Chapter

17

Phytochrome and Light Control of Plant Development

HAVE YOU EVER LIFTED UP A BOARD that has been lying on a lawn for a few weeks and noticed that the grass growing underneath was much paler and spindlier than the surrounding grass? The reason this happens is that the board is opaque, keeping the underlying grass in darkness. Seedlings grown in the dark have a pale, unusually tall and spindly appearance. This form of growth, known as **etiolated growth**, is dramatically different from the stockier, green appearance of seedlings grown in the light (Figure 17.1).

Given the key role of photosynthesis in plant metabolism, one might be tempted to attribute much of this contrast to differences in the availability of light-derived metabolic energy. However, it takes very little light or time to initiate the transformation from the etiolated to the green state. So in the change from dark to light growth, light acts as a developmental trigger rather than a direct energy source.

If you were to remove the board and expose the pale patch of grass to light, it would appear almost the same shade of green as the surrounding grass within a week or so. Although not visible to the naked eye, these changes actually start almost immediately after exposure to light. For example, within hours of applying a single flash of relatively dim light to a dark-grown bean seedling in the laboratory, one can measure several developmental changes: a decrease in the rate of stem elongation, the beginning of apical-hook straightening, and the initiation of the synthesis of pigments that are characteristic of green plants.

Light has acted as a signal to induce a change in the form of the seedling, from one that facilitates growth beneath the soil, to one that is more adaptive to growth above ground. In the absence of light, the seedling uses primarily stored seed reserves for etiolated growth. However, seed plants, including grasses, don't store enough energy to sustain growth indefinitely. They require light energy not only to fuel photosynthesis, but to initiate the developmental switch from dark to light growth.

Photosynthesis cannot be the driving force of this transformation because chlorophyll is not present during this time. Full de-etiolation

FIGURE 17.1 Corn (*Zea mays*) (A and B) and bean (*Phaseolus vulgaris*) (C and D) seedlings grown either in the light (A and C) or the dark (B and D). Symptoms of etiolation in corn, a monocot, include the absence of greening, reduction in leaf size, failure of leaves to unroll, and elongation of the coleoptile and mesocotyl. In bean, a dicot, etiolation symptoms include absence of greening, reduced leaf size, hypocotyl elongation, and maintenance of the apical hook. (Photos © M. B. Wilkins.)

does require some photosynthesis, but the initial rapid changes are induced by a distinctly different light response, called **photomorphogenesis** (from Latin, meaning literally "light form begins").

Among the different pigments that can promote photomorphogenic responses in plants, the most important are those that absorb red and blue light. The blue-light photoreceptors will be discussed in relation to guard cells and phototropism in Chapter 18. The focus of this chapter is **phytochrome**, a protein pigment that absorbs red and far-red light most strongly, but that also absorbs blue light. As we will see in this chapter and in Chapter 24, phytochrome plays a key role in light-regulated vegetative and reproductive development.

We begin with the discovery of phytochrome and the phenomenon of red/far-red photoreversibility. Next we will discuss the biochemical and photochemical properties of phytochrome, and the conformational changes induced by light. Different types of phytochromes are encoded by different members of a multigene family, and different phytochromes regulate distinct processes in the plant. These different phytochrome responses can be classified according to the amount of light and light quality required to produce the effect. Finally, we will examine what is known about the mechanism of phytochrome action at the cellular and molecular levels, including signal transduction pathways and gene regulation.

THE PHOTOCHEMICAL AND BIOCHEMICAL PROPERTIES OF PHYTOCHROME

Phytochrome, a blue protein pigment with a molecular mass of about 125 kDa (kilodaltons), was not identified as a unique chemical species until 1959, mainly because of technical difficulties in isolating and purifying the protein. However, many of the biological properties of phytochrome had been established earlier in studies of whole plants.

The first clues regarding the role of phytochrome in plant development came from studies that began in the 1930s on red light–induced morphogenic responses, especially seed germination. The list of such responses is now enormous and includes one or more responses at almost every stage in the life history of a wide range of different green plants (Table 17.1).

A key breakthrough in the history of phytochrome was the discovery that the effects of *red light* (650–680 nm) on morphogenesis could be reversed by a subsequent irradiation with light of longer wavelengths (710–740 nm), called *far-red light*. This phenomenon was first demonstrated in germinating seeds, but was also observed in relation to stem and leaf growth, as well as floral induction (see Chapter 24).

The initial observation was that the germination of lettuce seeds is stimulated by red light and inhibited by far-red light. But the real breakthrough was made many years later when lettuce seeds were exposed to alternating treatments of red and far-red light. Nearly 100% of the seeds that received red light as the final treatment germinated; in seeds that received far-red light as the final treatment, however, germination was strongly inhibited (Figure 17.2) (Flint 1936).

Two interpretations of these results were possible. One is that there are two pigments, a red light–absorbing pigment and a far-red light–absorbing pigment, and the two pigments act antagonistically in the regulation of seed germination. Alternatively, there might be a single pigment that can exist in two interconvertible forms: a red

TABLE 17.1
Typical photoreversible responses induced by phytochrome in a variety of higher and lower plants

Group	Genus	Stage of development	Effect of red light
Angiosperms	*Lactuca* (lettuce)	Seed	Promotes germination
	Avena (oat)	Seedling (etiolated)	Promotes de-etiolation (e.g., leaf unrolling)
	Sinapis (mustard)	Seedling	Promotes formation of leaf primordia, development of primary leaves, and production of anthocyanin
	Pisum (pea)	Adult	Inhibits internode elongation
	Xanthium (cocklebur)	Adult	Inhibits flowering (photoperiodic response)
Gymnosperms	*Pinus* (pine)	Seedling	Enhances rate of chlorophyll accumulation
Pteridophytes	*Onoclea* (sensitive fern)	Young gametophyte	Promotes growth
Bryophytes	*Polytrichum* (moss)	Germling	Promotes replication of plastids
Chlorophytes	*Mougeotia* (alga)	Mature gametophyte	Promotes orientation of chloroplasts to directional dim light

light–absorbing form and a far-red light–absorbing form (Borthwick et al. 1952).

The model chosen—the one-pigment model—was the more radical of the two because there was no precedent for such a photoreversible pigment. Several years later phytochrome was demonstrated in plant extracts for the first time, and its unique photoreversible properties were exhibited in vitro, confirming the prediction (Butler et al. 1959).

In this section we will consider three broad topics:

1. Photoreversibility and its relationship to phytochrome responses
2. The structure of phytochrome, its synthesis and assembly, and the conformational changes associated with the interconversions of the two main forms of phytochrome: Pr and Pfr
3. The phytochrome gene family, the members of which have different functions in photomorphogenesis

Phytochrome Can Interconvert between Pr and Pfr Forms

In dark-grown or etiolated plants, phytochrome is present in a red light–absorbing form, referred to as **Pr** because it

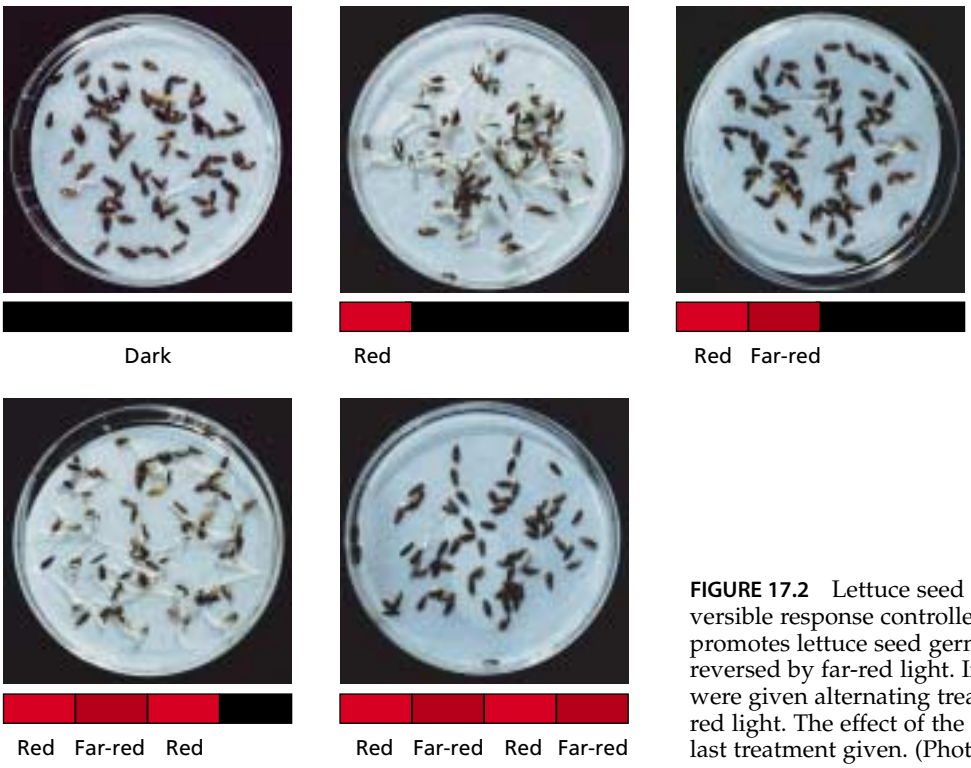


FIGURE 17.2 Lettuce seed germination is a typical photoreversible response controlled by phytochrome. Red light promotes lettuce seed germination, but this effect is reversed by far-red light. Imbibed (water-moistened) seeds were given alternating treatments of red followed by far-red light. The effect of the light treatment depended on the last treatment given. (Photos © M. B. Wilkins.)

is synthesized in this form. Pr, which to the human eye is blue, is converted by red light to a far-red light–absorbing form called **Pfr**, which is blue-green. Pfr, in turn, can be converted back to Pr by far-red light.

Known as **photoreversibility**, this conversion/reconversion property is the most distinctive property of phytochrome, and it may be expressed in abbreviated form as follows:

$$\text{Pr} \underset{\text{Far-red light}}{\overset{\text{Red light}}{\rightleftharpoons}} \text{Pfr}$$

The interconversion of the Pr and Pfr forms can be measured in vivo or in vitro. In fact, most of the spectral properties of carefully purified phytochrome measured in vitro are the same as those observed in vivo.

When Pr molecules are exposed to red light, most of them absorb it and are converted to Pfr, but some of the Pfr also absorbs the red light and is converted back to Pr because both Pr and Pfr absorb red light (Figure 17.3). Thus the proportion of phytochrome in the Pfr form after saturating irradiation by red light is only about 85%. Similarly, the very small amount of far-red light absorbed by Pr makes it impossible to convert Pfr entirely to Pr by broad-spectrum far-red light. Instead, an equilibrium of 97% Pr and 3% Pfr is achieved. This equilibrium is termed the **photostationary state**.

In addition to absorbing red light, both forms of phytochrome absorb light in the blue region of the spectrum (see Figure 17.3). Therefore, phytochrome effects can be elicited also by blue light, which can convert Pr to Pfr and vice versa. Blue-light responses can also result from the action of one or more specific blue-light photoreceptors (see Chapter 18). Whether phytochrome is involved in a response to blue light is often determined by a test of the ability of far-red light to reverse the response, since only phytochrome-induced responses are reversed by far-red light. Another way to discriminate between photoreceptors is to study mutants that are deficient in one of the photoreceptors.

