors through the RAN1 protein.

be blocked by biosynthesis inhibitors or by antagonists. AVG (aminoethoxy-vinylglycine) and AOA (aminooxy-acetic acid) inhibit the synthesis of ethylene; carbon dioxide, silver ions, *trans*-cyclooctene, and MCP inhibit ethylene action. Ethylene can be detected and measured by gas chromatography.

Ethylene regulates fruit ripening and other processes associated with leaf and flower senescence, leaf and fruit abscission, root hair development, seedling growth, and hook opening. Ethylene also regulates the expression of various genes, including ripening-related genes and pathogenesis-related genes.

The ethylene receptor is encoded by a family of genes that encode proteins similar to bacterial two-component histidine kinases. Ethylene binds to these receptors in a transmembrane domain through a copper cofactor. Downstream signal transduction components include CTR1, a member of the RAF family of protein kinases; and EIN2, a channel-like transmembrane protein. The pathway activates a cascade of transcription factors, including the EIN3 and EREBP families, which then modulate gene expression.

Web Material

Web Topics

22.1 Cloning of ACC Synthase

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

22.2 Cloning of the ACC Oxidase Gene

The ACC oxidase gene was cloned by a circuitous route using antisense DNA.

22.3 ACC Synthase Gene Expression and Biotechnology

A discussion of the use of the ACC synthase gene in biotechnology.

22.4 Abscission and the Dawn of Agriculture

A short essay on the domestication of modern cereals based on artificial selection for non-shattering rachises.

22.5 Ethylene Binding to ETR1 and Seedling Response to Ethylene

Ethylene-binding to its receptor ETR1 was first demonstrated by expressing the gene in yeast.

22.6 Conservation of Ethylene Signaling Components in Other Plant Species

The evidence suggests that ethylene signaling is similar in all plant species.

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