Chapter

18

Blue-Light Responses: Stomatal Movements and Morphogenesis

MOST OF US are familiar with the observation that house plants placed near a window have branches that grow toward the incoming light. This response, called *phototropism*, is an example of how plants alter their growth patterns in response to the direction of incident radiation. This response to light is intrinsically different from light trapping by photosynthesis. In photosynthesis, plants harness light and convert it into chemical energy (see Chapters 7 and 8). In contrast, phototropism is an example of the use of light as an *environmental signal*. There are two major families of plant responses to light signals: the phytochrome responses, which were covered in Chapter 17, and the **blue-light responses**.

Some blue-light responses were introduced in Chapter 9—for example, chloroplast movement within cells in response to incident photon fluxes, and sun tracking by leaves. As with the family of the phytochrome responses, there are numerous plant responses to blue light. Besides phototropism, they include inhibition of hypocotyl elongation, stimulation of chlorophyll and carotenoid synthesis, activation of gene expression, stomatal movements, phototaxis (the movement of motile unicellular organisms such as algae and bacteria toward or away from light), enhancement of respiration, and anion uptake in algae (Senger 1984). Blue-light responses have been reported in higher plants, algae, ferns, fungi, and prokaryotes.

Some responses, such as electrical events at the plasma membrane, can be detected within seconds of irradiation by blue light. More complex metabolic or morphogenetic responses, such as blue light–stimulated pigment biosynthesis in the fungus *Neurospora* or branching in the alga *Vaucheria*, might require minutes, hours, or even days (Horwitz 1994).

Readers may be puzzled by the different approaches to naming phytochrome and blue-light responses. The former are identified by a specific photoreceptor (phytochrome), the latter by the blue-light region of the visible spectrum. In the case of phytochrome, several of its spectroscopic and biochemical properties, particularly its red/far-red reversibil-

ity, made possible its early identification, and hundreds of photobiological responses of plants can be clearly attributed to the phytochrome photoreceptor (see Chapter 17).

In contrast, the spectroscopy of blue-light responses is complex. Both chlorophylls and phytochrome absorb blue light (400–500 nm) from the visible spectrum, and other chromophores and some amino acids, such as tryptophan, absorb light in the ultraviolet (250–400 nm) region. How, then, can we then distinguish specific responses to blue light? One important identification criterion is that in specific blue-light responses, blue light cannot be replaced by a red-light treatment, and there is no red/far-red reversibility. Red or far-red light would be effective if photosynthesis or phytochrome were involved.

Another key distinction is that *many blue-light responses of higher plants share a characteristic action spectrum*. You will recall from Chapter 7 that an action spectrum is a graph of the magnitude of the observed light response as a function of wavelength (see **Web Topic 7.1** for a detailed discussion of spectroscopy and action spectra). The action spectrum of the response can be compared with the *absorption spectra* of candidate photoreceptors. A close correspondence between action and absorption spectra provides a strong indication that the pigment under consideration is the photoreceptor mediating the light response under study (see Figure 7.8).

Action spectra for blue light–stimulated phototropism, stomatal movements, inhibition of hypocotyl elongation, and other key blue-light responses share a characteristic "three-finger" fine structure in the 400 to 500 nm region (Figure 18.1) that is not observed in spectra for responses to light that are mediated by photosynthesis, phytochrome, or other photoreceptors (Cosgrove 1994).

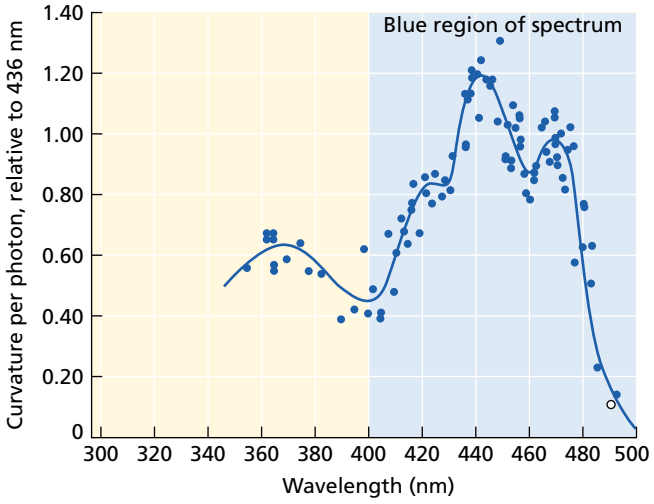


FIGURE 18.1 Action spectrum for blue light–stimulated phototropism in oat coleoptiles. An action spectrum shows the relationship between a biological response and the wavelengths of light absorbed. The "three-finger" pattern in the 400 to 500 nm region is characteristic of specific blue-light responses. (After Thimann and Curry 1960.)

In this chapter we will describe representative blue-light responses in plants: phototropism, inhibition of stem elongation, and stomatal movements. The stomatal responses to blue light are discussed in detail because of the importance of stomata in leaf gas exchange (see Chapter 9) and in plant acclimations and adaptations to their environment. We will also discuss blue-light photoreceptors and the signal transduction cascade that links light perception with the final expression of blue-light sensing in the organism.

THE PHOTOPHYSIOLOGY OF BLUE-LIGHT RESPONSES

Blue-light signals are utilized by the plant in many responses, allowing the plant to sense the presence of light and its direction. This section describes the major morphological, physiological, and biochemical changes associated with typical blue-light responses.

Blue Light Stimulates Asymmetric Growth and Bending

Directional growth toward (or in special circumstances away from) the light, is called **phototropism**. It can be observed in fungi, ferns, and higher plants. Phototropism is a **photomorphogenetic** response that is particularly dramatic in dark-grown seedlings of both monocots and dicots. Unilateral light is commonly used in experimental studies, but phototropism can also be observed when a seedling is exposed to two unequally bright light sources (Figure 18.2), a condition that can occur in nature.

As it grows through the soil, the shoot of a grass is protected by a modified leaf that covers it, called a **coleoptile** (Figure 18.3; see also Figure 19.1). As discussed in detail in Chapter 19, unequal light perception in the coleoptile results in unequal concentrations of auxin in the lighted and shaded sides of the coleoptile, unequal growth, and bending.

Keep in mind that phototropic bending occurs only in *growing* organs, and that coleoptiles and shoots that have stopped elongating will not bend when exposed to unilateral light. In grass seedlings growing in soil under sunlight, coleoptiles stop growing as soon as the shoot has emerged from the soil and the first true leaf has pierced the tip of the coleoptile.

On the other hand, dark-grown, *etiolated* coleoptiles continue to elongate at high rates for several days and, depending on the species, can attain several centimeters in length. The large phototropic response of these etiolated coleoptiles (see Figure 18.3) has made them a classic model for studies of phototropism (Firn 1994).

The action spectrum shown in Figure 18.1 was obtained through measurement of the angles of curvature from oat coleoptiles that were irradiated with light of different

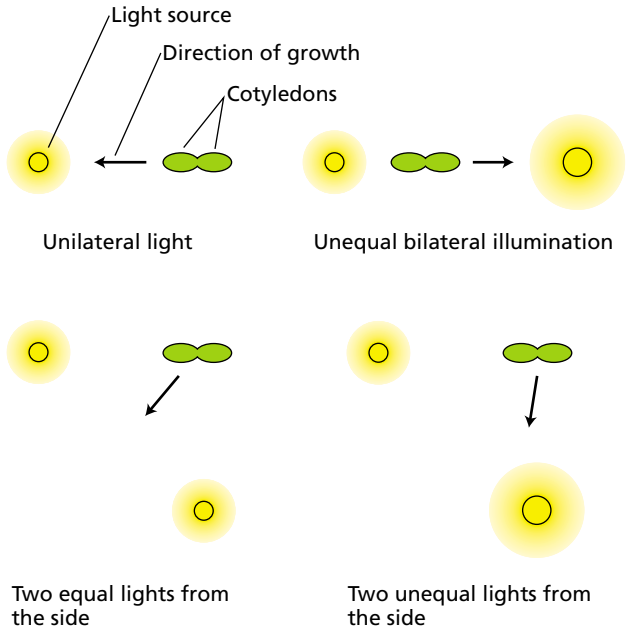


FIGURE 18.2 Relationship between direction of growth and unequal incident light. Cotyledons from a young seedling are shown as viewed from the top. The arrows indicate the direction of phototropic curvature. The diagrams illustrate how the direction of growth varies with the location and the intensity of the light source, but growth is always toward light. (After Firn 1994.)

wavelengths. The spectrum shows a peak at about 370 nm and the "three-finger" pattern in the 400 to 500 nm region discussed earlier. An action spectrum for phototropism in the dicot alfalfa (*Medicago sativa*) was found to be very similar to that of oat coleoptiles, suggesting that a common photoreceptor mediates phototropism in the two species.

Phototropism in sporangiophores of the mold *Phycomyces* has been studied to identify genes involved in phototropic responses. The sporangiophore consists of a sporangium (spore-bearing spherical structure) that develops on a stalk consisting of a long, single cell. Growth in the sporangiophore is restricted to a growing zone just below the sporangium.

When irradiated with unilateral blue light, the sporangiophore bends toward the light with an action spectrum similar to that of coleoptile phototropism (Cerda-Olmedo and Lipson 1987). These studies of *Phycomyces* have led to the isolation of many mutants with altered phototropic responses and the identification of several genes that are required for normal phototropism.

In recent years, phototropism of the stem of the small dicot *Arabidopsis* (Figure 18.4) has attracted much attention because of the ease with which advanced molecular techniques can be applied to *Arabidopsis* mutants. The genetics and the molecular biology of phototropism in *Arabidopsis* are discussed later in this chapter.

FIGURE 18.3 Time-lapse photograph of a corn coleoptile growing toward unilateral blue light given from the right. The consecutive exposures were made 30 minutes apart. Note the increasing angle of curvature as the coleoptile bends. (Courtesy of M. A. Quiñones.)

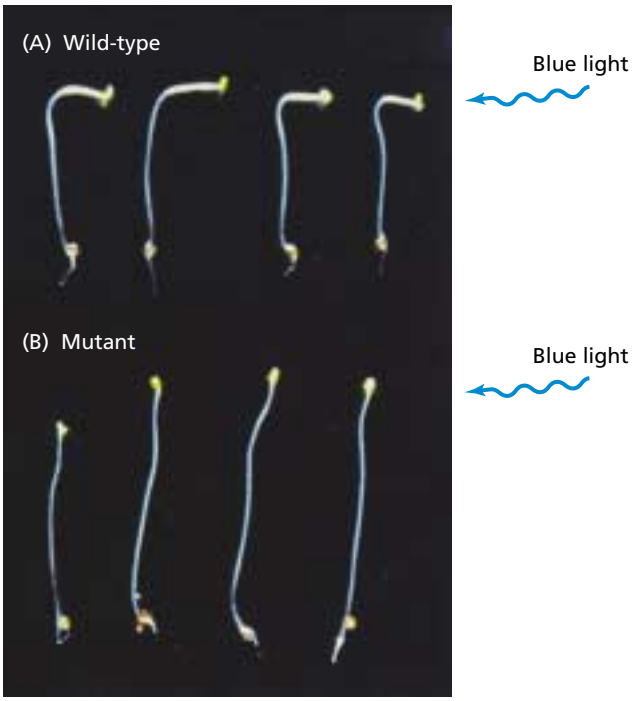


FIGURE 18.4 Phototropism in wild-type (A) and mutant (B) *Arabidopsis* seedlings. Unilateral light was applied from the right. (Courtesy of Dr. Eva Huala.)

How Do Plants Sense the Direction of the Light Signal?

Light gradients between lighted and shaded sides have been measured in coleoptiles and in hypocotyls from dicot seedlings irradiated with unilateral blue light. When a coleoptile is illuminated with 450 nm blue light, the ratio between the light that is incident to the surface of the illuminated side and the light that reaches the shaded side is 4:1 at the tip and the midregion of the coleoptile, and 8:1 at the base (Figure 18.5).

On the other hand, there is a *lens effect* in the sporangiophore of the mold *Phycomyces* irradiated with unilateral blue light, and as a result, the light measured at the distal cell surface of the sporangiophore is about twice the amount of light that is incident at the surface of the illuminated side. Light gradients and lens effects could play a role in how the bending organ senses the direction of the unilateral light (Vogelmann 1994).

Blue Light Rapidly Inhibits Stem Elongation

The stems of seedlings growing in the dark elongate very rapidly, and the inhibition of stem elongation by light is a key morphogenetic response of the seedling emerging from the soil surface (see Chapter 17). The conversion of Pr to Pfr (the red- and far red–absorbing forms of phytochrome, respectively) in etiolated seedlings causes a phytochrome-dependent, sharp decrease in elongation rates (see Figure 17.1).