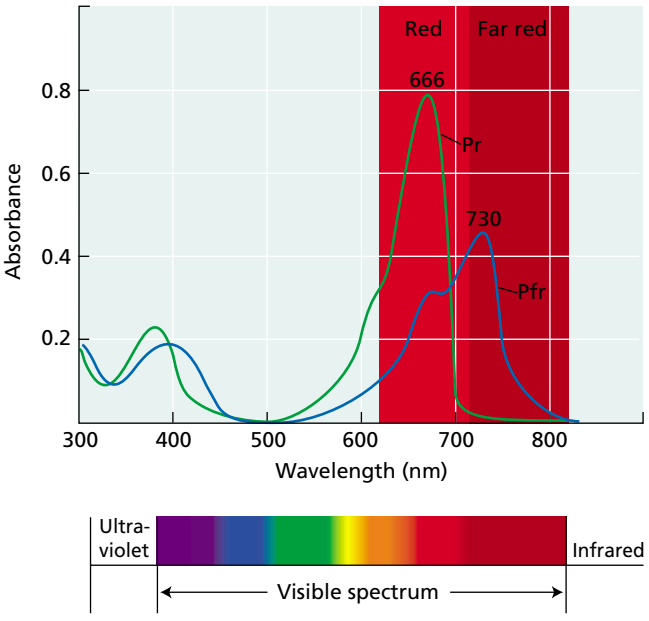


FIGURE 17.3 Absorption spectra of purified oat phytochrome in the Pr (green line) and Pfr (blue line) forms overlap. (After Vierstra and Quail 1983.)

Short-lived phytochrome intermediates. The photoconversions of Pr to Pfr, and of Pfr to Pr, are not one-step processes. By irradiating phytochrome with very brief flashes of light, we can observe absorption changes that occur in less than a millisecond.

Of course, sunlight includes a mixture of all visible wavelengths. Under such white-light conditions, both Pr and Pfr are excited, and phytochrome cycles continuously between the two. In this situation the intermediate forms of phytochrome accumulate and make up a significant fraction of the total phytochrome. Such intermediates could even play a role in initiating or amplifying phytochrome responses under natural sunlight, but this question has yet to be resolved.

Pfr Is the Physiologically Active Form of Phytochrome

Because phytochrome responses are induced by red light, they could in theory result either from the appearance of Pfr or from the disappearance of Pr. In most cases studied, a quantitative relationship holds between the magnitude of the physiological response and the amount of Pfr generated by light, but no such relationship holds between the physiological response and the loss of Pr.

Evidence such as this has led to the conclusion that Pfr is the physiologically active form of phytochrome. In cases in which it has been shown that a phytochrome response is not quantitatively related to the absolute amount of Pfr, it has been proposed that the ratio between Pfr and Pr, or between Pfr and the total amount of phytochrome, determines the magnitude of the response.

The conclusion that Pfr is the physiologically active form of phytochrome is supported by studies with mutants of *Arabidopsis* that are unable to synthesize phytochrome. In wild-type seedlings, hypocotyl elongation is strongly inhibited by white light, and phytochrome is one of the photoreceptors involved in this response. When grown under continuous white light, mutant seedlings with long hypocotyls were discovered and were termed *hy* mutants. Different *hy* mutants are designated by numbers: *hy1*, *hy2*, and so on. Because white light is a mixture of wavelengths (including red, far red, and blue), some, but not all, of the *hy* mutants have been shown to be deficient for one or more functional phytochrome(s).

The phenotypes of phytochrome-deficient mutants have been useful in identifying the physiologically active form of phytochrome. If the phytochrome-induced response to white light (hypocotyl growth inhibition) is caused by the absence of Pr, such phytochrome-deficient mutants (which have neither Pr nor Pfr) should have short hypocotyls in both darkness and white light. Instead, the opposite occurs; that is, they have long hypocotyls in both darkness and white light. It is the absence of Pfr that prevents the seedlings from responding to white light. In other words, Pfr brings about the physiological response.

Phytochrome Is a Dimer Composed of Two Polypeptides

Native phytochrome is a soluble protein with a molecular mass of about 250 kDa. It occurs as a dimer made up of two equivalent subunits. Each subunit consists of two components: a light-absorbing pigment molecule called the **chromophore**, and a polypeptide chain called the **apoprotein**. The apoprotein monomer has a molecular mass of about 125 kDa. Together, the apoprotein and its chromophore make up the **holoprotein**. In higher plants the chromophore of phytochrome is a linear tetrapyrrole termed **phytochromobilin**. There is only one chromophore per monomer of apoprotein, and it is attached to the protein through a thioether linkage to a cysteine residue (Figure 17.4).

Researchers have visualized the Pr form of phytochrome using electron microscopy and X-ray scattering, and the model shown in Figure 17.5 has been proposed (Nakasako et al. 1990). The polypeptide folds into two major domains separated by a "hinge" region. The larger N-terminal domain is approximately 70 kDa and bears the chromophore; the smaller C-terminal domain is approximately 55 kDa and contains the site where the two monomers associate with each other to form the dimer (see **Web Topic 17.1**).

Phytochromobilin Is Synthesized in Plastids

The phytochrome apoprotein alone cannot absorb red or far-red light. Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein. Phytochromobilin is synthesized inside plastids and is derived from 5-aminolevulinic acid via a pathway that branches from the chlorophyll biosynthetic pathway (see **Web Topic 7.11**). It is thought to leak out of the plastid into the cytosol by a passive process.

Assembly of the phytochrome apoprotein with its chromophore is **autocatalytic**; that is, it occurs spontaneously when purified phytochrome polypeptide is mixed with purified chromophore in the test tube, with no additional proteins or cofactors (Li and Lagarias 1992). The resultant holoprotein has spectral properties similar to those observed for the holoprotein purified from plants, and it exhibits red/far-red reversibility (Li and Lagarias 1992).

Mutant plants that lack the ability to synthesize the chromophore are defective in processes that require the action of phytochrome, even though the apoprotein polypeptides are present. For example, several of the *hy* mutants noted earlier, in which white light fails to suppress hypocotyl elongation, have defects in chromophore biosynthesis. In *hy1* and *hy2* mutant plants, phytochrome apoprotein levels are normal, but there is little or no spectrally

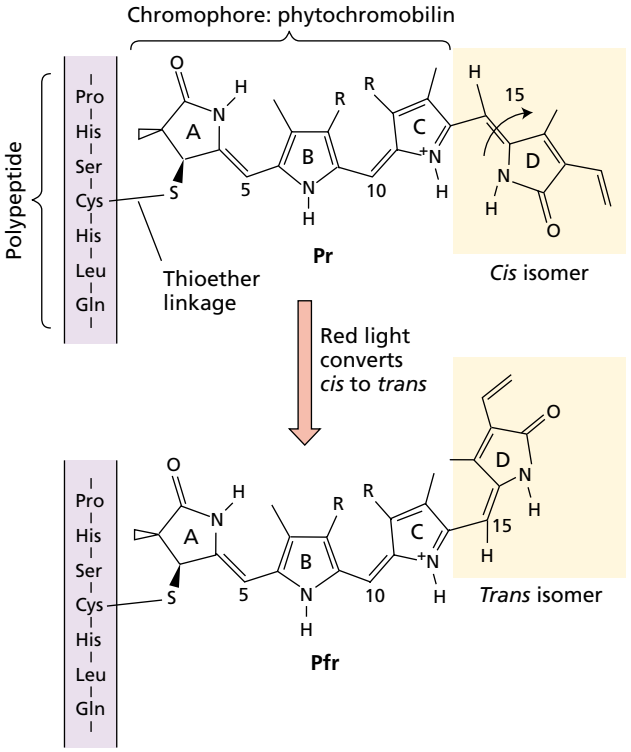


FIGURE 17.4 Structure of the Pr and Pfr forms of the chromophore (phytochromobilin) and the peptide region bound to the chromophore through a thioether linkage. The chromophore undergoes a *cis–trans* isomerization at carbon 15 in response to red and far-red light. (After Andel et al. 1997.)

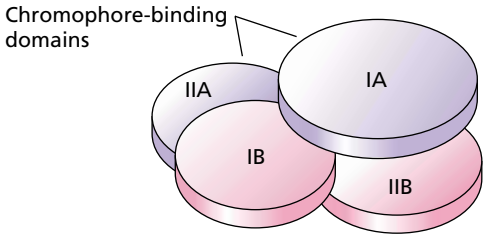


FIGURE 17.5 Structure of the phytochrome dimer. The monomers are labeled I and II. Each monomer consists of a chromophore-binding domain (A) and a smaller nonchromophore domain (B). The molecule as a whole has an ellipsoidal rather than globular shape. (After Tokutomi et al. 1989.)

active holoprotein. When a chromophore precursor is supplied to these seedlings, normal growth is restored.

The same type of mutation has been observed in other species. For example, the *yellow-green* mutant of tomato has properties similar to those of *hy* mutants, suggesting that it is also a chromophore mutant.

Both Chromophore and Protein Undergo Conformational Changes

Because the chromophore absorbs the light, conformational changes in the protein are initiated by changes in the chromophore. Upon absorption of light, the Pr chromophore undergoes a *cis–trans* isomerization of the double bond between carbons 15 and 16 and rotation of the C14–C15 single bond (see Figure 17.4) (Andel et al. 1997). During the conversion of Pr to Pfr, the protein moiety of the phytochrome holoprotein also undergoes a subtle conformational change.

Several lines of evidence suggest that the light-induced change in the conformation of the polypeptide occurs both in the N-terminal chromophore-binding domain and in the C-terminal region of the protein.

Two Types of Phytochromes Have Been Identified

Phytochrome is most abundant in etiolated seedlings; thus most biochemical studies have been carried out on phytochrome purified from nongreen tissues. Very little phytochrome is extractable from green tissues, and a portion of the phytochrome that can be extracted differs in molecular mass from the abundant form of phytochrome found in etiolated plants.

Research has shown that there are two different classes of phytochrome with distinct properties. These have been termed Type I and Type II phytochromes (Furuya 1993). Type I is about nine times more abundant than Type II in dark-grown pea seedlings; in light-grown pea seedlings the amounts of the two types are about equal. More recently, the two types have been shown to be distinct proteins.

The cloning of genes that encode different phytochrome polypeptides has clarified the distinct nature of the phytochromes present in etiolated and green seedlings. Even in etiolated seedlings, phytochrome is a mixture of related proteins encoded by different genes.

Phytochrome Is Encoded by a Multigene Family

The cloning of phytochrome genes made it possible to carry out a detailed comparison of the amino acid sequences of the related proteins. It also allowed the study of their expression patterns, at both the mRNA and the protein levels.

The first phytochrome sequences cloned were from monocots. These studies and subsequent research indicated that phytochromes are soluble proteins—a finding that is consistent with previous purification studies. A complementary-DNA clone encoding phytochrome from the dicot zucchini (*Cucurbita pepo*) was used to identify five structurally related phytochrome genes in *Arabidopsis* (Sharrock and Quail 1989). This phytochrome gene family is named *PHY*, and its five individual members are *PHYA*, *PHYB*, *PHYC*, *PHYD*, and *PHYE*.

The apoprotein by itself (without the chromophore) is designated PHY; the holoprotein (with the chromophore) is designated phy. By convention, phytochrome sequences from other higher plants are named according to their homology with the *Arabidopsis PHY* genes. Monocots appear to have representatives of only the *PHYA* through *PHYC* families, while dicots have others derived by gene duplication (Mathews and Sharrock 1997).

Some of the *hy* mutants have turned out to be selectively deficient in specific phytochromes. For example, *hy3* is deficient in phyB, and *hy1* and *hy2* are deficient in chromophore. These and other *phy* mutants have been useful in determining the physiological functions of the different phytochromes (as discussed later in this chapter).