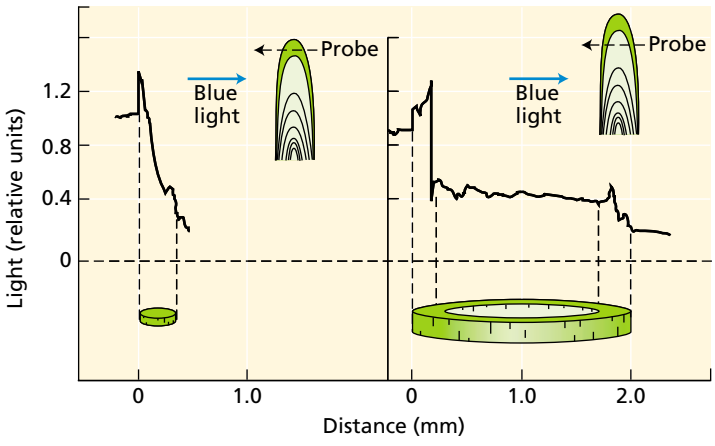


FIGURE 18.5 Distribution of transmitted, 450 nm blue light in an etiolated corn coleoptile. The diagram in the upper right of each frame shows the area of the coleoptile being measured by a fiber-optic probe. A cross section of the tissue appears at the bottom of each frame. The trace above it shows the amount of light sensed by the probe at each point. A sensing mechanism that depended on light gradients would sense the difference in the amount of light between the lighted and shaded sides of the coleoptile, and this information would be transduced into an unequal auxin concentration and bending. (After Vogelmann and Haupt 1985.)

However, action spectra for the decrease in elongation rate show strong activity in the blue region, which cannot be explained by the absorption properties of phytochrome (see Figure 17.9). In fact, the 400 to 500 nm blue region of the action spectrum for the inhibition of stem elongation closely resembles that of phototropism (compare the action spectra in Figures 17.10 and 18.1).

There are several ways to experimentally separate a reduction in elongation rates mediated by phytochrome from a reduction mediated by a specific blue-light response. If lettuce seedlings are given low fluence rates of blue light under a strong background of yellow light, their hypocotyl elongation rate is reduced by more than 50%. The background yellow light establishes a well-defined Pr:Pfr ratio (see Chapter 17). In such conditions, the low fluence rates of blue light added are too small to significantly change this ratio, ruling out a phytochrome effect on the reduction in elongation rate observed upon the addition of blue light.

Blue light– and phytochrome-mediated hypocotyl responses can also be distinguished by the swiftness of the response. Whereas phytochrome-mediated changes in elongation rates can be detected within 8 to 90 minutes, depending on the species, blue-light responses are rapid, and can be measured within 15 to 30 s (Figure 18.6). Interactions between phytochrome and the blue light–dependent sensory transduction cascade in the regulation of elongation rates will be described later in the chapter.

Another fast response elicited by blue light is a depolarization of the membrane of hypocotyl cells that precedes the inhibition of growth rate (see Figure 18.6). The membrane depolarization is caused by the activation of anion channels (see Chapter 6), which facilitates the efflux of anions such as chloride. Use of an anion channel blocker prevents the blue light–dependent membrane depolarization and decreases the inhibitory effect of blue light on hypocotyl elongation (Parks et al. 1998).

Blue Light Regulates Gene Expression

Blue light also regulates the expression of genes involved in several important morphogenetic processes. Some of these light-activated genes have been studied in detail—for example, the genes that code for the enzyme chalcone synthase, which catalyzes the first committed step in flavonoid biosynthesis, for the small subunit of rubisco, and for the proteins that bind chlorophylls *a* and *b* (see Chapters 13, 8, and 7, respectively). Most of the studies on light-activated genes show sensitivity to both blue and red light, as well as red/far-red reversibility, implicating both phytochrome and specific blue-light responses.

A recent study reported that *SIG5*, one of six *SIG* nuclear genes in *Arabidopsis* that play a regulatory role in the transcription of the chloroplast gene

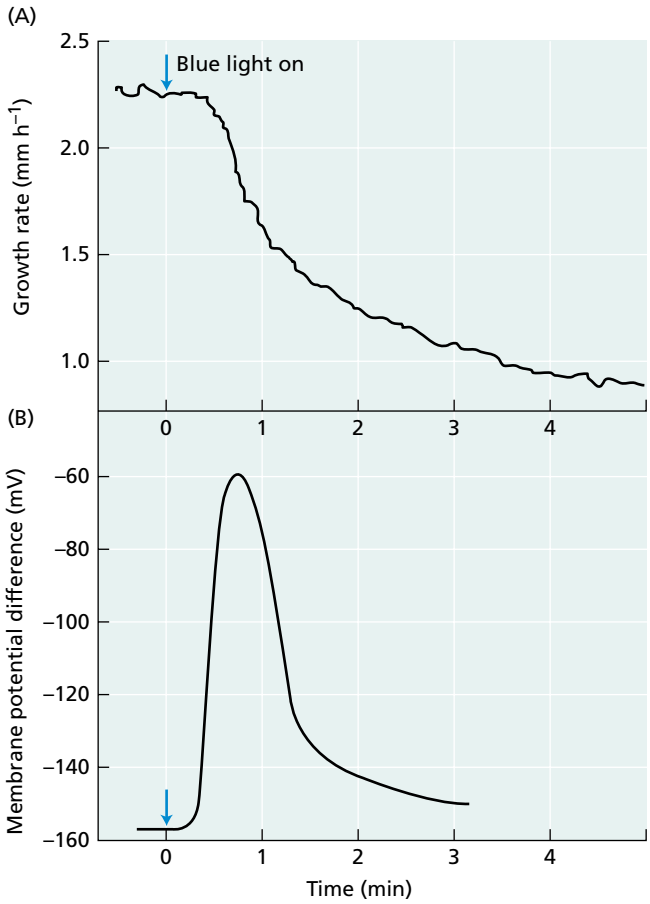


FIGURE 18.6 Blue light–induced (A) changes in elongation rates of etiolated cucumber seedlings and (B) transient membrane depolarization of hypocotyl cells. As the membrane depolarization (measured with intracellular electrodes) reaches its maximum, growth rate (measured with position transducers) declines sharply. Comparison of the two curves shows that the membrane starts to depolarize before the growth rate begins to decline, suggesting a cause–effect relation between the two phenomena. (After Spalding and Cosgrove 1989.)

psbD, which encodes the D2 subunit of the PSII reaction center (see Chapter 7), is specifically activated by blue light (Tsunoyama et al. 2002). In contrast, the other five *SIG* genes are activated by both blue and red light.

Another well-documented instance of gene expression that is mediated solely by a blue light–sensing system involves the *GSA* gene in the photosynthetic unicellular alga *Chlamydomonas reinhardtii* (Matters and Beale 1995). This gene encodes the enzyme glutamate-1-semialdehyde aminotransferase (GSA), a key enzyme in the chlorophyll biosynthesis pathway (see Chapter 7). The absence of phytochrome in *C. reinhardtii* simplifies the analysis of blue-light responses in this experimental system.

In synchronized cultures of *C. reinhardtii*, levels of *GSA* mRNA are strictly regulated by blue light, and 2 hours after the onset of illumination, *GSA* mRNA levels are 26-fold higher than they are in the dark (Figure 18.7). These blue light–mediated mRNA increases precede increases in chlorophyll content, indicating that chlorophyll biosynthesis is being regulated by activation of the *GSA* gene.

Blue Light Stimulates Stomatal Opening

We now turn our attention to the stomatal response to blue light. Stomata have a major regulatory role in gas exchange in leaves (see Chapter 9), and they can often affect yields of agricultural crops (see Chapter 25). Several characteristics of blue light–dependent stomatal movements make guard cells a valuable experimental system for the study of blue-light responses:

- The stomatal response to blue light is rapid and reversible, and it is localized in a single cell type, the guard cell.
- The stomatal response to blue light regulates stomatal movements throughout the life of the plant. This is unlike phototropism or hypocotyl elongation, which are functionally important at early stages of development.
- The signal transduction cascade that links the perception of blue light with the opening of stomata is understood in considerable detail.

In the following sections we will discuss two central aspects of the stomatal response to light, the osmoregulatory mechanisms that drive stomatal movements, and the role of a blue light–activated H^+-ATPase in ion uptake by guard cells.

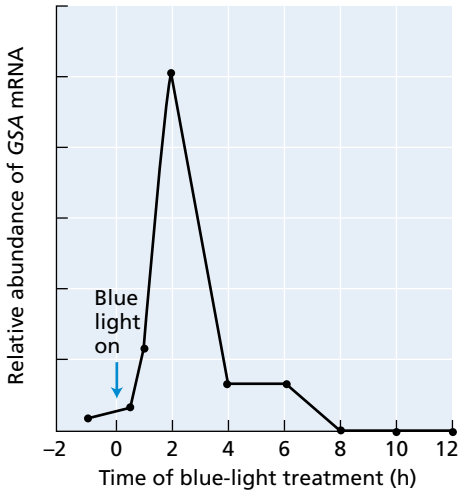


FIGURE 18.7 Time course of blue light–dependent gene expression in *Chlamydomonas reinhardtii*. The *GSA* gene encodes the enzyme glutamate-1-semialdehyde aminotransferase, which regulates an early step in chlorophyll biosynthesis. (After Matters and Beale 1995.)

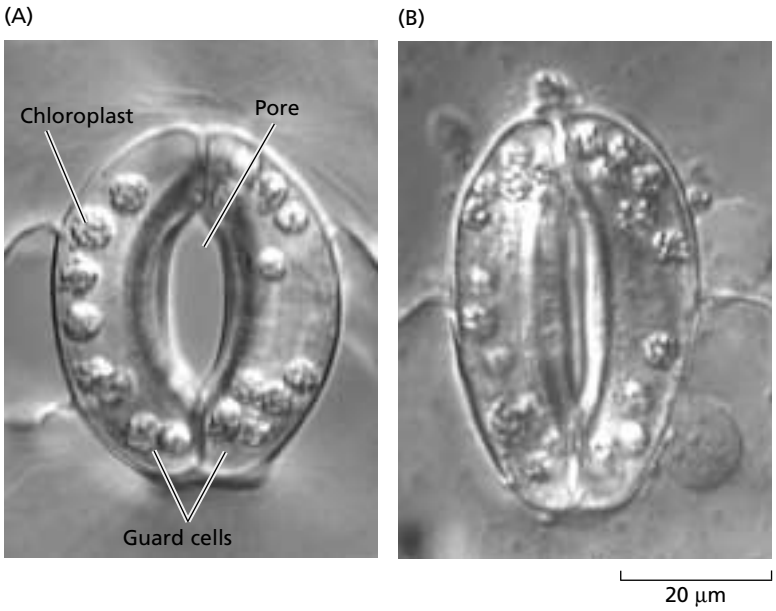


FIGURE 18.8 Light-stimulated stomatal opening in detached epidermis of *Vicia faba*. Open, light-treated stoma (A), is shown in the dark-treated, closed state in (B). Stomatal opening is quantified by microscopic measurement of the width of the stomatal pore. (Courtesy of E. Raveh.)

Light is the dominant environmental signal controlling stomatal movements in leaves of well-watered plants growing in natural environments. Stomata open as light levels reaching the leaf surface increase, and close as they decrease (Figure 18.8). In greenhouse-grown leaves of broad bean (*Vicia faba*), stomatal movements closely track incident solar radiation at the leaf surface (Figure 18.9).

Early studies of the stomatal response to light showed that DCMU (dichlorophenyldimethylurea), an inhibitor of photosynthetic electron transport (see Figure 7.31), causes a partial inhibition of light-stimulated stomatal opening. These results indicated that photosynthesis in the guard cell chloroplast plays a role in light-dependent stomatal opening, but the observation that the inhibition was only partial pointed to a nonphotosynthetic component of the stomatal response to light. Detailed studies of the light response of stomata have shown that light activates two distinct responses of guard cells: photosynthesis in the guard cell chloroplast (see **Web Essay 18.1**), and a specific blue-light response.

The specific stomatal response to blue light cannot be resolved properly under blue-light illumination because blue light simultaneously stimulates both the specific blue-light response and guard cell photosynthesis (for the photosynthetic response to blue light, see the action spectrum for photosynthesis in Figure 7.8). A clear-cut separation of the responses of the two light responses can be obtained in dual-beam experiments. High fluence rates of red light are used to *saturate* the photosynthetic response, and low photon fluxes of blue light are added after the response to the saturating red light has been completed (Figure 18.10). The addition of blue light causes substantial further stomatal opening that cannot be explained as a further stimulation of guard cell photosynthesis because photosynthesis is saturated by the background red light.

An action spectrum for the stomatal response to blue light under background red illumination shows the three-finger pattern discussed earlier (Figure 18.11). This action spectrum, typical of blue-light responses and distinctly different from the action spectrum for photosynthesis, further indicates that, in addition to photosynthesis, guard cells respond specifically to blue light.

When guard cells are treated with cellulolytic enzymes that digest the cell walls, *guard cell protoplasts* are released. Guard cell protoplasts *swell* when illuminated with blue light (Figure 18.12), indicating that blue light is sensed within the guard cells proper. The swelling of guard cell

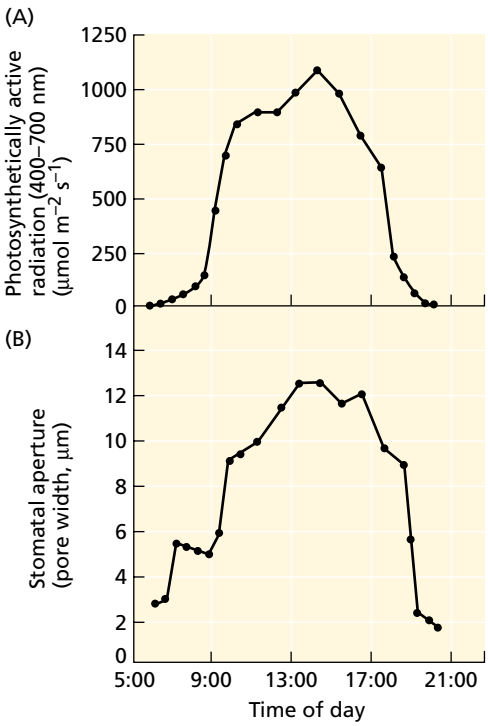


FIGURE 18.9 Stomatal opening tracks photosynthetic active radiation at the leaf surface. Stomatal opening in the lower surface of leaves of *Vicia faba* grown in a greenhouse, measured as the width of the stomatal pore (A), closely follows the levels of photosynthetically active radiation (400–700 nm) incident to the leaf (B), indicating that the response to light was the dominant response regulating stomatal opening. (After Srivastava and Zeiger 1995a.)

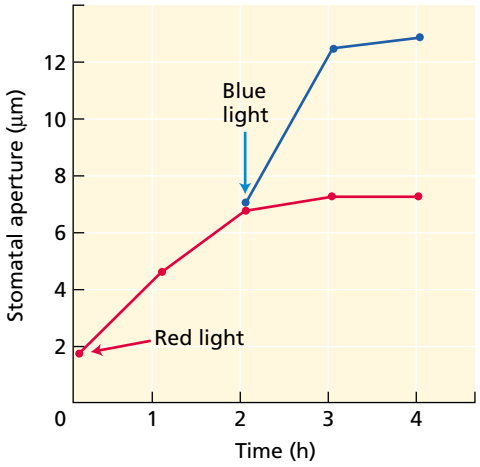


FIGURE 18.10 The response of stomata to blue light under a red-light background. Stomata from detached epidermis of *Commelina communis* (common dayflower) were treated with saturating photon fluxes of red light (red trace). In a parallel treatment, stomata illuminated with red light were also illuminated with blue light, as indicated by the arrow (blue trace). The increase in stomatal opening above the level reached in the presence of saturating red light indicates that a different photoreceptor system, stimulated by blue light, is mediating the additional increases in opening. (From Schwartz and Zeiger 1984.)

protoplasts also illustrates how intact guard cells function. The light-stimulated uptake of ions and the accumulation of organic solutes decrease the cell's osmotic potential (increase the osmotic pressure). Water flows in as a result, leading to an increase in turgor that in guard cells with intact walls is mechanically transduced into an increase in stomatal apertures (see Chapter 4). In the absence of a cell wall, the blue light–mediated increase in osmotic pressure causes the guard cell protoplast to swell.

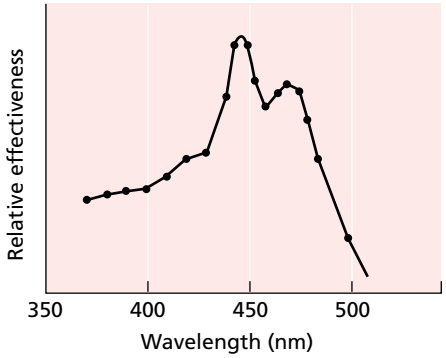
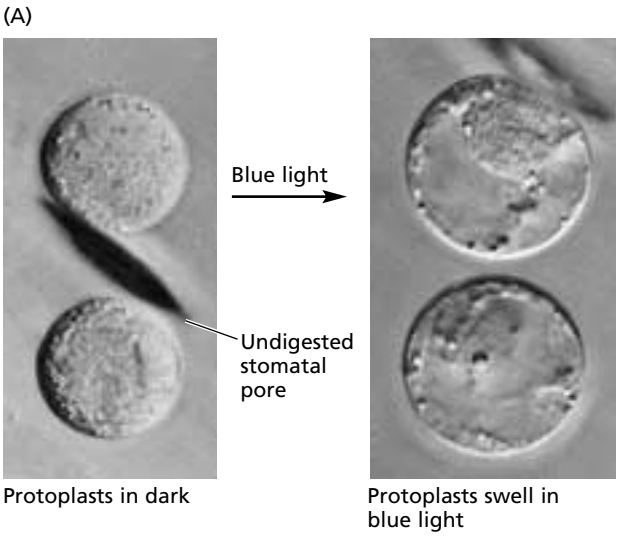


FIGURE 18.11 The action spectrum for blue light–stimulated stomatal opening (under a red-light background). (After Karlsson 1986.)



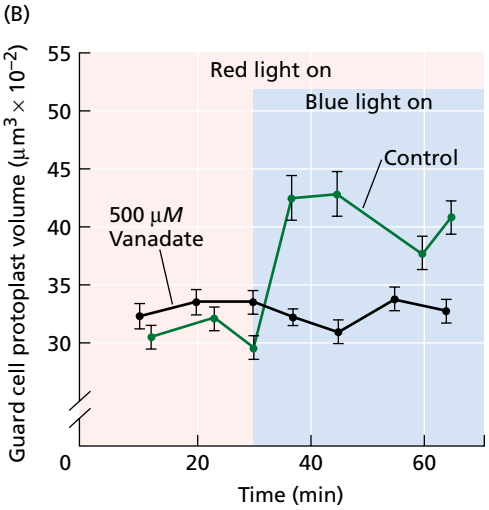


FIGURE 18.12 Blue light–stimulated swelling of guard cell protoplasts. (A) In the absence of a rigid cell wall, guard cell protoplasts of onion (*Allium* cepa) swell. (B) Blue light stimulates the swelling of guard cell protoplasts of broad bean (*Vicia faba*), and vanadate, an inhibitor of the H^+-ATPase, inhibits this swelling. Blue light stimulates ion and water uptake in the guard cell protoplasts, which in the intact guard cells provides a mechanical force that drives increases in stomatal apertures. (A from Zeiger and Hepler 1977; B after Amodeo et al. 1992.)

Blue Light Activates a Proton Pump at the Guard Cell Plasma Membrane

When guard cell protoplasts from broad bean (*Vicia faba*) are irradiated with blue light under background red-light illumination, the pH of the suspension medium becomes more acidic (Figure 18.13). This blue light–induced acidification is blocked by inhibitors that dissipate pH gradients, such as CCCP (discussed shortly), and by inhibitors of the proton-pumping H^+-ATPase, such as vanadate (see Figure 18.12C; see also Chapter 6).

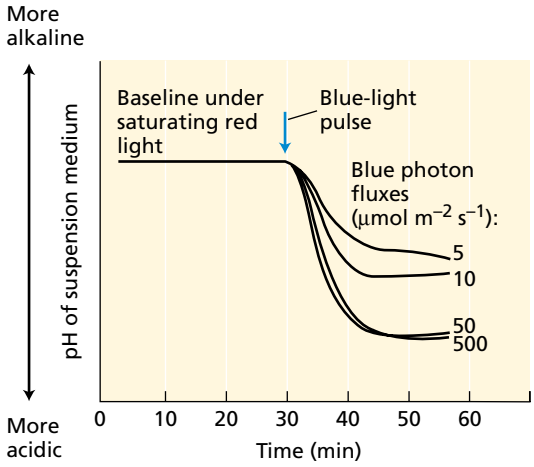


FIGURE 18.13 Acidification of a suspension medium of guard cell protoplasts of *Vicia faba* stimulated by a 30 s pulse of blue light. The acidification results from the stimulation of an H^+-ATPase at the plasma membrane by blue light, and it is associated with protoplast swelling (see Figure 18.12). (After Shimazaki et al. 1986.)

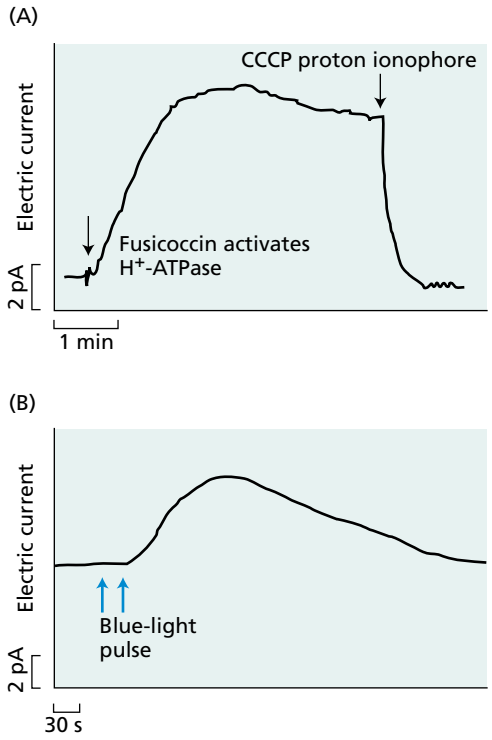


FIGURE 18.14 Activation of the H^+-ATPase at the plasma membrane of guard cell protoplasts by fusiccocin and blue light can be measured as electric current in patch clamp experiments. (A) Outward electric current (measured in picoamps, pA) at the plasma membrane of a guard cell protoplast stimulated by the fungal toxin fusicoccin, an activator of the H^+-ATPase. The current is abolished by the proton ionophore CCCP (carbonyl cyanide *m*-chlorophenylhydrazone). (B) Outward electric current at the plasma membrane of a guard cell protoplast stimulated by a blue-light pulse. These results indicate that blue light stimulates the H^+-ATPase. (A after Serrano et al. 1988; B after Assmann et al. 1985.)

This indicates that *the acidification results from the activation by blue light of a proton-pumping ATPase in the guard cell plasma membrane* that extrudes protons into the protoplast suspension medium and lowers its pH. In the intact leaf, this blue-light stimulation of proton pumping lowers the pH of the apoplastic space surrounding the guard cells. The plasma membrane ATPase from guard cells has been isolated and extensively characterized (Kinoshita et al. 2001).

The activation of electrogenic pumps such as the proton-pumping ATPase can be measured in patch-clamping experiments as an outward electric current at the plasma membrane (see **Web Topic 6.2** for a description of patch clamping). A patch clamp recording of a guard cell protoplast treated with the fungal toxin fusicoccin, a well-characterized activator of plasma membrane ATPases, is shown in Figure 18.14A. Exposure to fusicoccin stimulates an outward electric current, which is abolished by the proton ionophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). This proton ionophore makes the plasma membrane highly permeable to protons, thus precluding the formation of a proton gradient across the membrane and abolishing net proton efflux.

The relationship between proton pumping at the guard cell plasma membrane and stomatal opening is evident from the observation that fusicoccin stimulates both proton extrusion from guard cell protoplasts and stomatal opening, and that CCCP inhibits the fusiccocin-stimulated opening. The increase in proton-pumping rates as a function of fluence rates of blue light (see Figure 18.13) indicates that the increasing rates of blue photons in the solar radiation reaching the leaf cause a larger stomatal opening.

The close relationship among the number of incident blue-light photons, proton pumping at the guard cell plasma membrane, and stomatal opening further suggests that the blue-light response of stomata might function as a sensor of photon fluxes reaching the guard cell.

Pulses of blue light given under a saturating red-light background also stimulate an outward electric current from guard cell protoplasts (see Figure 18.14B). The acidification measurements shown in Figure 18.13 indicate that the outward electric current measured in patch clamp experiments is carried by protons.

Blue-Light Responses Have Characteristic Kinetics and Lag Times

Some of the characteristics of the responses to blue-light pulses underscore some important properties of blue-light responses: the persistence of the response after the light sig-

nal has been switched off, and a significant lag time separating the onset of the light signal and the beginning of the response.

In contrast to typical photosynthetic responses, which are activated very quickly after a "light on" signal, and cease when the light goes off (see, for instance, Figure 7.13), blue-light responses proceed at maximal rates for several minutes after application of the pulse (see Figure 18.14B). This property can be explained by a physiologically inactive form of the blue-light photoreceptor that is converted to an active form by blue light, with the active form reverting slowly to the physiologically inactive form in the absence of blue light (Iino et al. 1985). The rate of the response to a blue-light pulse would thus depend on the time course of the reversion of the active form to the inactive one.

Another property of the response to blue-light pulses is a lag time, which lasts about 25 s in both the acidification response and the outward electric currents stimulated by blue light (see Figures 18.13 and 18.14). This amount of time is probably required for the signal transduction cascade to proceed from the photoreceptor site to the proton-pumping ATPase and for the proton gradient to form. Similar lag times have been measured for blue light–dependent inhibition of hypocotyl elongation, which was discussed earlier.