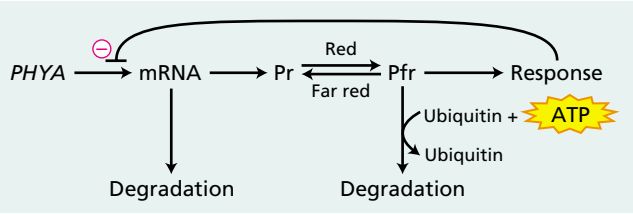
PHY Genes Encode Two Types of Phytochrome

On the basis of their expression patterns, the products of members of the *PHY* gene family can be classified as either Type I or Type II phytochromes. *PHYA* is the only gene that encodes a Type I phytochrome. This conclusion is based on the expression pattern of the *PHYA* promoter, as well as on the accumulation of its mRNA and polypeptide in response to light. Additional studies of plants that contain mutated forms of the *PHYA* gene (termed *phyA* alleles) have confirmed this conclusion and have given some clues about the role of this phytochrome in whole plants.

The *PHYA* gene is transcriptionally active in dark-grown seedlings, but its expression is strongly inhibited in the light in monocots. In dark-grown oat, treatment with red light reduces phytochrome synthesis because the Pfr form of phytochrome inhibits the expression of its own gene. In addition, the *PHYA* mRNA is unstable, so once etiolated oat seedlings are transferred to the light, *PHYA* mRNA rapidly disappears. The inhibitory effect of light on *PHYA* transcription is less dramatic in dicots, and in *Arabidopsis* red light has no measurable effect on *PHYA*.

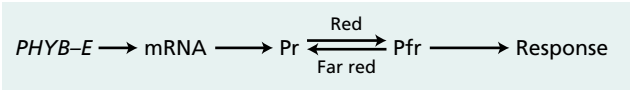
The amount of phyA in the cell is also regulated by protein destruction. The Pfr form of the protein encoded by the *PHYA* gene, called **PfrA**, is unstable. There is evidence that PfrA may become marked or tagged for destruction by the ubiquitin system (Vierstra 1994). As discussed in Chapter 14 on the web site, *ubiquitin* is a small polypeptide that binds covalently to proteins and serves as a recognition site for a large proteolytic complex, the *proteasome*.

Therefore, oats and other monocots rapidly lose most of their Type I phytochrome (phyA) in the light as a result of a combination of factors: inhibition of transcription, mRNA degradation, and proteolysis:



In dicots, phyA levels also decline in the light as a result of proteolysis, but not as dramatically.

The remaining *PHY* genes (*PHYB* through *PHYE*) encode the Type II phytochromes. Although detected in green plants, these phytochromes are also present in etiolated plants. The reason is that the expression of their mRNAs is not significantly changed by light, and the encoded phyB through phyE proteins are more stable in the Pfr form than is PfrA.



LOCALIZATION OF PHYTOCHROME IN TISSUES AND CELLS

Valuable insights into the function of a protein can be gained from a determination of where it is located. It is not surprising, therefore, that much effort has been devoted to the localization of phytochrome in organs and tissues, and within individual cells.

Phytochrome Can Be Detected in Tissues Spectrophotometrically

The unique photoreversible properties of phytochrome can be used to quantify the pigment in whole plants through the use of a spectrophotometer. Because its color is masked by chlorophyll, phytochrome is difficult to detect in green tissue. In dark-grown plants, where there is no chlorophyll, phytochrome has been detected in many angiosperm tissues—both monocot and dicot—as well as in gymnosperms, ferns, mosses, and algae.

In etiolated seedlings the highest phytochrome levels are usually found in meristematic regions or in regions that were recently meristematic, such as the bud and first node of pea (Figure 17.6), or the tip and node regions of the coleoptile in oat. However, differences in expression patterns between monocots and dicots and between Type I and Type II phytochromes are apparent when other, more sensitive methods are used.

Phytochrome Is Differentially Expressed In Different Tissues

The cloning of individual *PHY* genes has enabled researchers to determine the patterns of expression of individual phytochromes in specific tissues by several methods. The sequences can be used directly to probe mRNAs isolated from different tissues or to analyze transcriptional activity by means of a reporter gene, which visually reveals sites of gene expression. In the latter approach, the promoter of a *PHYA* or *PHYB* gene is joined to the coding portion of a reporter gene, such as the gene for the enzyme β-glucuronidase, which is

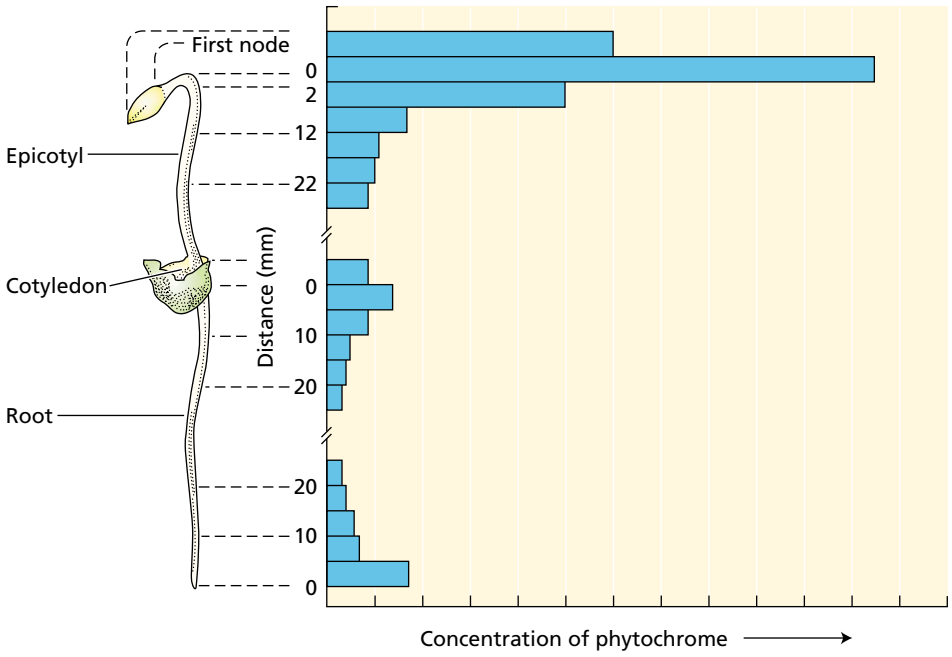


FIGURE 17.6 Phytochrome is most heavily concentrated in the regions where dramatic developmental changes are occurring: the apical meristems of the epicotyl and root. Shown here is the distribution of phytochrome in an etiolated pea seedling, as measured spectrophotometrically. (From Kendrick and Frankland 1983.)

called *GUS* (recall that the promoter is the sequence upstream of the gene that is required for transcription).

The advantage of using the *GUS* sequence is that it encodes an enzyme that, even in very small amounts, converts a colorless substrate to a colored precipitate when the substrate is supplied to the plant. Thus, cells in which the *PHYA* promoter is active will be stained blue, and other cells will be colorless. The hybrid, or fused, gene is then placed back into the plant through use of the Ti plasmid of *Agrobacterium tumefaciens* as a vector (see **Web Topic 21.5**).

When this method was used to examine the transcription of two different *PHYA* genes in tobacco, dark-grown seedlings were found to contain the highest amount of stain in the apical hook and the root tips, in keeping with earlier immunological studies (Adam et al. 1994). The pattern of staining in light-grown seedlings was similar but, as might be expected, was of much lower intensity. Similar studies with *Arabidopsis PHYA–GUS* and *PHYB–GUS* fusions placed back in *Arabidopsis* confirmed the *PHYA* results for tobacco and indicated that *PHYB–GUS* is expressed at much lower levels than *PHYA–GUS* in all tissues (Somers and Quail 1995).

A recent study comparing the expression patterns of *PHYB–GUS*, *PHYD–GUS*, and *PHYE–GUS* fusions in *Arabidopsis* has revealed that although these Type II promoters are less active than the Type I promoters, they do show distinct expression patterns (Goosey et al. 1997). Thus the general picture emerging from these studies is that the phytochromes are expressed in distinct but overlapping patterns.

In summary, phytochromes are most abundant in young, undifferentiated tissues, in the cells where the mRNAs are most abundant and the promoters are most active. The strong correlation between phytochrome abundance and cells that have the potential for dynamic developmental changes is consistent with the important role of phytochromes in controlling such developmental changes. However, note that the studies discussed here do not address whether the phytochromes are photoactive as apoproteins or holoproteins.

Because the expression patterns of individual phytochromes overlap, it is not surprising that they function cooperatively, although they probably also use distinct signal transduction pathways. Support for this idea also comes from the study of phytochrome mutants, which we will discuss later in this chapter.

CHARACTERISTICS OF PHYTOCHROME-INDUCED WHOLE-PLANT RESPONSES

The variety of different phytochrome responses in intact plants is extensive, in terms of both the kinds of responses (see Table 17.1) and the quantity of light needed to induce the responses. A survey of this variety will show how diversely the effects of a single photoevent—the absorption of light by Pr—are manifested throughout the plant. For ease of discussion, phytochrome-induced responses may be logically grouped into two types:

1. Rapid biochemical events
2. Slower morphological changes, including movements and growth

Some of the early biochemical reactions affect later developmental responses. The nature of these early biochemical events, which comprise signal transduction pathways, will be treated in detail later in the chapter. Here we will focus on the effects of phytochrome on whole-plant responses. As we will see, such responses can be classified into various types, depending on the amount and duration of light required, and on their action spectra.

Phytochrome Responses Vary in Lag Time and Escape Time

Morphological responses to the photoactivation of phytochrome may be observed visually after a *lag time*—the time between a stimulation and an observed response. The lag time may be as brief as a few minutes or as long as several weeks. The more rapid of these responses are usually reversible movements of organelles (see **Web Topic 17.2**) or reversible volume changes (swelling, shrinking) in cells, but even some growth responses are remarkably fast.

Red-light inhibition of the stem elongation rate of light-grown pigweed (*Chenopodium album*) is observed within 8 minutes after its relative level of Pfr is increased. Kinetic studies using *Arabidopsis* have confirmed this observation and further shown that phyA acts within minutes after exposure to red light (Parks and Spalding 1999). In these studies the primary contribution of phyA was found to be over by 3 hours, at which time phyA protein was no longer detectable through the use of antibodies, and the contribution of phyB increased (Morgan and Smith 1978). Longer lag times of several weeks are observed for the induction of flowering (see Chapter 24).

Information about the lag time for a phytochrome response helps researchers evaluate the kinds of biochemical events that could precede and cause the induction of that response. The shorter the lag time, the more limited the range of biochemical events that could have been involved.

Variety in phytochrome responses can also be seen in the phenomenon called **escape from photoreversibility**. Red light–induced events are reversible by far-red light for only a limited period of time, after which the response is said to have "escaped" from reversal control by light.

A model to explain this phenomenon assumes that phytochrome-controlled morphological responses are the result of a step-by-step sequence of linked biochemical reactions in the responding cells. Each of these sequences has a point of no return beyond which it proceeds irrevocably to the response. The escape time for different responses ranges from less than a minute to, remarkably, hours.

Phytochrome Responses Can Be Distinguished by the Amount of Light Required

In addition to being distinguished by lag times and escape times, phytochrome responses can be distinguished by the amount of light required to induce them. The amount of light is referred to as the **fluence**,[1] which is defined as the number of photons impinging on a unit surface area (see Chapter 9 and Web Topic 9.1). The most commonly used units for fluence are moles of quanta per square meter (mol m^{-2}). In addition to the fluence, some phytochrome responses are sensitive to the **irradiance**,[2] or *fluence rate*, of light. The units of irradiance in terms of photons are moles of quanta per square meter per second (mol m^{-2} s^{-1}).