Blue Light Regulates Osmotic Relations of Guard Cells

Blue light modulates guard cell osmoregulation via its activation of proton pumping (described earlier) and via the stimulation of the synthesis of organic solutes. Before discussing these blue-light responses, let us briefly describe the major osmotically active solutes in guard cells.

The botanist Hugo von Mohl proposed in 1856 that turgor changes in guard cells provide the mechanical force for changes in stomatal apertures. The plant physiologist F. E. Lloyd hypothesized in 1908 that guard cell turgor is regulated by osmotic changes resulting from starch–sugar interconversions, a concept that led to a starch–sugar hypothesis of stomatal movements. The discovery of the changes in potassium concentrations in guard cells in the 1960s led to the modern theory of guard cell osmoregulation by potassium and its counterions.

Potassium concentration in guard cells increases severalfold when stomata open, from 100 m*M* in the closed state to 400 to 800 m*M* in the open state, depending on the plant species and the experimental conditions. These large concentration changes in the positively charged potassium ions are electrically balanced by the anions Cl^- and $malate^{2-}$ (Figure 18.15A). In species of the genus *Allium*, such as onion (*Allium cepa*), K^+ ions are balanced solely by Cl^-. In most species, however, potassium fluxes are balanced by varying amounts of Cl^- and the organic anion $malate^{2-}$ (Talbott et al. 1996).

The Cl^- ion is taken up into the guard cells during stomatal opening and extruded during stomatal closing. Malate, on the other hand, is synthesized in the guard cell cytosol, in a metabolic pathway that uses carbon skeletons generated by starch hydrolysis (see Figure 18.15B). The malate content of guard cells decreases during stomatal closing, but it remains to be established whether malate is catabolized in mitochondrial respiration or is extruded into the apoplast.

Potassium and chloride are taken up into guard cells via secondary transport mechanisms driven by the gradient of electrochemical potential for H^+, $\Delta\mu_{H^+}$, generated by the proton pump (see Chapter 6) discussed earlier in the chapter. Proton extrusion makes the electric-potential difference across the guard cell plasma membrane more negative; light-dependent hyperpolarizations as high as 50 mV have been measured. In addition, proton pumping generates a pH gradient of about 0.5 to 1 pH unit.

The electrical component of the proton gradient provides a driving force for the passive uptake of potassium ions via voltage-regulated potassium channels (see Chapter 6) (Schroeder et al. 2001). Chloride is thought to be taken up through anion channels. Thus, blue light–dependent stimulation of proton pumping plays a key role in guard cell osmoregulation during light-dependent stomatal movements

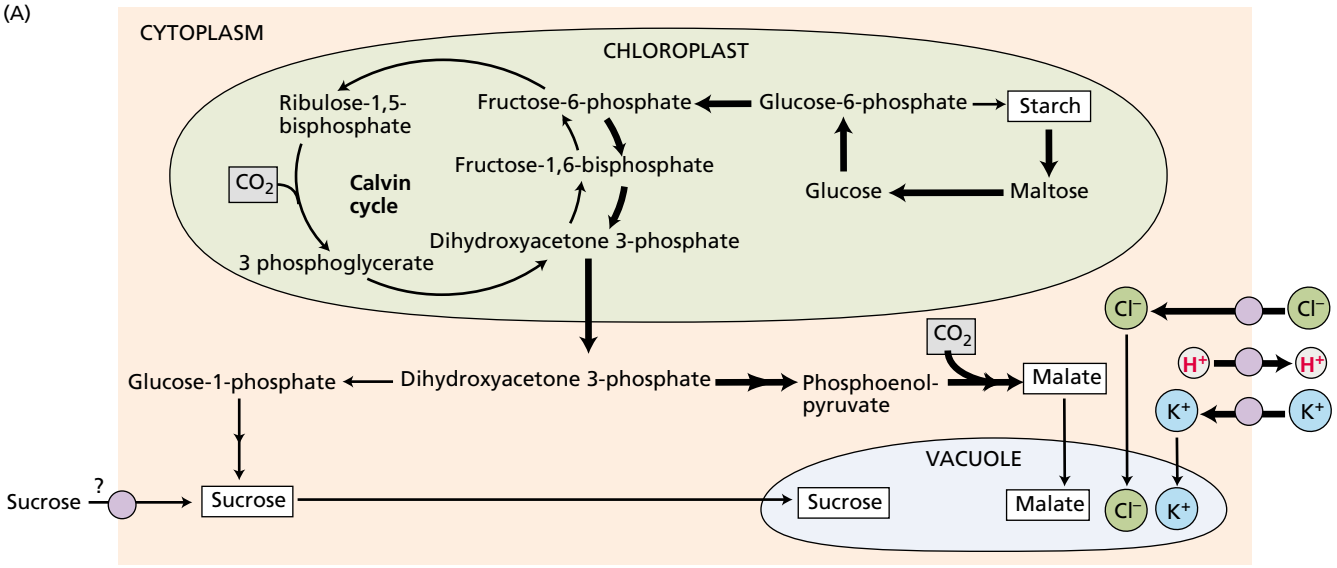
Guard cell chloroplasts (see Figure 18.8) contain large starch grains, and their starch content decreases during stomatal opening and increases during closing. Starch, an insoluble, high-molecular-weight polymer of glucose, does not contribute to the cell's osmotic potential, but the hydrolysis of starch into soluble sugars causes a decrease in the osmotic potential (or increase in osmotic pressure) of guard cells. In the reverse process, starch synthesis decreases the sugar concentration, resulting in an increase of the cell's osmotic potential, which the starch–sugar hypothesis predicted to be associated with stomatal closing.

With the discovery of the major role of potassium and its counterion in guard cell osmoregulation, the sugar–starch hypothesis was no longer considered important (Outlaw 1983). Recent studies, however, described in the next section, have characterized a major osmoregulatory phase of guard cells in which sucrose is the dominant osmotically active solute.

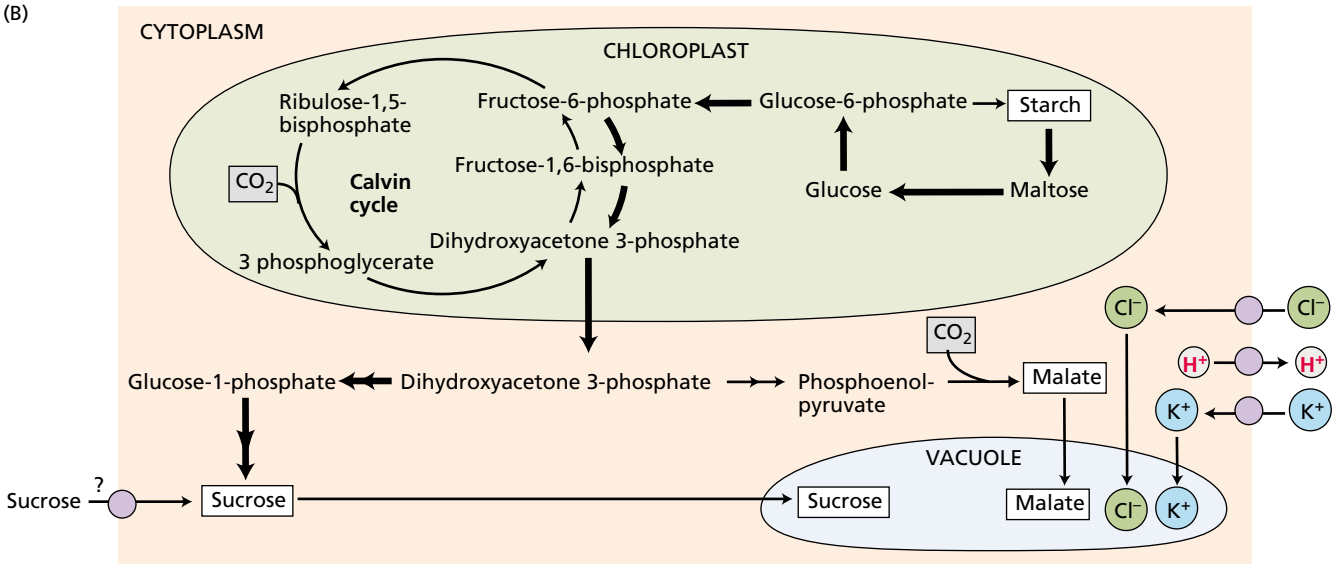
Sucrose Is an Osmotically Active Solute in Guard Cells

Studies of daily courses of stomatal movements in intact leaves have shown that the potassium content in guard cells increases in parallel with early-morning opening, but it decreases in the early afternoon under conditions in which apertures continue to increase. The sucrose content of guard cells increases slowly in the morning, but upon potassium efflux, sucrose becomes the dominant osmoti-

(A)


CYTOPLASM
CHLOROPLAST
Ribulose-1,5-bisphosphate
Fructose-6-phosphate
Glucose-6-phosphate
Starch
CO2
Calvin cycle
Fructose-1,6-bisphosphate
Glucose
Maltose
Dihydroxyacetone 3-phosphate
3 phosphoglycerate
Cl−
H+
K+
CO2
Glucose-1-phosphate
Dihydroxyacetone 3-phosphate
Phosphoenol-pyruvate
Malate
VACUOLE
Sucrose
?
Sucrose
Sucrose
Malate

(B)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CHLOROPLAST
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Fructose-6-phosphate
Glucose-6-phosphate
Starch
CO2
Calvin cycle
Fructose-1,6-bisphosphate
Glucose
Maltose
Dihydroxyacetone 3-phosphate
3 phosphoglycerate
Cl−
H+
K+
CO2
Glucose-1-phosphate
Dihydroxyacetone 3-phosphate
Phosphoenol-pyruvate
Malate
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Sucrose
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Sucrose
Sucrose
Malate

(C)

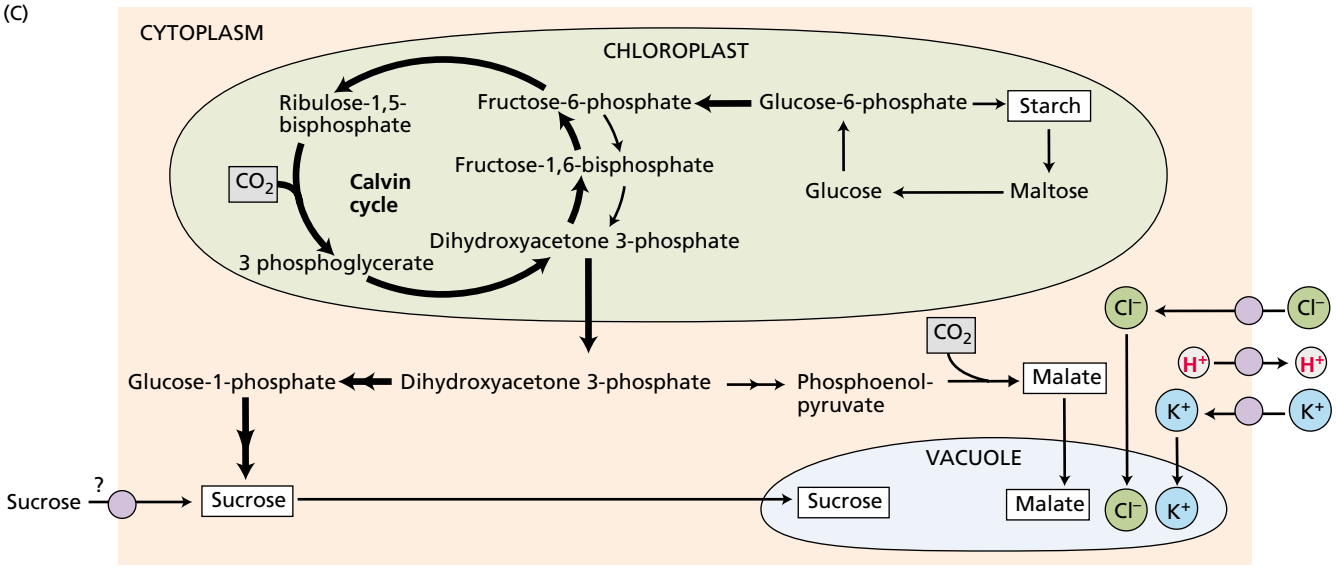
CYTOPLASM
CHLOROPLAST
Ribulose-1,5-bisphosphate
Fructose-6-phosphate
Glucose-6-phosphate
Starch
CO2
Calvin cycle
Fructose-1,6-bisphosphate
Glucose
Maltose
Dihydroxyacetone 3-phosphate
3 phosphoglycerate
Cl−
H+
K+
CO2
Glucose-1-phosphate
Dihydroxyacetone 3-phosphate
Phosphoenol-pyruvate
Malate
VACUOLE
Sucrose
?
Sucrose
Sucrose
Malate

FIGURE 18.15 Three distinct osmoregulatory pathways in guard cells. The dark arrows identify the major metabolic steps of each pathway that lead to the accumulation of osmotically active solutes in the guard cells. (A) Potassium and its counterions. Potassium and chloride are taken up in secondary transport processes driven by a proton gradient; malate is formed from the hydrolysis of starch. (B) Accumulation of sucrose from starch hydrolysis. (C) Accumulation of sucrose from photosynthetic carbon fixation. The possible uptake of apoplastic sucrose is also indicated. (From Talbott and Zeiger 1998.)

cally active solute, and stomatal closing at the end of the day parallels a decrease in the sucrose content of guard cells (Figure 18.16) (Talbott and Zeiger 1998).

These osmoregulatory features indicate that stomatal opening is associated primarily with K^+ uptake, and closing is associated with a decrease in sucrose content (see Figure 18.16). The need for distinct potassium- and sucrose-dominated osmoregulatory phases is unclear, but it might underlie regulatory aspects of stomatal function. Potassium might be the solute of choice for the consistent daily opening that occurs at sunrise. The sucrose phase might be associated with the coordination of stomatal movements in the epidermis with rates of photosynthesis in the mesophyll.

Where do osmotically active solutes originate? Four distinct metabolic pathways that can supply osmotically active solutes to guard cells have been characterized (see Figure 18.15):

1. The uptake of K^+ and Cl^- coupled to the biosynthesis of malate^{2-}
2. The production of sucrose from starch hydrolysis
3. The production of sucrose by photosynthetic carbon fixation in the guard cell chloroplast
4. The uptake of apoplastic sucrose generated by mesophyll photosynthesis

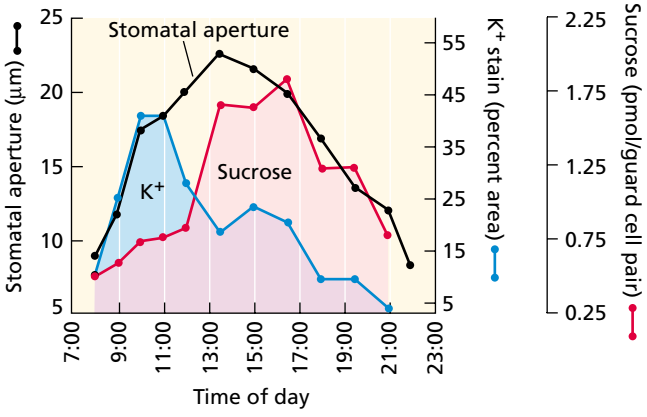

FIGURE 18.16 Daily course of changes in stomatal aperture, and in potassium and sucrose content, of guard cells from intact leaves of broad bean (*Vicia faba*). These results indicate that the changes in osmotic potential required for stomatal opening in the morning are mediated by potassium and its counterions, whereas the afternoon changes are mediated by sucrose. (After Talbott and Zeiger 1998.)

Depending on environmental conditions, one or several pathways may be activated. For instance, red light–stimulated stomatal opening in detached epidermis depends solely on sucrose generated by guard cell photosynthesis, with no detectable K^+ uptake. The other osmoregulatory pathways can be selectively activated under different experimental conditions (see **Web Topic 18.1**). Current studies are beginning to unravel the mysteries of guard cell osmoregulation in the intact leaf (Dietrich et al. 2001).

BLUE-LIGHT PHOTORECEPTORS

Experiments carried out by Charles Darwin and his son Francis in the nineteenth century determined that the site of photoreception in blue light–stimulated phototropism is in the coleoptile tip. Early hypotheses about blue-light photoreceptors focused on carotenoids and flavins (for a historical account of early research on blue-light photoreceptors, see **Web Topic 18.2**). Despite active research efforts, no significant advances toward the identification of blue-light photoreceptors were made until the early 1990s. In the case of phototropism and the inhibition of stem elongation, progress resulted from the identification of mutants for key blue-light responses, and the subsequent isolation of the relevant gene.

Cloning of the gene led to the identification and characterization of the protein encoded by the gene. In the case of stomatal guard cells, the carotenoid zeaxanthin has been postulated to be the chromophore of a blue-light photoreceptor, whereas the identity of the apoprotein remains to be established. For a detailed discussion of the basic differences between carotenoid and flavin photoreceptors, see **Web Topic 18.3**. In the following section we will describe the three photoreceptors associated with blue-light responses: cryptochromes, phototropins, and zeaxanthin.

Cryptochromes Are Involved in the Inhibition of Stem Elongation

The *hy4* mutant of *Arabidopsis* lacks the blue light–stimulated inhibition of hypocotyl elongation described earlier in the chapter. As a result of this genetic defect, *hy4* plants show an elongated hypocotyl when irradiated with blue light. Isolation of the *HY4* gene showed that it encodes a 75 kDa protein with significant sequence homology to microbial DNA **photolyase**, a blue light–activated enzyme that repairs pyrimidine dimers in DNA formed as a result of exposure to ultraviolet radiation (Ahmad and Cashmore 1993). In view of this sequence similarity, the hy4 protein, later renamed **cryptochrome 1** (**cry1**), was proposed to be a blue-light photoreceptor mediating the inhibition of stem elongation.

Photolyases are pigment proteins that contain a flavin adenine dinucleotide (FAD; see Figure 11.2B) and a pterin.

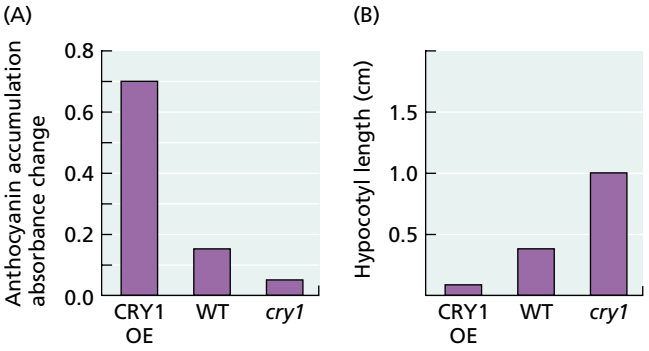

FIGURE 18.17 Blue light stimulates the accumulation of anthocyanin (A) and the inhibition of stem elongation (B) in transgenic and mutant seedlings of *Arabidopsis*. These bar graphs show a transgenic phenotype overexpressing the gene that encodes CRY1 (CRY1 OE), the wild type (WT), and *cry1* mutants. The enhanced blue-light response of the transgenic plant overexpressing the gene that encodes CRY1 demonstrates the important role of this gene product in stimulating anthocyanin biosynthesis and inhibiting stem elongation. (After Ahmad et al. 1998.)

Pterins are light-absorbing, pteridine derivatives that often function as pigments in insects, fishes, and birds (see Chapter 12 for pterin structure). When expressed in *Escherichia coli*, the cry1 protein binds FAD and a pterin, but it lacks detectable photolyase activity. No information is available on the chromophore(s) bound to cry1 in vivo, or on the nature of the photochemical reactions involving cry1, that would start the postulated sensory transduction cascade mediating the several blue-light responses mediated by cry1.

The most important evidence for a role of cry1 in blue light–mediated inhibition of stem elongation comes from overexpression studies. Overexpression of the CRY1 protein in transgenic tobacco or *Arabidopsis* plants results in a stronger blue light–stimulated inhibition of hypocotyl elongation than in the wild type, as well as increased production of anthocyanin, another blue-light response (Figure 18.17). Thus, overexpression of CRY1 caused an enhanced sensitivity to blue light in transgenic plants. Other blue-light responses, such as phototropism and blue light–dependent stomatal movements, appear to be normal in the *cry1* mutant phenotype.

A second gene product homologous to CRY1, named CRY2, has been isolated from *Arabidopsis* (Lin 2000). Both CRY1 and CRY2 appear ubiquitous throughout the plant kingdom. A major difference between them is that CRY2 is rapidly degraded in the light, whereas CRY1 is stable in light-grown seedlings.

Transgenic plants overexpressing the gene that encodes CRY2 show a small enhancement of the inhibition of hypocotyl elongation, indicating that unlike CRY1, CRY2 does not play a primary role in inhibiting stem elongation. On the other hand, the transgenic plants overexpressing the gene that encodes CRY2 show a large increase in blue light–stimulated cotyledon expansion, yet another blue-light response. In addition, CRY1 has been shown to be involved in the setting of the circadian clock in *Arabidopsis* (see Chapter 17), and both CRY1 and CRY2 have been shown to play a role in the induction of flowering (see Chapter 24). Cryptochrome homologs have been found to regulate the circadian clock in *Drosophila*, mouse, and humans.

Phototropins Are Involved in Phototropism and Chloroplast Movements

Some recently isolated *Arabidopsis* mutants impaired in blue light–dependent phototropism of the hypocotyl have provided valuable information about cellular events preceding bending. One of these mutants, the *nph1* (*n*on*p*hototropic *h*ypocotyl) mutant has been found to be genetically independent of the *hy4* (*cry1*) mutant discussed earlier: The *nph1* mutant lacks a phototropic response in the hypocotyl but has normal blue light–stimulated inhibition of hypocotyl elongation, while *hy4* has the converse phenotype. Recently the *nph1* gene was renamed *phot1*, and the protein it encodes was named **phototropin** (Briggs and Christie 2002).

The C-terminal half of phototropin is a serine/threonine kinase. The N-terminal half contains two repeated domains, of about 100 amino acids each, that have sequence similarities to other proteins involved in signaling in bacteria and mammals. Proteins with sequence similarity to the N terminus of phototropin bind flavin cofactors. These proteins are oxygen sensors in *Escherichia coli* and *Azotobacter*, and voltage sensors in potassium channels of *Drosophila* and vertebrates.

When expressed in insect cells, the N-terminal half of phototropin binds flavin mononucleotide (FMN) (see Figure 11.2B and **Web Essay 18.2**) and shows a blue light–dependent autophosphorylation reaction. This reaction resembles the blue light–dependent phosphorylation of a 120 kDa membrane protein found in growing regions of etiolated seedlings.

The *Arabidopsis* genome contains a second gene, *phot2*, which is related to *phot1*. The *phot1* mutant lacks hypocotyl phototropism in response to low-intensity blue light (0.01–1 μmol mol^{-2} s^{-1}) but retains a phototropic response at higher intensities (1–10 μmol m^{-2} s^{-1}). The *phot2* mutant has a normal phototropic response, but the *phot1/phot2* double mutant is severely impaired at both low and high intensities. These data indicate that both *phot1* and *phot2* are involved in the phototropic response, with *phot2* functioning at high light fluence rates.

Blue light–activated chloroplast movement. Leaves show an adaptive feature that can alter the intracellular distribution of their chloroplasts in order to control light absorption and prevent photodamage (see Figure 9.5). The action spectrum for chloroplast movement shows the "three finger" fine structure typical of blue-light responses. When incident radiation is weak, chloroplasts gather at the upper and lower surfaces of the mesophyll cells (the "accu-

mulation" response; see Figure 9.5B), thus maximizing light absorption.

Under strong light, the chloroplasts move to the cell surfaces that are parallel to the incident light (the "avoidance" response; see Figure 9.5C), thus minimizing light absorption. Recent studies have shown that mesophyll cells of the *phot1* mutant have a normal avoidance response and a rudimentary accumulation response. Cells from the *phot2* mutant show a normal accumulation response but lack the avoidance response. Cells from the *phot1/phot2* double mutant lack both the avoidance and accumulation responses (Sakai et al. 2001). These results indicate that *phot2* plays a key role in the avoidance response, and that both *phot1* and *phot2* contribute to the accumulation response.

The Carotenoid Zeaxanthin Mediates Blue-Light Photoreception in Guard Cells

The carotenoid zeaxanthin has been implicated as a photoreceptor in blue light–stimulated stomatal opening. Recall from Chapters 7 and 9 that zeaxanthin is one of the three components of the xanthophyll cycle of chloroplasts, which protects photosynthetic pigments from excess excitation energy. In guard cells, however, the changes in zeaxanthin content as a function of incident radiation are distinctly different from the changes in mesophyll cells (Figure 18.18).

In sun plants such as *Vicia faba*, zeaxanthin accumulation in the mesophyll begins at about 200 µmol m^{-2} s^{-1}, and there is no detectable zeaxanthin in the early morning or late afternoon. In contrast, the zeaxanthin content in guard cells closely follows incident solar radiation at the leaf surface throughout the day, and it is nearly linearly proportional to incident photon fluxes in the early morning and late afternoon. Several key characteristics of the guard cell chloroplast strongly indicate that the primary function of the guard cell chloroplast is sensory transduction and not carbon fixation (Zeiger et al. 2002).