Each phytochrome response has a characteristic range of light fluences over which the magnitude of the response is proportional to the fluence. As Figure 17.7 shows, these responses fall into three major categories based on the amount of light required: very-low-fluence responses (VLFRs), low-fluence responses (LFRs), and high-irradiance responses (HIRs).

Very-Low-Fluence Responses Are Nonphotoreversible

Some phytochrome responses can be initiated by fluences as low as 0.0001 µmol m^{-2} (one-tenth of the amount of light emitted from a firefly in a single flash), and they saturate (i.e., reach a maximum) at about 0.05 µmol m^{-2}. For example, in dark-grown oat seedlings, red light can stimulate the growth of the coleoptile and inhibit the growth of the mesocotyl (the elongated axis between the coleoptile and the root) at such low fluences. *Arabidopsis* seeds can be induced to germinate with red light in the range of 0.001 to 0.1 µmol m^{-2}. These remarkable effects of vanishingly low levels of illumination are called **very-low-fluence responses** (**VLFRs**).

The minute amount of light needed to induce VLFRs converts less than 0.02% of the total phytochrome to Pfr. Because the far-red light that would normally reverse a red-light effect converts 97% of the Pfr to Pr (as discussed earlier), about 3% of the phytochrome remains as Pfr—significantly more than is needed to induce VLFRs (Mandoli and Briggs 1984). Thus, far-red light cannot reverse VLFRs. The VLFR action spectrum matches the absorption spectrum of Pr, supporting the view that Pfr is the active form for these responses (Shinomura et al. 1996).

Ecological implications of the VLFR in seed germination are discussed in Web Essay 17.1

[1] For definitions of *fluence*, *irradiance*, and other terms involved in light measurement, see Web Topic 9.1.

[2] Irradiance is sometimes loosely equated with light intensity. The term *intensity*, however, refers to light emitted by the source, whereas *irradiance* refers to light that is incident on the object.

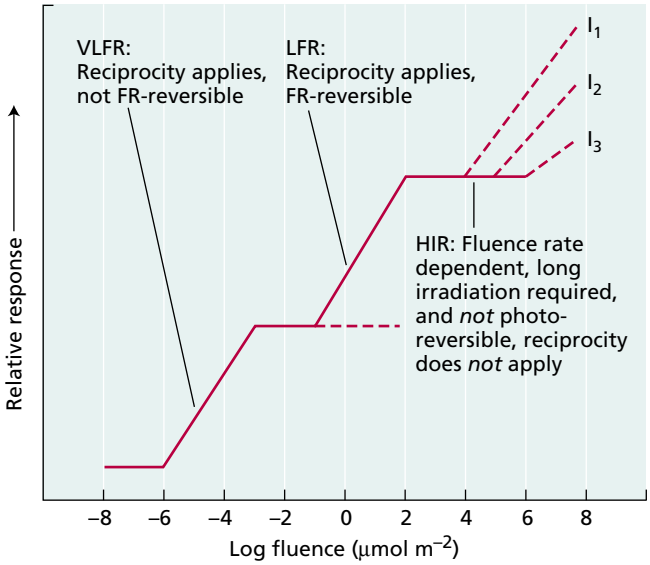


FIGURE 17.7 Three types of phytochrome responses, based on their sensitivities to fluence. The relative magnitudes of representative responses are plotted against increasing fluences of red light. Short light pulses activate VLFRs and LFRs. Because HIRs are also proportional to the irradiance, the effects of three different irradiances given continuously are illustrated ($I_1 > I_2 > I_3$). (From Briggs et al. 1984.)

Low-Fluence Responses Are Photoreversible

Another set of phytochrome responses cannot be initiated until the fluence reaches 1.0 µmol m^{-2}, and they are saturated at 1000 µmol m^{-2}. These responses are referred to as **low-fluence responses** (**LFRs**), and they include most of the red/far-red photoreversible responses, such as the promotion of lettuce seed germination and the regulation of leaf movements, that are mentioned in Table 17.1. The LFR action spectrum for *Arabidopsis* seed germination is shown in Figure 17.8. LFR spectra include a main peak for stimulation in the red region (660 nm), and a major peak for inhibition in the far-red region (720 nm).

Both VLFRs and LFRs can be induced by brief pulses of light, provided that the total amount of light energy adds up to the required fluence. The total fluence is a function of two factors: the fluence rate (mol m^{-2} s^{-1}) and the irradiation time. Thus a brief pulse of red light will induce a response, provided that the light is sufficiently bright, and conversely, very dim light will work if the irradiation time is long enough. This reciprocal relationship between fluence rate and time is known as the **law of reciprocity**, which was first formulated by R. W. Bunsen and H. E. Roscoe in 1850. VLFRs and LFRs both obey the law of reciprocity.

High-Irradiance Responses Are Proportional to the Irradiance and the Duration

Phytochrome responses of the third type are termed **high-irradiance responses** (**HIRs**), several of which are listed in

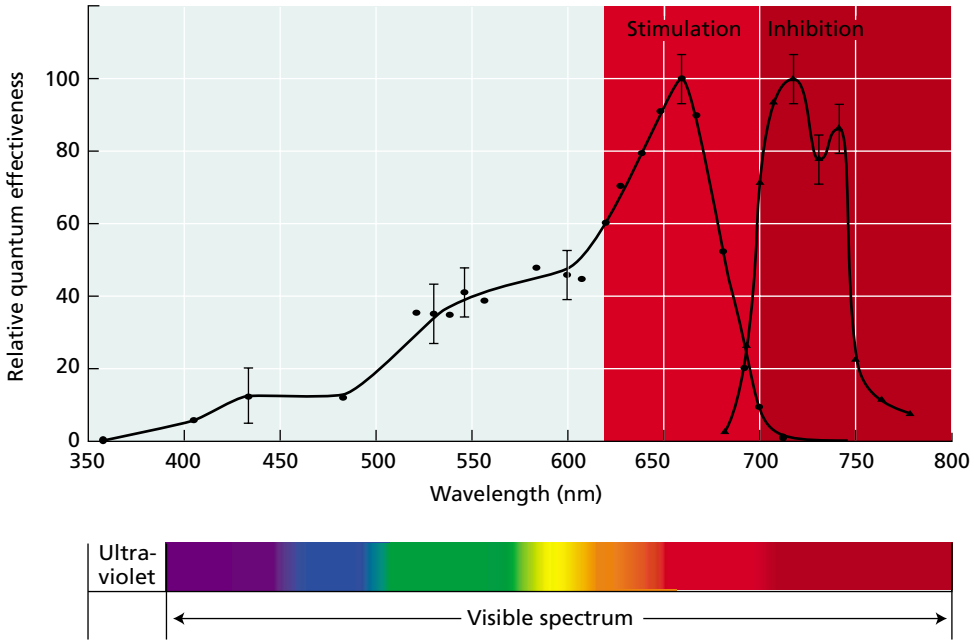


FIGURE 17.8 LFR action spectra for the photoreversible stimulation and inhibition of seed germination in *Arabidopsis*. (After Shropshire et al. 1961.)

Table 17.2. HIRs require prolonged or continuous exposure to light of relatively high irradiance, and the response is proportional to the irradiance within a certain range.

The reason that these responses are called high-irradiance responses rather than high-fluence responses is that they are proportional to irradiance (loosely speaking, the brightness of the light) rather than to fluence. HIRs saturate at much higher fluences than LFRs—at least 100 times higher—and are not photoreversible. Because neither continuous exposure to dim light nor transient exposure to bright light can induce HIRs, HIRs do not obey the law of reciprocity.

Many of the photoreversible LFRs listed in Table 17.1, particularly those involved in de-etiolation, also qualify as HIRs. For example, at low fluences the action spectrum for anthocyanin production in seedlings of white mustard (*Sinapis alba*) shows a single peak in the red region of the spectrum, the effect is reversible with far-red light, and the response obeys the law of reciprocity. However, if the dark-grown seedlings are instead exposed to high-irradiance light for several hours, the action spectrum now includes peaks in the far-red and blue regions (see the next section), the effect is no longer photoreversible, and the response becomes proportional to the irradiance. Thus the same effect can be either an LFR or an HIR, depending on its history of exposure to light.

TABLE 17.2
Some plant photomorphogenic responses induced by high irradiances

Synthesis of anthocyanin in various dicot seedings and in apple skin segments
Inhibition of hypocotyl elongation in mustard, lettuce, and petunia seedlings
Induction of flowering in henbane (*Hyoscyamus*)
Plumular hook opening in lettuce
Enlargement of cotyledons in mustard
Production of ethylene in sorghum

The HIR Action Spectrum of Etiolated Seedlings Has Peaks in the Far-Red, Blue, and UV-A Regions

HIRs, such as the inhibition of stem or hypocotyl growth, have usually been studied in dark-grown, etiolated seedlings. The HIR action spectrum for the inhibition of hypocotyl elongation in dark-grown lettuce seedlings is shown in Figure 17.9. For HIRs the main peak of activity is in the far-red region between the absorption maxima of Pr and Pfr, and there are peaks in the blue and UV-A regions as well. Because the absence of a peak in the red region is unusual for a phytochrome-mediated response, at first researchers believed that another pigment might be involved.

A large body of evidence now supports the view that phytochrome is one of the photoreceptors involved in HIRs (see **Web Topic 17.3**). However, it has long been suspected that the peaks in the UV-A and blue regions are due to a separate photoreceptor that absorbs UV-A and blue light.

As a test of this hypothesis, the HIR action spectrum for the inhibition of hypocotyl elongation was determined in dark-grown *hy2* mutants of *Arabidopsis*, which have little or no phytochrome holoprotein. As expected, the wild-type seedlings exhibited peaks in the UV-A, blue, and far-red regions of the spectrum. In contrast, the *hy2* mutant failed to respond to either far-red or red light. Although the phytochrome-deficient *hy2* mutant exhibited no peak in the far-red region, it showed a normal response to UV-A and blue light (Goto et al. 1993).

These results demonstrate that phytochrome is not involved in the HIR to either UV-A or blue light, and that a separate blue/UV-A photoreceptor is responsible for the response to these

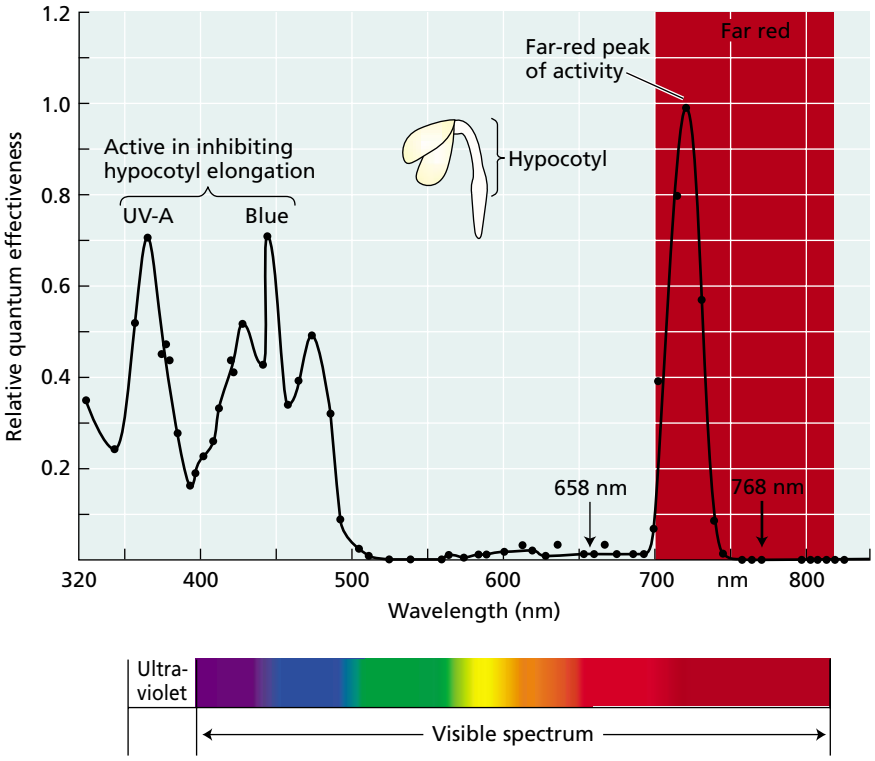


FIGURE 17.9 HIR action spectrum for the inhibition of hypocotyl elongation of dark-grown lettuce seedlings. The peaks of activity for the inhibition of hypocotyl elongation occur in the UV-A, blue, and far-red regions of the spectrum. (After Hartmann 1967.)

wavelengths. More recent studies indicate that the blue-light photoreceptors CRY1 and CRY2 are involved in blue-light inhibition of hypocotyl elongation.