Compelling evidence indicates that zeaxanthin is a blue-light photoreceptor in guard cells:

- The absorption spectrum of zeaxanthin (Figure 18.19) closely matches the action spectrum for blue light–stimulated stomatal opening (see Figure 18.11).
- In daily courses of stomatal opening in intact leaves grown in a greenhouse, incident radiation, zeaxanthin content of guard cells, and stomatal apertures are closely related (see Figure 18.18).
- The blue-light sensitivity of guard cells increases as a function of their zeaxanthin concentration. Experimentally, zeaxanthin concentration in guard cells can be varied with increasing fluence rates of red light. When guard cells from epidermal peels illuminated with increasing fluence rates of red light are exposed to blue light, the resulting blue light–stimulated stomatal opening is linearly related to the fluence rate of background red-light irradiation (see the wild-type treatment in Figure 18.20) and to

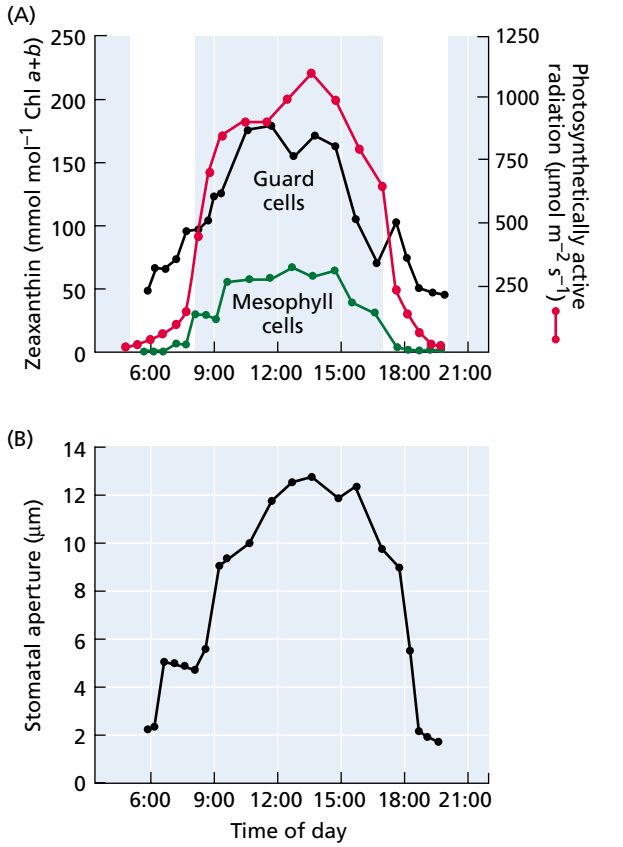

FIGURE 18.18 The zeaxanthin content of guard cells closely tracks photosynthetic active radiation and stomatal apertures. (A) Daily course of photosynthetic active radiation reaching the leaf surface, and of zeaxanthin content of guard cells and mesophyll cells of *Vicia faba* leaves grown in a greenhouse. The white areas within the graph highlight the contrasting sensitivity of the xanthophyll cycle in mesophyll and guard cell chloroplasts under the low irradiances prevailing early and late in the day. (B) Stomatal apertures in the same leaves used to measure guard cell zeaxanthin content. (After Srivastava and Zeiger 1995a.)

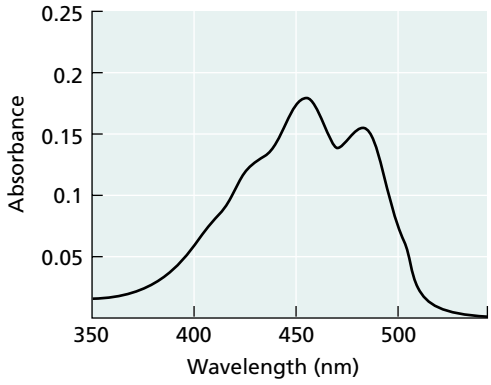

FIGURE 18.19 The absorption spectrum of zeaxanthin in ethanol.

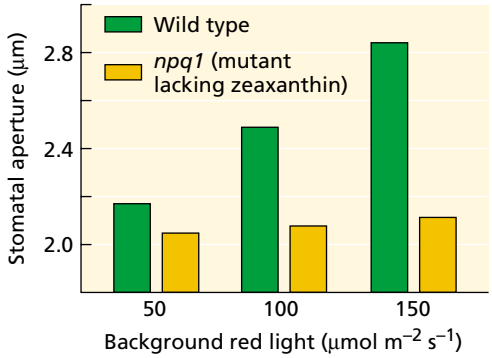

FIGURE 18.20 Stomatal responses to blue light in the wild type and *npq1*, an *Arabidopsis* mutant that lacks zeaxanthin. Stomata in detached epidermis were irradiated with red light for 2 hours, and 20 μmol m^{-2} s^{-1} of blue light was added for one additional hour. Stomatal opening in the wild type is proportional to the fluence rates of background red light. In contrast, *npq1* stomata lacked this response and showed reduced opening under both blue and red light, probably mediated by guard cell photosynthesis. (From Frechilla et al. 1999.)

zeaxanthin content (Srivastava and Zeiger 1995b). The same relationship among background red light, zeaxanthin content, and blue-light sensitivity has been found in blue light–stimulated phototropism of corn coleoptiles (see **Web Topic 18.4**).

- Blue light–stimulated stomatal opening is completely inhibited by 3 m*M* dithiothreitol (DTT), and the inhibition is concentration dependent. Zeaxanthin formation is blocked by DTT, a reducing agent that reduces S—S bonds to –SH groups and effectively inhibits the enzyme that converts violaxanthin into zeaxanthin. The specificity of the inhibition of blue light–stimulated stomatal opening by DTT, and its concentration dependence, indicate that guard cell zeaxanthin is required for the stomatal response to blue light.
- In the facultative CAM species *Mesembryanthemum crystallinum* (see Chapters 8 and 25), salt accumulation

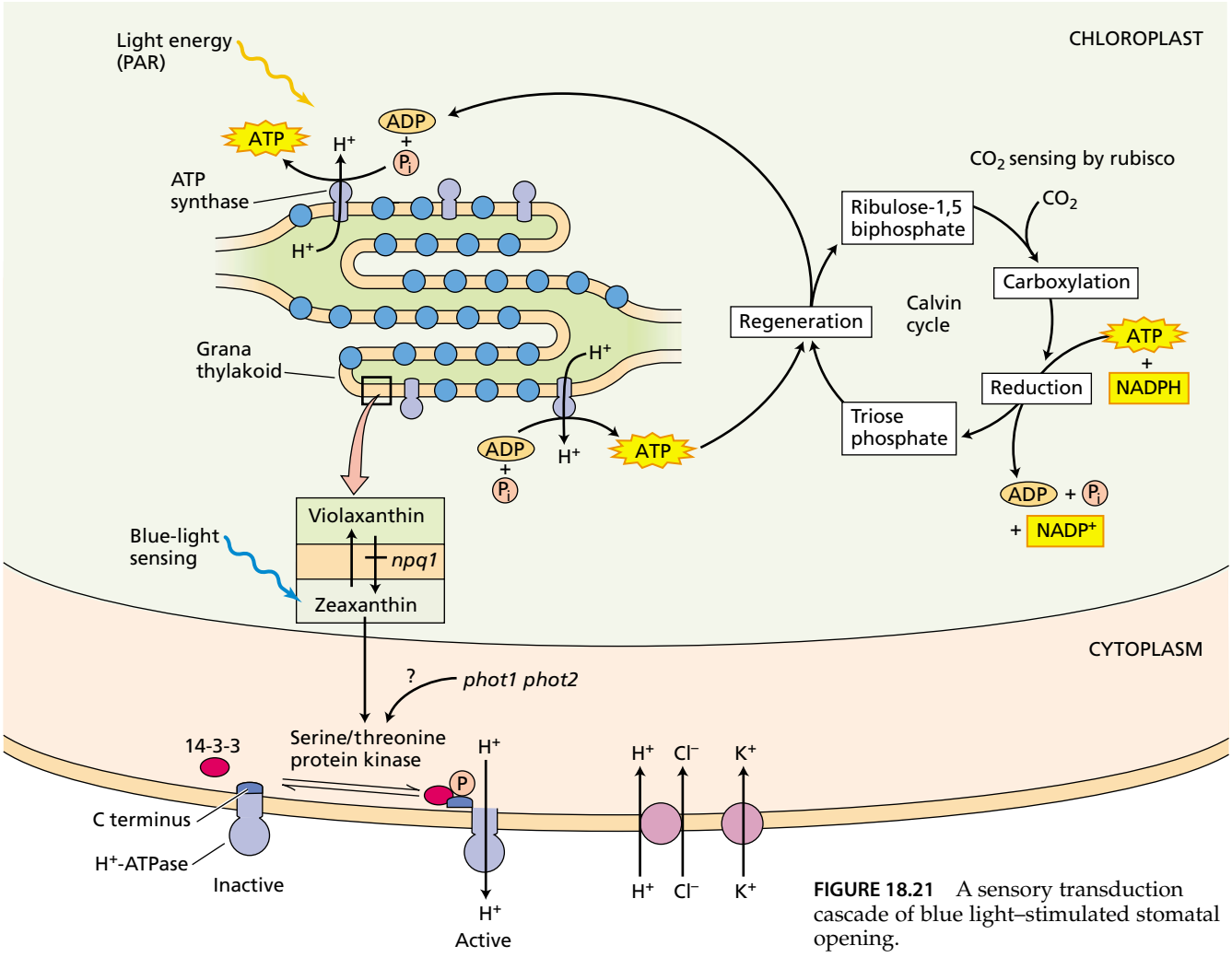

FIGURE 18.21 A sensory transduction cascade of blue light–stimulated stomatal opening.

shifts its carbon metabolism from C_3 to CAM mode. In the C_3 mode, stomata accumulate zeaxanthin and show a blue-light response. CAM induction inhibits the ability of guard cells to accumulate zeaxanthin, and to respond to blue light (Tallman et al. 1997).

***The blue-light response of the* Arabidopsis *mutant* npq1.** The *Arabidopsis* mutant *npq1* (*n*on*p*hotochemical *q*uenching), has a genetic lesion in the enzyme that converts violaxanthin into zeaxanthin (see Figure 18.21) (Niyogi et al. 1998). Because of this mutation, neither mesophyll nor guard cell chloroplasts of *npq1* accumulate zeaxanthin (Frechilla et al. 1999). Availability of this mutant made it possible to test the zeaxanthin hypothesis with guard cells in which zeaxanthin accumulation is genetically blocked.

Because photosynthesis in the guard cell chloroplast is stimulated by blue light (see Figure 18.10), an adequate test for the blue-light response of the zeaxanthin-less *npq1* mutant requires an experimental design ensuring that any observed response to blue light is blue light specific and not mediated by photosynthesis. As discussed earlier in the chapter, action spectra provide a stringent test of specificity, but determination of action spectra is time-consuming and labor-intensive.

Another option is to test the enhancement of blue-light sensitivity by background red light, a specific characteristic of blue light–stimulated stomatal movements (Assmann 1988), discussed earlier. In experiments testing the enhancement of the blue-light response in *npq1* by background red light, the zeaxanthin-less stomata showed baseline apertures in response to blue or red light, driven by guard cell photosynthesis, and failed to show any increases in the blue-light response.

The close relationship between incident solar radiation and zeaxanthin content in guard cells, and the role of zeaxanthin in blue-light photoreception suggest that the blue-light component of the stomatal response to light functions as a light sensor that couples stomatal apertures to incident photon fluxes at the leaf surface. The photosynthetic component, on the other hand, could function in the coupling of the stomatal responses with photosynthetic rates in the mesophyll (see Chapter 9).

The* phot1/phot2 *mutant lacks blue light–stimulated opening. Stomata from the *phot1/phot2* double mutant fail to exhibit a specific blue-light response, whereas in the single *phot1* or *phot2* mutant the blue-light response is only slightly affected (Kinoshita et al. 2001). These findings implicate phototropin in the blue-light response of stomata (Figure 18.21). It will be of great interest to determine whether phototropin is a second blue-light photoreceptor in guard cells or plays a regulatory role in later steps of the sensory transduction cascade.

SIGNAL TRANSDUCTION

Sensory transduction cascades for the blue-light responses encompass the sequence of events linking the initial absorption of blue light by a chromophore and the final expression of a blue-light response, such as stomatal opening or phototropism. In this section we will discuss available information on signal transduction cascades for cryptochromes, phototropin, and zeaxanthin.

Cryptochromes Accumulate in the Nucleus

The sequence similarity of cry1 and cry2 to photolyase suggests that like photolyase, cryptochromes initiate their sensory transduction cascade by the reduction of a flavin chromophore by light, and a subsequent electron transfer reaction to an electron acceptor (see Figure 11.2). However, there is no experimental evidence for an involvement of cry1 or cry2 in redox reactions.

Recent studies have shown that cry2, and to a lesser extent cry1, accumulates in the nucleus. This suggests that both proteins might be involved in the regulation of gene expression. But some of the cryptochrome action in response to blue light seems to occur in the cytoplasm because one of the earliest detected defects in *cry1* mutant seedlings is impaired activation of anion channels at the plasma membrane. In addition, cry1 and cry2 have been shown to interact with phytochrome A in vivo, and to be phosphorylated by phytochrome A in vitro (see Chapter 17 and **Web Essay 18.3**).