The HIR Action Spectrum of Green Plants Has a Major Red Peak

During studies of the HIR of etiolated seedlings, it was observed that the response to continuous far-red light declines rapidly as the seedlings begin to green. For example, the action spectrum for the inhibition of hypocotyl growth of light-grown green *Sinapis alba* (white mustard) seedlings is shown in Figure 17.10. In general, HIR action spectra for light-grown plants exhibit a single major peak in the red, similar to the action spectra of LFRs (see Figure 17.8), except that the effect is nonphotoreversible.

The loss of responsiveness to continuous far-red light is strongly correlated with the depletion of the light-labile pool of Type I phytochrome, which consists mostly of phyA. This finding suggests that the HIR of etiolated seedlings to far-red light is mediated by phyA, whereas the HIR of green seedlings to red light is mediated by the Type II phytochrome phyB and possibly others.

ECOLOGICAL FUNCTIONS: SHADE AVOIDANCE

Thus far we have discussed phytochrome-regulated responses as studied in the laboratory. However, phytochrome plays important ecological roles for plants growing in the environment. In the discussion that follows we will learn how plants sense and respond to shading by other plants, and how phytochrome is involved in regulating various daily rhythms. We will also examine the specialized functions of the different phytochrome gene family members in these processes.

Phytochrome Enables Plants to Adapt to Changing Light Conditions

The presence of a red/far-red reversible pigment in all green plants, from algae to dicots, suggests that these wavelengths of light provide information that helps plants adjust to their environment. What environmental conditions change the relative levels of these two wavelengths of light in natural radiation?

The ratio of red light (R) to far-red light (FR) varies remarkably in different environments. This ratio can be defined as follows:

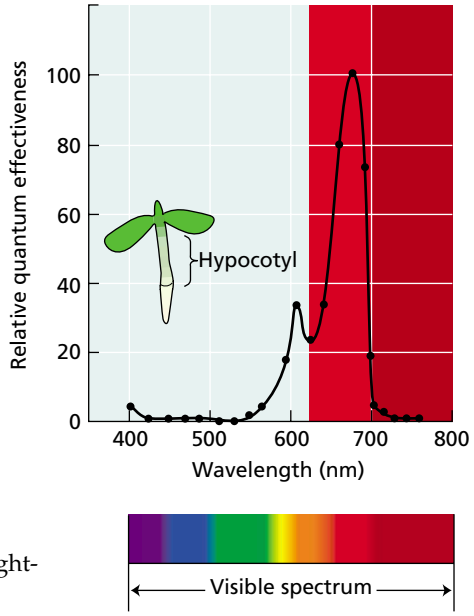


FIGURE 17.10 HIR action spectra for the inhibition of hypocotyl elongation of light-grown white mustard (*Sinapis alba*) seedlings. (After Beggs et al. 1980.)

$$\text{R/FR} = \frac{\text{Photon fluence rate in 10 nm band centered on 660 nm}}{\text{Photon fluence rate in 10 nm band centered on 730 nm}}$$

Table 17.3 compares both the total light intensity in photons (400–800 nm) and the R/FR values in eight natural environments. Both parameters vary greatly in different environments.

Compared with direct daylight, there is relatively more far-red light during sunset, under 5 mm of soil, or under the canopy of other plants (as on the floor of a forest). The canopy phenomenon results from the fact that green leaves absorb red light because of their high chlorophyll content but are relatively transparent to far-red light.

The R:FR ratio and shading. An important function of phytochrome is that it enables plants to sense shading by other plants. Plants that increase stem extension in response to shading are said to exhibit a **shade avoidance response**. As shading increases, the R:FR ratio decreases. The greater proportion of far-red light converts more Pfr to Pr, and the ratio of Pfr to total phytochrome (Pfr/Ptotal) decreases. When simulated natural radiation was used to vary the far-red content, it was found that for so-called sun plants (plants that normally grow in an open-field habitat), the higher the far-red content (i.e., the lower the Pfr:Ptotal ratio), the higher the rate of stem extension (Figure 17.11).

In other words, simulated canopy shading (high levels of far-red light) induced these plants to allocate more of their resources to growing taller. This correlation did not hold for "shade plants," which normally grow in a shaded environment. Shade plants showed little or no reduction in their stem extension rate as they were exposed to higher R/FR values (see Figure 17.11). Thus there appears to be a systematic relationship between phytochrome-controlled growth and species habitat. Such results are taken as an indication of the involvement of phytochrome in shade perception.

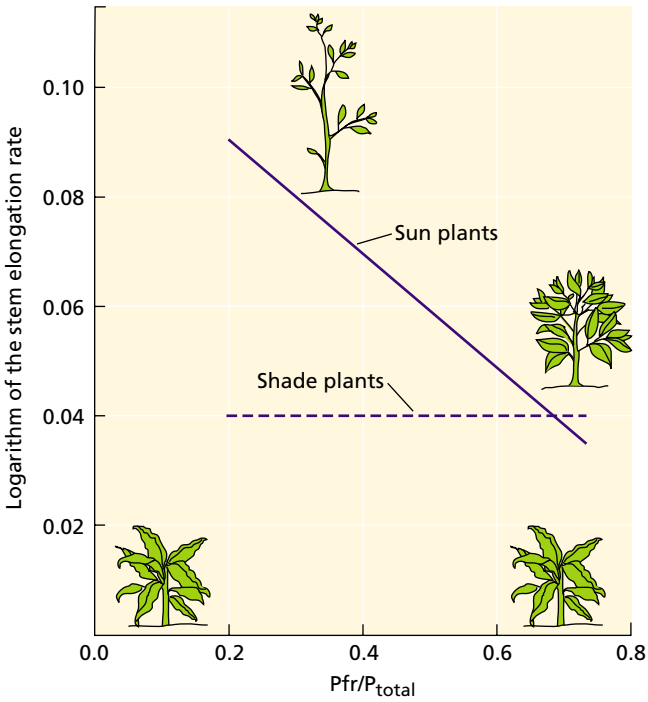


FIGURE 17.11 Role of phytochrome in shade perception in sun plants (solid line) versus shade plants (dashed line). (After Morgan and Smith 1979.)

For a "sun plant" or "shade-avoiding plant" there is a clear adaptive value in allocating its resources toward more rapid extension growth when it is shaded by another plant. In this way it can enhance its chances of growing above the canopy and acquiring a greater share of unfiltered, photosynthetically active light. The price for favoring internode elongation is usually reduced leaf area and reduced branching, but at least in the short run this adaptation to canopy shade seems to work.

The R:FR ratio and seed germination. Light quality also plays a role in regulating the germination of some seeds. As discussed earlier, phytochrome was discovered in studies of light-dependent lettuce seed germination.

In general, large-seeded species, with ample food reserves to sustain prolonged seedling growth in darkness (e.g., underground), do not require light for germination. However, a light requirement is

TABLE 17.3
Ecologically important light parameters

	Photon flux density (μmol m^{-2} s^{-1})	R/FR[a]
Daylight	1900	1.19
Sunset	26.5	0.96
Moonlight	0.005	0.94
Ivy canopy	17.7	0.13
Lakes, at a depth of 1 m		
Black Loch	680	17.2
Loch Leven	300	3.1
Loch Borralie	1200	1.2
Soil, at a depth of 5 mm	8.6	0.88

Source: Smith 1982, p. 493.

Note: The light intensity factor (400–800 nm) is given as the photon flux density, and phytochrome-active light is given as the R:FR ratio.

[a]Absolute values taken from spectroradiometer scans; the values should be taken to indicate the relationships between the various natural conditions and not as actual environmental means.

often observed in the small seeds of herbaceous and grassland species, many of which remain dormant, even while hydrated, if they are buried below the depth to which light penetrates. Even when such seeds are on or near the soil surface, their level of shading by the vegetation canopy (i.e., the R:FR ratio they receive) is likely to affect their germination. For example, it is well documented that far-red enrichment imparted by a leaf canopy inhibits germination in a range of small-seeded species.

For the small seeds of the tropical species trumpet tree (*Cecropia obtusifolia*) and Veracruz pepper (*Piper auritum*) planted on the floor of a deeply shaded forest, this inhibition can be reversed if a light filter is placed immediately above the seeds that permits the red component of the canopy-shaded light to pass through while blocking the far-red component. Although the canopy transmits very little red light, the level is enough to stimulate the seeds to germinate, probably because most of the inhibitory far-red light is excluded by the filter and the R:FR ratio is very high. These seeds would also be more likely to germinate in spaces receiving sunlight through gaps in the canopy than in densely shaded spaces. The sunlight would help ensure that the seedlings became photosynthetically self-sustaining before their seed food reserves were exhausted.

As will be discussed later in the chapter, recent studies on light-dependent lettuce seeds have shown that red light–induced germination is the result of an increase in the level of the biologically active form of the hormone gibberellin. Thus, phytochrome may promote seed germination through its effects on gibberellin biosynthesis (see Chapter 20).

ECOLOGICAL FUNCTIONS: CIRCADIAN RHYTHMS

Various metabolic processes in plants, such as oxygen evolution and respiration, cycle alternately through high-activity and low-activity phases with a regular periodicity of about 24 hours. These rhythmic changes are referred to as **circadian rhythms** (from the Latin *circa diem*, meaning "approximately a day"). The **period** of a rhythm is the time that elapses between successive peaks or troughs in the cycle, and because the rhythm persists in the absence of external controlling factors, it is considered to be **endogenous**.

The endogenous nature of circadian rhythms suggests that they are governed by an internal pacemaker, called an **oscillator**. The endogenous oscillator is coupled to a variety of physiological processes. An important feature of the oscillator is that it is unaffected by temperature, which enables the clock to function normally under a wide variety of seasonal and climatic conditions. The clock is said to exhibit **temperature compensation**.

Light is a strong modulator of rhythms in both plants and animals. Although circadian rhythms that persist under controlled laboratory conditions usually have periods one or more hours longer or shorter than 24 hours, in nature their periods tend to be uniformly closer to 24 hours because of the synchronizing effects of light at daybreak, referred to as **entrainment**. Both red and blue light are effective in entrainment. The red-light effect is photoreversible by far-red light, indicative of phytochrome; the blue-light effect is mediated by blue-light photoreceptor(s).

Phytochrome Regulates the Sleep Movements of Leaves

The sleep movements of leaves, referred to as **nyctinasty**, are a well-described example of a plant circadian rhythm that is regulated by light. In nyctinasty, leaves and/or leaflets extend horizontally (open) to face the light during the day and fold together vertically (close) at night (Figure 17.12). Nyctinastic leaf movements are exhibited by many legumes, such as *Mimosa*, *Albizia*, and *Samanea*, as well as members of the oxalis family. The change in leaf or leaflet angle is caused by rhythmic turgor changes in the cells of the **pulvinus** (plural *pulvini*), a specialized structure at the base of the petiole.

Once initiated, the rhythm of opening and closing persists even in constant darkness, both in whole plants and in isolated leaflets (Figure 17.13). The phase of the rhythm (see Chapter 24), however, can be shifted by various exogenous signals, including red or blue light.

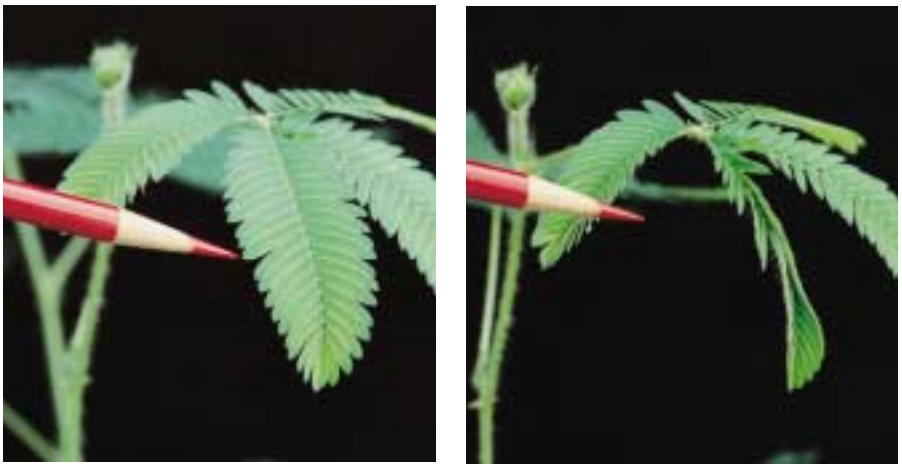


FIGURE 17.12 Nyctinastic leaf movements of *Mimosa pudica*. (A) Leaflets open. (B) Leaflets closed. (Photos © David Sieren/Visuals Unlimited.)

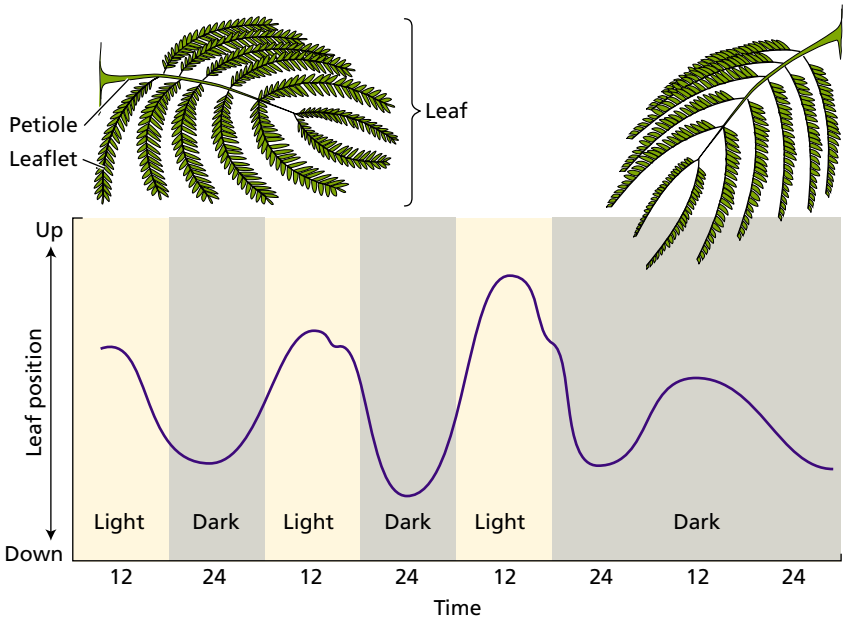


FIGURE 17.13 Circadian rhythm in the diurnal movements of *Albizia* leaves. The leaves are elevated in the morning and lowered in the evening. In parallel with the raising and lowering of the leaves, the leaflets open and close. The rhythm persists at a lower amplitude for a limited time in total darkness.

Light also directly affects movement: Blue light stimulates closed leaflets to open, and red light followed by darkness causes open leaflets to close. The leaflets begin to close within 5 minutes after being transferred to darkness, and closure is complete in 30 minutes. Because the effect of red light can be canceled by far-red light, phytochrome regulates leaflet closure.

The physiological mechanism of leaf movement is well understood. It results from turgor changes in cells located on opposite sides of the pulvinus, called **ventral motor cells** and **dorsal motor cells** (Figure 17.14). These changes in turgor pressure depend on K^+ and Cl^- fluxes across the plasma membranes of the dorsal and ventral motor cells. Leaflets open when the dorsal motor cells accumulate K^+ and Cl^-, causing them to swell, while the ventral motor cells release K^+ and Cl^-, causing them to shrink. Reversal of this process results in leaflet closure. Leaflet closure is therefore an example of a rapid response to phytochrome involving ion fluxes across membranes.

Gene expression and circadian rhythms. Phytochrome can also interact with circadian rhythms at the level of gene expression. The expression of genes in the *LHCB* family, encoding the light-harvesting chlorophyll *a/b*–binding proteins of photosystem II, is regulated at the transcriptional level by both circadian rhythms and phytochrome.

In leaves of pea and wheat, the level of *LHCB* mRNA has been found to oscillate during daily light–dark cycles, rising in the morning and falling in the evening. Since the rhythm persists even in continuous darkness, it appears to be a circadian rhythm. But phytochrome can perturb this cyclical pattern of expression.

When wheat plants are transferred from a cycle of 12 hours light and 12 hours dark to continuous darkness, the rhythm persists for a while, but it slowly *damps out* (i.e., reduces in amplitude until no peaks or troughs are discernible). If, however, the plants are given a pulse of red light before they are transferred to continuous darkness, no damping occurs (i.e., the levels of *LHCB* mRNA continue to oscillate as they do during the light–dark cycles).

In contrast, a far-red flash at the end of the day prevents the expression of *LHCB* in continuous darkness, and the effect of far red is reversed by red light. Note that it is not the oscillator that damps out under constant conditions, but the coupling of the oscillator to the physiological event being monitored. Red light restores the coupling between the oscillator and the physiological process.

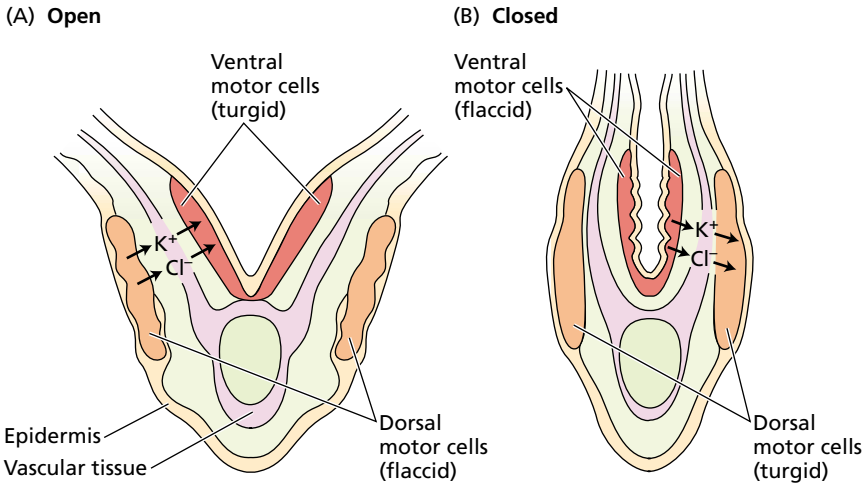


FIGURE 17.14 Ion fluxes between the dorsal and ventral motor cells of *Albizia pulvini* regulate leaflet opening and closing. (After Galston 1994.)

Circadian Clock Genes of *Arabidopsis* Have Been Identified

The isolation of clock mutants has been an important tool for the identification of clock genes in other organisms. Isolating clock mutants in plants requires a convenient assay that allows monitoring of the circadian rhythms of many thousands of individual plants to detect the rare abnormal phenotype.

To allow screening for clock mutants in *Arabidopsis*, the promoter region of the *LHCB* gene was fused to the gene that encodes luciferase, an enzyme that emits light in the presence of its substrate, luciferin. This reporter gene construct was then used to transform *Arabidopsis* with the Ti plasmid of *Agrobacterium* as a vector. Investigators were then able to monitor the temporal and spatial regulation of bioluminescence in individual seedlings in real time using a video camera (Millar et al. 1995).

A total of 21 independent ***toc*** (*t*iming *o*f *C*AB [*LHCB*] expression) mutants have been isolated, including both short-period and long-period lines. The *toc1* mutant in particular has been implicated in the core oscillator mechanism (Strayer et al. 2001). A model for the endogenous oscillator will be discussed later in the chapter.

ECOLOGICAL FUNCTIONS: PHYTOCHROME SPECIALIZATION

Phytochrome is encoded by a multigene family: *PHYA* through *PHYE*. Despite the great similarity in their structures, each of these phytochromes performs distinct roles in the life of the plant. In this section we will discuss the current state of our knowledge of the ecological functions of the different phytochromes, focusing primarily on phyA and phyB.

Phytochrome B Mediates Responses to Continuous Red or White Light

Phytochrome B was first suspected to play a role in responses to continuous light because the *hy3* mutant (now called *phyB*), which has long hypocotyls under continuous white light, was found to have an altered *PHYB* gene. In these mutants, *PHYB* mRNA was reduced in amount or was absent, and little or no phyB protein could be detected. In contrast, the levels of *PHYA* mRNA and phyA protein were normal.

Phytochrome B mediates shade avoidance by regulating hypocotyl length in response to red light given in low-fluence pulses or continuously, and as might be expected, the *phyB* mutant is unable to respond to shading by increasing hypocotyl extension. In addition, these plants do not extend their hypocotyls in response to far-red light given at the end of each photoperiod (called the *end-of-day far-red response*). Both of these responses are likely to involve perception of the Pfr:Ptotal ratio and occur in the low-fluence region of the spectrum. Although phyB is centrally involved in the shade avoidance response, evidence suggests that other phytochromes play important roles as well (Smith and Whitelam 1997).

The *phyB* mutant is deficient in chlorophyll and in some mRNAs that encode chloroplast proteins, and it is impaired in its ability to respond to plant hormones. Since a mutation in *PHYB* results in impaired perception of continuous red light, the presence of the other phytochromes must not be sufficient to confer responsiveness to continuous red or white light.

Phytochrome B also appears to regulate photoreversible seed germination, the phenomenon that originally led to the discovery of phytochrome. Wild-type *Arabidopsis* seeds require light for germination, and the response shows red/far-red reversibility in the low-fluence range. Mutants that lack phyA respond normally to red light; mutants deficient in phyB are unable to respond to low-fluence red light (Shinomura et al. 1996). This experimental evidence strongly suggests that phyB mediates photoreversible seed germination.

Phytochrome A Is Required for the Response to Continuous Far-Red Light

No phytochrome gene mutations other than *phyB* were found in the original *hy* collection, so the identification of *phyA* mutants required the development of more ingenious screens. As discussed previously, because the far-red HIRs were known to require light-labile (Type I) phytochrome, it was suspected that phyA must be the photoreceptor involved in the perception of continuous far-red light. If this is true, then the phyA mutants should fail to respond to continuous far-red light and grow tall and spindly under these light conditions. However, mutants lacking chromophore would also look like this because phyA can detect far-red light only when assembled with the chromophore into holophytochrome.