Phototropin Binds FMN

As discussed earlier, the products of the *phot1* and *phot2* genes expressed in vitro bind FMN and undergo photophosphorylation in response to blue light. Recent spectroscopic studies have shown that the blue light–induced spectral changes of phototropin-bound FMN resemble those typical of the binding of FMN to a cysteine residue of phototropin (Figure 18.22; see also **Web Essay 18.2**) (Swartz et al. 2001). This reaction is reversed by a dark treatment.

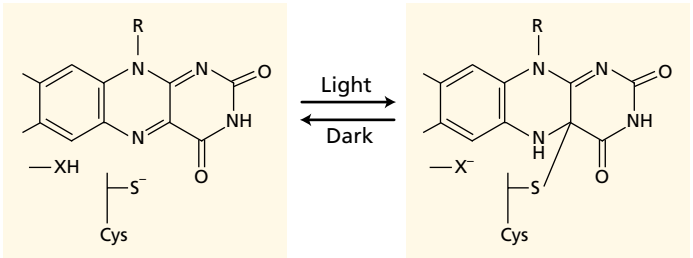

FIGURE 18.22 Proposed adduct formation of FMN and a cysteine residue of phototropin protein upon blue-light irradiation. XH and X^- represent an unidentified, proton donor acceptor. (After Briggs and Christie 2002.)

These results suggest that blue irradiation of the protein-bound FMN in intact cells causes a conformational change of phototropin that triggers autophosphorylation and starts the sensory transduction cascade. The cellular events that follow the autophosphorylation remain unknown.

High-resolution analysis of the changes in growth rate mediating the inhibition of hypocotyl elongation by blue light has provided valuable information about the interactions among phototropin, cry1, cry2, and the phytochrome phyA (Parks et al. 2001). After a lag of 30 s, blue light–treated, wild-type *Arabidopsis* seedlings show a rapid decrease in elongation rates during the first 30 minutes, and then they grow very slowly for several days (Figure 18.23).

Analysis of the same response in *phot1*, *cry1*, *cry2*, and *phyA* mutants has shown that suppression of stem elongation by blue light during seedling de-etiolation is initiated by *phot1*, with *cry1*, and to a limited extent *cry2*, modulating the response after 30 minutes. The slow growth rate of stems in blue light–treated seedlings is primarily a result of the persistent action of cry1, and this is the reason that *cry1* mutants of *Arabidopsis* show a long hypocotyl, compared to the short hypocotyl of the wild type. There is also a role for phytochrome A in at least the early stages of blue light–regulated growth because growth inhibition does not progress normally in *phyA* mutants.

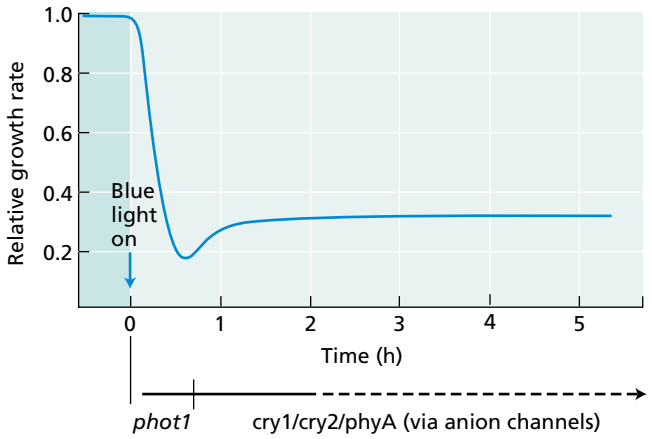

FIGURE 18.23 Sensory transduction cascade of blue light–stimulated inhibition of stem elongation in *Arabidopsis*. Elongation rates in the dark (0.25 mm h^{-1}) were normalized to 1. Within 30 s of the onset of blue-light irradiation, growth rates decreased and approached zero within 30 minutes, then continued at very reduced rates for several days. If blue light is applied to a *phot1* mutant, dark-growth rates remain unchanged for the first 30 minutes, indicating that the inhibition of elongation in the first 30 minutes is under phototropin control. Similar experiments with *cry1*, *cry2*, and *phyA* mutants indicate that the respective gene products control elongation rates at later times. (After Parks et al. 2001.)

Zeaxanthin Isomerization Might Start a Cascade Mediating Blue Light–Stimulated Stomatal Opening

Several key steps in the sensory transduction cascade for blue light–stimulated stomatal opening have been characterized (see Figure 18.21). The C terminus of the H^+-ATPase (see Figure 6.15) has an autoinhibitory domain that regulates the activity of the enzyme. If this autoinhibitory domain is experimentally removed by a protease, the H^+-ATPase becomes *irreversibly activated*. The autoinhibitory domain of the C terminus is thought to lower the activity of the enzyme by blocking its catalytic site. Conversely, fusiccocin appears to activate the enzyme by moving the autoinhibitory domain away from the catalytic site.

Upon blue-light irradiation, the H^+-ATPase shows a lower K_m for ATP and a higher V_{max} (see Chapter 6), indicating that blue light activates the H^+-ATPase. Activation of the enzyme involves the phosphorylation of serine and threonine residues of the C-terminal domain of the H^+-ATPase (Kinoshita and Shimazaki 1999). Blue light–stimulated proton pumping and stomatal opening are prevented by inhibitors of protein kinases, which might block phosphorylation of the H^+-ATPase. As with fusiccocin, phosphorylation of the C-terminal domain appears also to displace the autoinhibitory domain of the C-terminal from the catalytic site of the enzyme.

A **14-3-3 protein** has been found to bind to the phosphorylated C terminus of the guard cell H^+-ATPase, but not the nonphosphorylated one. The family of 14-3-3 proteins was originally discovered in brain tissue, and its members were found to be ubiquitous regulatory proteins in eukaryotic organisms. In plants, 14-3-3 proteins regulate transcription by binding to activators in the nucleus, and they regulate metabolic enzymes such as nitrate reductase.

Only one of the four 14-3-3 isoforms found in guard cells binds to the H^+-ATPase, so the binding appears to be specific (Emi et al. 2001). The same 14-3-3 isoform binds to the guard cell H^+-ATPase in response to both fusiccocin and blue-light treatments. The 14-3-3 protein seems to dissociate from the H^+-ATPase upon dephosphorylation of the C-terminal domain.

Proton-pumping rates of guard cells increase with fluence rates of blue light (see Figure 18.13), and the electrochemical gradient generated by the proton pump drives ion uptake into the guard cells, increasing turgor and turgor-mediated stomatal apertures. Taken together, these steps define the major sensory transducing steps linking the activation of a serine/threonine protein kinase by blue light and blue light–stimulated stomatal opening (see Figure 18.21).

The zeaxanthin hypothesis postulates that excitation of zeaxanthin in the antenna bed of the guard cell chloroplast by blue light starts the sensory transduction cascade that activates the serine/threonine kinase in the cytosol. Isomerization is the predominant photochemical reaction of

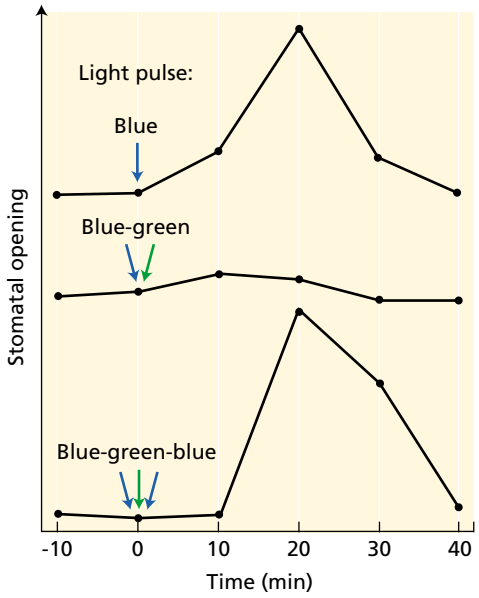

FIGURE 18.24 Blue/green reversibility of stomatal movements. Stomata open when given a 30 s blue-light pulse (1800 µmol m^{-2} s^{-1}) under a background of continuous red light (120 µmol m^{-2} s^{-1}). A green-light pulse (3600 µmol m^{-2} s^{-1}) applied after the blue-light pulse blocks the blue-light response, and the opening is restored upon application of a second blue-light pulse given after the green-light pulse. (After Frechilla et al. 2000.)

carotenoids, so blue light would isomerize zeaxanthin and the conformational change would start the transducing cascade.

The reversal of blue light–stimulated opening by green light. A reversal of blue light–stimulated stomatal opening by green light has been recently discovered. Stomata in epidermal strips open in response to a 30 s blue-light pulse (Figure 18.24), but the opening is not observed if the blue-light pulse is followed by a green-light pulse. The opening is restored if the green pulse is followed by a second blue-light pulse, in a response analogous to the red/far-red reversibility of phytochrome responses. (Frechilla et al. 2000.)

The blue/green reversibility response has been reported in stomata of several species, and in blue light–stimulated, coleoptile phototropism (see **Web Essay 18.4**). The role of the blue/green reversal of stomatal movements under natural conditions remains to be established, but it could be related to the sensing of environmental conditions such as sun and shade.

The action spectrum for the green reversal of blue light–stimulated opening shows a maximum at 540 nm, and two minor peaks at 490 and 580 nm. Such an action spectrum rules out the involvement of phytochrome or chlorophylls in the response. Rather, the action spectrum is remarkably similar to the action spectrum for blue light–stimulated stomatal opening (see Figure 18.11), but red-shifted (displaced toward the longer, red wave band of the spectrum) by about 90 nm.

Such spectral red shifts have been observed upon the isomerization of carotenoids in a protein environment (see **Web Essay 18.4**). In reconstituted vesicles containing chlorophyll *a/b*–binding protein and the xanthophylls zeaxanthin, violaxanthin, and neoxanthin, blue/green reversible absorption spectrum changes have been associated with zeaxanthin isomerization.

The blue/green reversal of stomatal movements and the absorption spectrum changes elicited by blue and green light suggest that a physiologically inactive, *trans* isomer of zeaxanthin is converted to a *cis* isomer by blue light, and that the isomerization starts the sensory transduction cascade. Available data suggest that green light converts the *cis* isomer into the physiologically inactive *trans* form, and therefore reverses the blue light–stimulated opening signal. Results from a previous study further indicate that after a blue pulse, the *cis* form slowly reverts to the *trans* form in the dark (Iino et al. 1985).

The Xanthophyll Cycle Confers Plasticity to the Stomatal Responses to Light

Zeaxanthin concentration in guard cells varies with the activity of the xanthophyll cycle. The enzyme that converts violaxanthin to zeaxanthin is an integral thylakoid protein showing a pH optimum at pH 5.2 (Yamamoto 1979). Acidification of lumen pH stimulates zeaxanthin formation, and lumen alkalinization favors violaxanthin formation.

Lumen pH depends on levels of incident photosynthetic active radiation (most effective at blue and red wavelengths; see Chapter 7), and on the rate of ATP synthesis, that dissipates the pH gradient across the thylakoid. Thus, photosynthetic activity in the guard cell chloroplast, lumen pH, zeaxanthin content, blue-light sensitivity, and stomatal apertures are tightly coupled.

Some unique properties of the guard cell chloroplast appear optimally geared for its sensory transducing function. Compared with their mesophyll counterparts, guard cell chloroplasts are enriched in photosystem II, and they have unusually high rates of photosynthetic electron transport and low rates of photosynthetic carbon fixation (Zeiger et al. 2002). These properties favor lumen acidification at low photon fluxes, and they explain zeaxanthin formation in the guard cell chloroplast early in the day (see Figure 18.18).

The regulation of zeaxanthin content by lumen pH, and the tight coupling between lumen pH and Calvin cycle activity in the guard cell chloroplast (see Figure 18.21) further suggest that zeaxanthin can also operate as a CO_2 sensor in guard cells (see **Web Essay 18.5**).

The remarkable progress achieved by the recent discoveries in the molecular biology of blue-light responses has

dramatically increased our understanding of the subject. The identification of cryptochromes, phototropin, and zeaxanthin as putative blue-light photoreceptors in plant cells has stimulated great interest in this aspect of plant photobiology. Current and future work is addressing important open questions, such as the detailed sequence of the sensory transduction cascades and the precise localization and composition of the pigment proteins involved. Ongoing research on the subject virtually ensures rapid further progress.

SUMMARY

Plants utilize light as a source of energy and as a signal that provides information about their environment. A large family of blue-light responses is used to sense light quantity and direction. These blue-light signals are transduced into electrical, metabolic, and genetic processes that allow plants to alter growth, development, and function in order to acclimate to changing environmental conditions. Blue-light responses include phototropism, stomatal movements, inhibition of stem elongation, gene activation, pigment biosynthesis, tracking of the sun by leaves, and chloroplast movements within cells.