To select for just the phyA mutants, the seedlings that grew tall in continuous far-red light were then grown under continuous red light. The phyA-deficient mutants can grow normally under this regimen, but a chromophore-deficient mutant, which also lacks functional phyB, does not respond. The *phyA* mutant seedlings selected in this screen had no obvious phenotype when grown in normal white light, confirming that phyA has no discernible role in sensing white light.

This also explains why *phyA* mutants were not detected in the original long-hypocotyl screen. Thus, phyA appears to have a limited role in photomorphogenesis, restricted primarily to de-etiolation and far-red responses. For example, phyA would be important when seeds germinate under a canopy, which filters out much of the red light.

It is also clear from this constant far-red light phenotype that none of the other phytochromes is sufficient for the perception of constant far-red light, and despite the ability of all phytochromes to absorb red and far-red light, at least phyA and phyB have distinct roles in this regard.

TABLE 17.4
Comparison of the very-low-fluence (VLFR), low-fluence (LFR), and high-irradiance responses (HIR)

Type of Response	Photoreversibility	Reciprocity	Peaks of action spectra[a]	Photoreceptor
VLFR	No	Yes	Red, Blue	phyA, phyE[a]
LFR	Yes	Yes	Red, far red	phyB, phyD, phyE
HIR	No	No	Dark-grown: far red, blue, UV-A Light-grown: red	Dark-grown: phyA, cryptochrome Light-grown: phyB

[a] phyE is required for seed germination but not for other VLFR responses mediated by phyA

Phytochrome A also appears to be involved in the germination VLFR of *Arabidopsis* seeds. Thus, mutants lacking phyA cannot germinate in response to red light in the very-low-fluence range, but they show a normal response to red light in the low-fluence range (Shinomura et al. 1996). This result demonstrates that phyA functions as the primary photoreceptor for this VLFR, although recent evidence suggests that phyE is required for this component of seed germination (Hennig et al. 2002).

Table 17.4 summarizes the different roles of phyA, phyB, and other photoreceptors in the various phytochrome-mediated responses.

Developmental Roles for Phytochromes C, D, and E Are Also Emerging

Some of the roles of other phytochromes in plant growth and development have recently begun to be elucidated through experiments on mutant plants. Because these phytochromes have functions that overlap with those of phyA and phyB, it was necessary to screen for mutants in *phyAB* null mutant backgrounds to uncover mutations. For example, both phyD and phyE help mediate the shade avoidance response—a response mediated primarily by phyB.

The creation of double and triple mutants has made it possible to assess the relative role of each phytochrome in a given response. Thus it was found that, like phyB, phyD plays a role in regulating leaf petiole elongation, as well as in flowering time (see Chapter 24). Similar analyses support the idea that phyE acts redundantly with phyB and phyD in these processes, but also acts redundantly with phyA and phyB in inhibition of internode elongation.

Of the *Arabidopsis* phytochromes, phyC is the least well characterized. However, although *phyAphyBphyDphyE* quadruple mutants appear to have normal responses to the red:far red ratio, there are differences in phytochrome-regulated gene expression.

In summary, phyC, phyD, and phyE appear to play roles that are for the most part redundant with those of phyA and phyB. Whereas phyB appears to be involved in regulating all stages of development, the functions of the other phytochromes are restricted to specific developmental steps or responses.

Phytochrome Interactions Are Important Early in Germination

Figure 17.15A shows the action of constant red and far-red light absorbed separately by the phyA and phyB systems. Continuous red light absorbed by phyB stimulates de-eti-

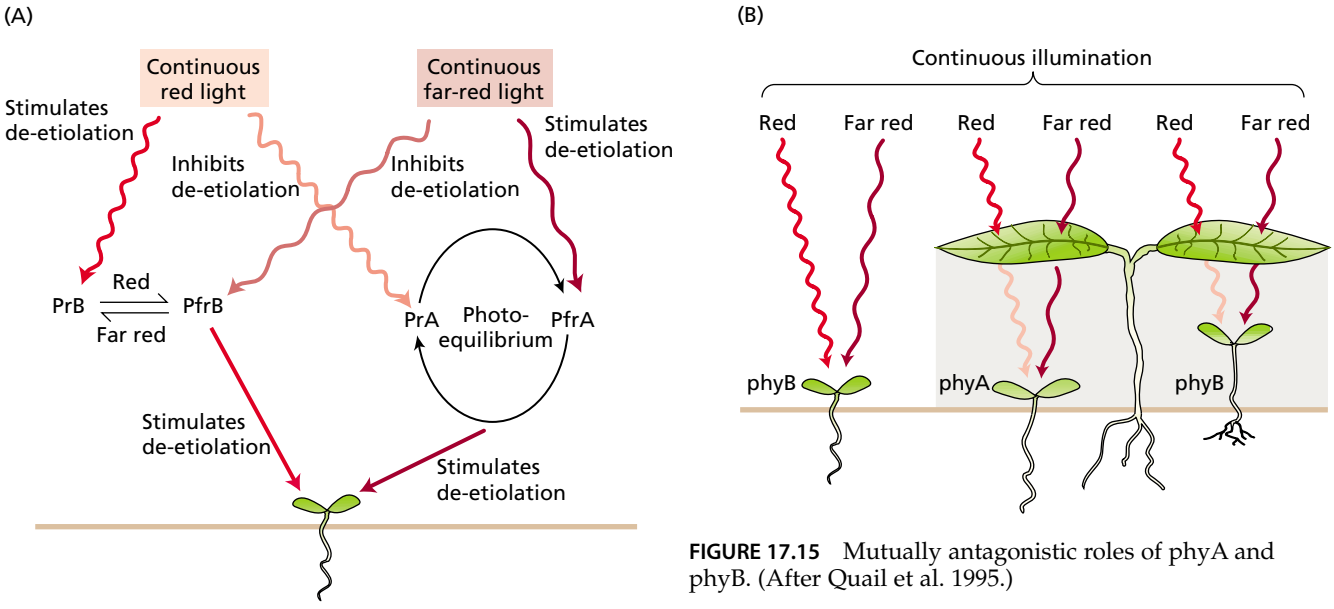


FIGURE 17.15 Mutually antagonistic roles of phyA and phyB. (After Quail et al. 1995.)

olation by maintaining high levels of PfrB. Continuous far-red light absorbed by PfrB prevents this stimulation by reducing the amount of PfrB. The stimulation of de-etiolation by phyA depends on the photostationary state of phytochrome (indicated in Figure 17.15A by the circular arrows). Continuous far-red light stimulates de-etiolation when absorbed by the phyA system; continuous red light inhibits the response.

The effects of phyA and phyB on seedling development in sunlight versus canopy shade (enriched in far-red light) are shown in Figure 17.15B. In open sunlight, which is enriched in red light compared with canopy shade, de-etiolation is mediated primarily by the phyB system (on the left in the figure). A seedling emerging under canopy shade, enriched in far-red light, initiates de-etiolation primarily through the phyA system (center). Because phyA is labile, however, the response is taken over by phyB (right). In switching over to phyB, the stem is released from growth inhibition (see Figure 17.15A), allowing for the accelerated rate of stem elongation that is part of the shade avoidance response (see **Web Topic 17.4**).

For a discussion of how plants sense their neighbors using reflected light, see **Web Essay 17.2**.

PHYTOCHROME FUNCTIONAL DOMAINS

Prior to the identification of the multiple forms of phytochrome, it was difficult to understand how a single photoreceptor could regulate such diverse processes in the cell. However, the discovery that phytochrome is encoded by members of a multigene family, each with its own pattern of expression, provided a more plausible alternative explanation: Each phytochrome-mediated response is regulated by a specific phytochrome, or by interactions between specific phytochromes. As discussed earlier, this hypothesis was supported by the phenotypes of mutants deficient in either phyA or phyB.

As a corollary to this hypothesis, it was further postulated that specific regions of the PHY proteins must be specialized to allow them to perform their distinct functions. Molecular biology provides the tools to answer such difficult questions. In this section we will describe what is known about the functional domains of the phytochrome holoprotein.

Just as mutations *reducing* the amount of a particular phytochrome have yielded information about its role, plants genetically engineered to *overexpress* a specific phytochrome are also useful. First, they allow an extension of the range of phytochrome levels testable in relation to function. Second, as we will see, a particular phytochrome sequence can be changed and reintroduced into a normal plant to test its phenotypic effects.

Usually plants overexpressing an introduced *PHYA* or *PHYB* gene have a dramatically altered phenotype. Such transgenic plants are often dwarfed, are dark green because of elevated chlorophyll levels, and show reduced apical dominance. This phenotype requires elevated levels of an intact, photoactive holoprotein because overexpression of a mutated form of phytochrome that is unable to combine with its chromophore has a normal phenotype. Similarly, plants expressing only the N-terminal domain of each phytochrome have a normal phenotype, even though elevated levels of the photoactive fragment accumulate.

Although protein overexpression greatly perturbs the normal metabolism of a cell and is therefore subject to certain artifacts, such studies of structure and function have helped build a picture of phytochrome as a molecule having two domains linked by a hinge: an N-terminal light-sensing domain in which the light specificity and stability reside, and a C-terminal domain that contains the signal-transmitting sequences (Figure 17.16).

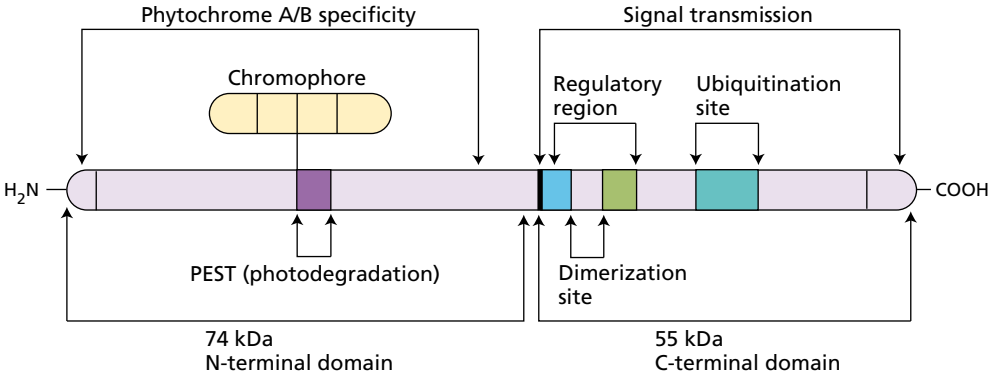


FIGURE 17.16 Schematic diagram of the phytochrome holoprotein, showing the various functional domains. The chromophore-binding site and PEST sequence are located in the N-terminal domain, which confers photosensory specificity to the molecule—that is, whether it responds to continuous red or far-red light. The C-terminal domain contains a dimerization site, a ubiquitination site, and a regulatory region. The C-terminal domain transmits signals to proteins that act downstream of phytochrome.

The C-terminal domain also contains the site for the formation of phytochrome dimers and the site for the addition of ubiquitin, a tag for degradation. (For a more detailed description of experiments that helped map the functional domains of phytochrome, see **Web Topic 17.5**.)

CELLULAR AND MOLECULAR MECHANISMS

All phytochrome-regulated changes in plants begin with absorption of light by the pigment. After light absorption, the molecular properties of phytochrome are altered, probably causing the signal-transmitting sequences in the C terminus to interact with one or more components of a signal transduction pathway that ultimately bring about changes in the growth, development, or position of an organ (see Table 17.1).

Some of the signal-transmitting motifs appear to interact with multiple signal transduction pathways; others appear to be unique to a specific pathway. Furthermore, it is reasonable to assume that the different phytochrome proteins utilize different sets of signal transduction pathways.