Specific blue-light responses can be distinguished from other responses that have some sensitivity to blue light by a characteristic "three-finger" action spectrum in the 400 to 500 nm region.

The physiology of blue-light responses varies broadly. In phototropism, stems grow toward unilateral light sources by asymmetric growth on their shaded side. In the inhibition of stem elongation, perception of blue light depolarizes the membrane potential of elongating cells, and the rate of elongation rapidly decreases. In gene activation, blue light stimulates transcription and translation, leading to the accumulation of gene products that are required for the morphogenetic response to light.

Blue light–stimulated stomatal movements are driven by blue light–dependent changes in the osmoregulation of guard cells. Blue light stimulates an H^+-ATPase at the guard cell plasma membrane, and the resulting pumping of protons across the membrane generates an electrochemical-potential gradient that provides a driving force for ion uptake. Blue light also stimulates starch degradation and malate biosynthesis. Solute accumulation within the guard cells leads to stomatal opening. Guard cells also utilize sucrose as a major osmotically active solute, and light quality can change the activity of different osmoregulatory pathways that modulate stomatal movements.

Cry1 and *cry2* are two *Arabidopsis* genes involved in blue light–dependent inhibition of stem elongation, cotyledon expansion, anthocyanin synthesis, the control of flowering, and the setting of circadian rhythms. It has been proposed that CRY1 and CRY2 are apoproteins of flavin-containing pigment proteins that mediate blue-light photoreception.

The cry1 and cry2 gene products have sequence similarity to photolyase but no photolyase activity. The cry1 protein, and to a lesser extent cry2, accumulates in the nucleus and might be involved in gene expression. The cry1 protein also regulates anion channel activity at the plasma membrane.

The protein phototropin has a major role in the regulation of phototropism. The C-terminal half of phototropin is a serine/threonine kinase, and the N-terminal half has two flavin-binding domains. In vitro, phototropin binds the flavin FMN and autophosphorylates in response to blue light. Mutants called *phot1* and *phot2* are defective in phototropism and in chloroplast movements. The *phot1/phot2* double mutant lacks blue light–stimulated stomatal opening.

The chloroplastic carotenoid zeaxanthin has been implicated in blue-light photoreception in guard cells. Blue light–stimulated stomatal opening is blocked if zeaxanthin accumulation in guard cells is prevented by genetic or biochemical means. Manipulation of zeaxanthin content in guard cells makes it possible to regulate their response to blue light. The signal transduction cascade for the blue-light response of guard cells comprises blue-light perception in the guard cell chloroplast, transduction of the blue-light signal across the chloroplast envelope, activation of the H^+-ATPase, turgor buildup, and stomatal opening.

Web Material

Web Topics

18.1 Guard Cell Osmoregulation and a Blue Light–Activated Metabolic Switch
Blue light controls major osmoregulatory pathways in guard cells and unicellular algae.

18.2 Historical Notes on the Research of Blue-Light Photoreceptors
Carotenoids and flavins have been the main candidates for blue-light photoreceptors.

18.3 Comparing Flavins and Carotenoids
Flavin and carotenoid photoreceptors have contrasting functional properties.

18.4 The Coleoptile Chloroplast
Both the coleoptile and the guard cell chloroplasts specialize in sensory transduction.

Web Essays

18.1 Guard Cell Photosynthesis
Photosynthesis in the guard cell chloroplast shows unique regulatory features.

18.2 Phototropins
Phototropins regulate several light responses in plants.

18.3 The Sensory Transduction of the Inhibition of Stem Elongation by Blue Light

The regulation of stem elongation rates by blue light has critical importance for plant development.

18.4 The Blue/Green Reversibility of the Blue-Light Response of Stomata

The blue/green reversal of stomatal movements is a remarkable photobiological response.

18.5 Zeaxanthin and CO_2 Sensing in Guard Cells

The functional relationship between Calvin cycle activity and zeaxanthin content of guard cells couples blue light and CO_2 sensing during stomatal movements.

Chapter References

Ahmad, M., and Cashmore, A. R. (1993) *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue light photoreceptor. *Nature* 366: 162–166.

Ahmad, M., Jarillo, J. A., Smirnova, O., and Cashmore, A. R. (1998) Cryptochrome blue light photoreceptors of *Arabidopsis* implicated in phototropism. *Nature* 392: 720–723.

Amodeo, G., Srivastava, A., and Zeiger, E. (1992) Vanadate inhibits blue light–stimulated swelling of *Vicia* guard cell protoplasts. *Plant Physiol.* 100: 1567–1570.

Assmann, S. M. (1988) Enhancement of the stomatal response to blue light by red light, reduced intercellular concentrations of carbon dioxide and low vapor pressure differences. *Plant Physiol.* 87: 226–231.

Assmann, S. M., Simoncini, L., and Schroeder, J. I. (1985) Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. *Nature* 318: 285–287.

Briggs, W. R., and Christie, J. M. (2002) Phototropins 1 and 2: Versatile plant blue-light receptors. *Trends Plant Sci.* 7: 204–210.

Cerda-Olmedo, E., and Lipson, E. D. (1987) *Phycomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Cosgrove, D. J. (1994) Photomodulation of growth. In *Photomorphogenesis in Plants*, 2nd ed., R. E. Kendrick and G. H. M. Kronenberg, eds., Kluwer, Dordrecht, Netherlands, pp. 631–658.

Dietrich, P., Sanders, D., and Hedrich, R. (2001) The role of ion channels in light-dependent stomatal opening. *J. Exp. Bot.* 52: 1959–1967.

Emi, T., Kinoshita, T., and Shimazaki, K. (2001) Specific binding of vf14-3-3a isoform to the plasma membrane H+-ATPase in response to blue light and fusicoccin in guard cells of broad bean. *Plant Physiol.* 125: 1115–1125.

Firn, R. D. (1994) Phototropism. In *Photomorphogenesis in Plants*, 2nd ed., R. E. Kendrick and G. H. M. Kronenberg, eds., Kluwer, Dordrecht, Netherlands, pp. 659–681.

Frechilla, S., Talbott, L. D., Bogomolni, R. A., and Zeiger, E. (2000) Reversal of blue light-stimulated stomatal opening by green light. *Plant Cell Physiol.* 41: 171–176.

Frechilla, S., Zhu, J., Talbott, L. D., and Zeiger, E. (1999) Stomata from npq1, a zeaxanthin-less *Arabidopsis* mutant, lack a specific response to blue light. *Plant Cell Physiol.* 40: 949–954.

Horwitz, B. A. (1994) Properties and transduction chains of the UV and blue light photoreceptors. In *Photomorphogenesis in Plants*, 2nd ed., R. E. Kendrick and G. H. M. Kronenberg, eds., Kluwer, Dordrecht, Netherlands, pp. 327–350.

Iino, M., Ogawa, T., and Zeiger, E. (1985) Kinetic properties of the blue light response of stomata. *Proc. Natl. Acad. Sci. USA* 82: 8019–8023.

Karlsson, P. E. (1986) Blue light regulation of stomata in wheat seedlings. II. Action spectrum and search for action dichroism. *Physiol. Plant.* 66: 207–210.

Kinoshita, T., and Shimazaki, K. (1999) Blue light activates the plasma membrane H^+-ATPase by phosphorylation of the C-terminus in stomatal guard cells. *EMBO J.* 18: 5548–5558.

Kinoshita, T., and Shimazaki, K. (2001) Analysis of the phosphorylation level in guard-cell plasma membrane H^+-ATPase in response to fusicoccin. *Plant Cell Physiol.* 42: 424–432.

Kinoshita, T., Doi, M., Suetsugu, N., Kagawa, T., Wada, M., and Shimazaki, K. (2001) phot1 and phot2 mediate blue light regulation of stomatal opening. *Nature* 414: 656–660.

Lin, C. (2000) Plant blue-light receptors. *Trends Plant Sci.* 5: 337–342.

Matters, G. L., and Beale, S. I. (1995) Blue-light-regulated expression of genes for two early steps of chlorophyll biosynthesis in *Chlamydomonas reinhardtii*. *Plant Physiol.* 109: 471–479.

Niyogi, K. K., Grossman, A. R., and Björkman, O. (1998) *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *Plant Cell* 10: 1121–1134.

Outlaw, W. H., Jr. (1983) Current concepts on the role of potassium in stomatal movements. *Physiol. Plant.* 59: 302–311.

Parks, B. M., Cho, M. H., and Spalding, E. P. (1998) Two genetically separable phases of growth inhibition induced by blue light in Arabidopsis seedlings. *Plant Physiol.* 118: 609–615.

Parks, B. M., Folta, K. M., and Spalding, E. P. (2001) Photocontrol of stem growth. *Curr. Opin. Plant Biol.* 4: 436–440.

Sakai, T., Kagawa, T., Kasahara, M., Swartz, T. E., Christie, J. M., Briggs, W. R., Wada, M., and Okada, K. (2001) Arabidopsis nph1 and npl1: Blue light receptors that mediate both phototropism and chloroplast relocation. *Proc. Natl. Acad. Sci. USA* 98: 6969–6974.

Schroeder, J. I., Allen, G. J., Hugouvieux, V., Kwak, J. M., and Waner, D. (2001) Guard cell signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 627–658.

Schwartz, A., and Zeiger, E. (1984) Metabolic energy for stomatal opening. Roles of photophosphorylation and oxidative phosphorylation. *Planta* 161: 129–136.

Senger, H. (1984) *Blue Light Effects in Biological Systems*. Springer, Berlin.

Serrano, E. E., Zeiger, E., and Hagiwara, S. (1988) Red light stimulates an electrogenic proton pump in *Vicia* guard cell protoplasts. *Proc. Natl. Acad. Sci. USA* 85: 436–440.

Shimazaki, K., Iino, M., and Zeiger, E. (1986) Blue light–dependent proton extrusion by guard cell protoplasts of *Vicia faba*. *Nature* 319: 324–326.

Spalding, E. P., and Cosgrove, D. J. (1989) Large membrane depolarization precedes rapid blue-light induced growth inhibition in cucumber. *Planta* 178: 407–410.

Srivastava, A., and Zeiger, E. (1995a) Guard cell zeaxanthin tracks photosynthetic active radiation and stomatal apertures in *Vicia faba* leaves. *Plant Cell Environ.* 18: 813–817.

Srivastava, A., and Zeiger, E. (1995b) The inhibitor of zeaxanthin formation, dithiothreitol, inhibits blue-light-stimulated stomatal opening in *Vicia faba*. *Planta* 196: 445–449.

Swartz, T. E., Corchnoy, S. B., Christie, J. M., Lewis, J. W., Szundi, I., Briggs, W. R., and Bogomolni, R. A. (2001) The photocycle of a flavin-binding domain of the blue light photoreceptor phototropin. *J. Biol. Chem.* 276: 36493–36500.

Talbott, L. D., and Zeiger, E. (1998) The role of sucrose in guard cell osmoregulation. *J. Exp. Bot*. 49: 329–337.

Talbott, L. D., Srivastava, A., and Zeiger, E. (1996) Stomata from growth-chamber-grown *Vicia faba* have an enhanced sensitivity to CO_2. *Plant Cell Environ.* 19: 1188–1194.

Tallman, G., Zhu, J., Mawson, B. T., Amodeo, G., Nouhi, Z., Levy, K., and Zeiger, E. (1997) Induction of CAM in *Mesembryanthemum crystallinum* abolishes the stomatal response to blue light and light-dependent zeaxanthin formation in guard cell chloroplasts. *Plant Cell Physiol.* 38: 236–242.

Thimann, K. V., and Curry, G. M. (1960) Phototropism and phototaxis. In *Comparative Biochemistry*, Vol. 1, M. Florkin and H. S. Mason, eds., Academic Press, New York, pp. 243–306.

Tsunoyama, Y., Morikawa, K., Shiina, T., and Toyoshima, Y. (2002) Blue light specific and differential expression of a plastid sigma factor, Sig5 in *Arabidopsis thaliana*. *FEBS Lett.* 516: 225–228.

Vogelmann, T. C. (1994) Light within the plant. In *Photomorphogenesis in Plants*, 2nd ed., R. E. Kendrick and G. H. M. Kronenberg, eds., Kluwer, Dordrecht, Netherlands, pp. 491–533.

Vogelmann, T. C., and Haupt, W. (1985) The blue light gradient in unilaterally irradiated maize coleoptiles: Measurements with a fiber optic probe. *Photochem. Photobiol.* 41: 569–576.

Yamamoto, H. Y. (1979) Biochemistry of the violaxanthin cycle in higher plants. *Pure Appl. Chem.* 51: 639–648.

Zeiger, E., and Hepler, P. K. (1977) Light and stomatal function: Blue light stimulates swelling of guard cell protoplasts. *Science* 196: 887–889.

Zeiger, E., Talbott, L. D., Frechilla, S., Srivastava, A., and Zhu, J. X. (2002) The guard cell chloroplast: A perspective for the twenty-first century. *New Phytol.* 153: 415–424.