Molecular and biochemical techniques are helping to unravel the early steps in phytochrome action and the signal transduction pathways that lead to physiological or developmental responses. These responses fall into two general categories:

1. Relatively rapid turgor responses involving ion fluxes
2. Slower, long-term processes associated with photomorphogenesis, involving alterations in gene expression

In this section we will examine the effects of phytochrome on both membrane permeability and gene expression, as well as the possible chain of events constituting the signal transduction pathways that bring about these effects.

Phytochrome Regulates Membrane Potentials and Ion Fluxes

Phytochrome can rapidly alter the properties of membranes. We have already seen that low-fluence red light is required before the dark period to induce rapid leaflet closure during nyctinasty, and that fluxes of K^+ and Cl^- into and out of dorsal and ventral motor cells mediate the response. However, the rapidity of leaf closure in the dark (lag time about 5 minutes) would seem to rule out mechanisms based on gene expression. Instead, rapid phytochrome-induced changes in membrane permeability and transport appear to be involved.

During phytochrome-mediated leaflet closure, the apoplastic pH of the dorsal motor cells (the cells that swell during leaflet closure) decreases, while the apoplastic pH of the ventral motor cells (the cells that shrink during leaflet closure) increases. Thus the plasma membrane H^+ pump of the dorsal cells appears to be activated by darkness (provided that phytochrome is in the Pfr form), and the H^+ pump of the ventral cells appears to be deactivated under the same conditions (see Figure 17.14). The reverse pattern of apoplastic pH change is observed during leaflet opening.

Studies have also been carried out on phytochrome regulation of K^+ channels in isolated protoplasts (cells without their cell walls) of both dorsal and ventral motor cells from *Samanea* leaves (Kim et al. 1993). When the extracellular K^+ concentration was raised, K^+ entered the protoplasts and depolarized the membrane potential only if the K^+ channels were open. When the dorsal and ventral motor cell protoplasts were transferred to constant darkness, the state of the K^+ channels exhibited a circadian rhythmicity during a 21-hour incubation period, and the two cell types varied reciprocally, just as they do in vivo. That is, when the dorsal cell K^+ channels were open, the ventral cell K^+ channels were closed, and vice versa. Thus the circadian rhythm of leaf movements has its origins in the circadian rhythm of K^+ channel opening.

On the basis of the evidence thus far, we can conclude that phytochrome brings about leaflet closure by regulating the activities of the primary proton pumps and the K^+ channels of the dorsal and ventral motor cells. Although the effect is rapid, it is not instantaneous, and it is therefore unlikely to be due to a direct effect of phytochrome on the membrane. Instead, phytochrome acts indirectly via one or more signal transduction pathways, as in the case of the regulation of gene expression by phytochrome (see the next section).

However, some effects of red and far-red light on the membrane potential are so rapid that phytochrome may also interact directly with the membrane. Such rapid modulation has been measured in individual cells and has been inferred from the effects of red and far-red light on the surface potential of roots and oat (*Avena*) coleoptiles, where the lag between the production of Pfr and the onset of measurable potential changes is 4.5 s for hyperpolarization.

Changes in the bioelectric potential of cells imply changes in the flux of ions across the plasma membrane (see **Web Topic 17.6**). Membrane isolation studies provide evidence that a small portion of the total phytochrome is tightly bound to various organellar membranes.

These findings led some workers to suggest that membrane-bound phytochrome represents the physiologically active fraction, and that all the effects of phytochrome on gene expression are initiated by changes in membrane permeability. On the basis of sequence analysis, however, it is now clear that phytochrome is a hydrophilic protein without membrane-spanning domains. The current view is that it may be associated with microtubules located directly beneath the plasma membrane, at least in the case of the alga, *Mougeotia*, as described in **Web Topic 17.2**.

If phytochrome exerts its effects on membranes from some distance, no matter how small, involvement of a *second messenger* is implied, and calcium is a good candidate. Rapid changes in cytosolic free calcium have been implicated as second messengers in several signal transduction

pathways, and there is evidence that calcium plays a role in chloroplast movement in *Mougeotia*.

Phytochrome Regulates Gene Expression

As the term *photomorphogenesis* implies, plant development is profoundly influenced by light. Etiolation symptoms include spindly stems, small leaves (in dicots), and the absence of chlorophyll. Complete reversal of these symptoms by light involves major long-term alterations in metabolism that can be brought about only by changes in gene expression.

The stimulation and repression of transcription by light can be very rapid, with lag times as short as 5 minutes. Such early-gene expression is likely to be regulated by the direct activation of transcription factors by one or more phytochrome-initiated signal transduction pathways. The activated transcription factors then enter the nucleus, where they stimulate the transcription of specific genes.

Some of these early gene products are transcription factors themselves, which activate the expression of other genes. Expression of the early genes, also called **primary response genes**, is independent of protein synthesis; expression of the late genes, or **secondary response genes**, requires the synthesis of new proteins.

The photoregulation of gene expression has focused on the nuclear genes that encode messages for chloroplast proteins: the small subunit of ribulose-1,6-bisphosphate carboxylase/oxygenase (rubisco) and the major light-harvesting chlorophyll *a/b*–binding proteins associated with the light-harvesting complex of photosystem II (LHCIIb proteins). These proteins play important roles in chloroplast development and greening; hence their regulation by phytochrome has been studied in detail. The genes for both of these proteins—*RBCS* and *LHCB* (also called *CAB* in some studies)—are present in multiple copies in the genome.

We can demonstrate phytochrome regulation of mRNA abundance (e.g., *RBCS* mRNAs) experimentally by giving etiolated plants a brief pulse of low-fluence red or far-red light, returning them to darkness to allow the signal transduction pathway to operate, and then measuring the abundance of specific mRNAs in total RNA prepared from each set of plants. If its abundance is regulated by phytochrome, the mRNA is absent or present at low levels in etiolated plants but is increased by red light. The red light–induced increase in expression can be reversed by immediate treatment with far-red light, but far-red light alone has little effect on mRNA abundance. The expression of some other genes is down-regulated under these conditions.

Recently red-light stimulation of lettuce seed germination has been correlated with an increase in the biologically active form of the hormone gibberellin. Red light causes a large increase in the expression of the gene coding for a key enzyme in the gibberellin biosynthetic pathway (Toyomasu et al. 1998). The effect of red light is reversed by a treatment with far-red light, indicative of phytochrome. Since gibberellin can substitute for red light in promoting lettuce seed germination, it appears that phytochrome promotes seed germination by increasing the biosynthesis of the hormone. Gibberellins are discussed in detail in Chapter 20.

For an expanded discussion see **Web Topic 17.7**.

Both Phytochrome and the Circadian Rhythm Regulate *LHCB*

A MYB-related transcription factor whose mRNA level increases rapidly when *Arabidopsis* is transferred from the dark to the light is involved in phytochrome-mediated expression of *LHCB* genes (Figure 17.17). (For information on MYB, see Chapter 14 on the web site.)

This transcription factor appears to bind to the promoter of certain *LHCB* genes and regulate their transcription, which, as Figure 17.17 shows, occurs later than the increase in the MYB-related protein (Wang et al. 1997). The gene that encodes the MYB-related protein is therefore probably a primary response gene, and the *LHCB* gene itself is probably a secondary response gene.

Recent work has indicated that this MYB-related protein, now known as *c*ircadian *c*lock *a*ssociated *1* (CCA1), also plays a role in the circadian regulation of *LHCB* gene expression. A second but distinct MYB-related protein, *l*ate elongated *hy*pocotyl (LHY), has also been identified as a potential clock gene. Expression of *CCA1* and *LHY* oscillates with a circadian rhythm. Constitutive expression of *CCA1* abolishes several circadian rhythms and suppresses both *CCA1* and *LHY* expression. When the *CCA1* gene is mutated so that no functional protein is produced, circadian and phytochrome regulation of four genes, including *LHCB*, is affected. These observations suggest that *CCA1* and *LHY* are associated with the circadian clock.

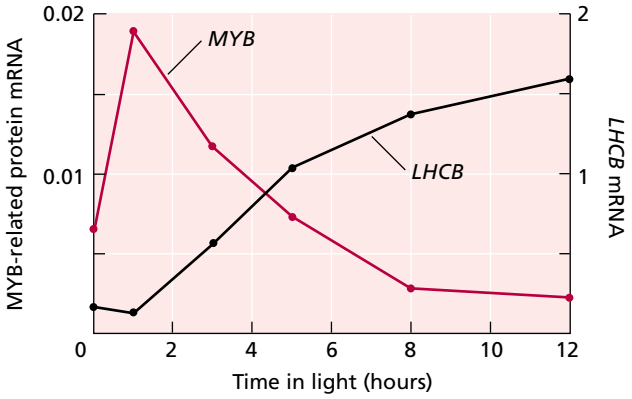


FIGURE 17.17 Time course for inducing transcription. Kinetics of the induction of transcripts for a MYB-related transcription factor (MYB) and the light-harvesting chlorophyll *a/b*–binding protein (LHCB) in *Arabidopsis* after transfer of the seedlings from darkness to continuous white light. (After Wang et al. 1997.)

A protein kinase (CK2) can interact with and phosphorylate CCA1. The CK2 kinase is a multisubunit protein with serine/threonine kinase activity. The regulatory subunit of CK2 (CKB3) has been shown to interact with, and phosphorylate, CCA1 in vitro. Mutations in CKB3 have also been found to perturb CK2 activity and, in turn, change the period of rhythmic expression of CCA1. These mutations affect many clock outputs, from gene expression to flowering time, suggesting that CK2 is involved in the regulation of the circadian clock via its interactions with CCA1 (Sugano et al. 1999).

The Circadian Oscillator Involves a Transcriptional Negative Feedback Loop

The circadian oscillators of cyanobacteria (*Synechococcus*), fungi (*Neurospora crassa*), insects (*Drosophila melanogaster*), and mouse (*Mus musculus*) have now been elucidated. In these four organisms, the oscillator is composed of several "clock genes" involved in a transcriptional–translational negative feedback loop.

So far, three major clock genes have been identified in *Arabidopsis*: *TOC1*, *LHY*, and *CCA1*. The protein products of these genes are all regulatory proteins. *TOC1* is not related to the clock genes of other organisms, suggesting that the plant oscillator is unique.

According to a recent model (Alabadi et al. 2001), light and the TOC1 regulatory protein activate *LHY* and *CCA1* expression at dawn (Figure 17.18). The increase in LHY and CCA1 represses the expression of the *TOC1* gene. Because TOC1 is a positive regulator of the *LHY* and *CCA1* genes, the repression of *TOC1* expression causes a progressive reduction in the levels of LHY and CCA1, which reach their minimum levels at the end of the day. As LHY and CCA1 levels decline, *TOC1* gene expression is released from inhibition. TOC1 reaches its maximum at the end of the day, when LHY and CCA1 are at their minimum. TOC1 then either directly or indirectly stimulates the expression of *LHY* and *CCA1*, and the cycle begins again.

The two MYB regulator proteins—LHY and CCA1—have dual functions. In addition to serving as components of the oscillator, they regulate the expression of other genes, such as *LHCB* and other "morning genes," and they repress genes expressed at night. Light acts to reinforce the effect of the *TOC1* gene in promoting *LHY* and *CCA1* expression. This reinforcement represents the underlying mechanism of *entrainment*. Other proteins, such as the CK2 kinase, affect the activity of CCA1, and thus regulate the clock. Phytochrome and the blue-light photoreceptor CRY2 (see Chapter 18) mediate the effects of red and blue light, respectively.

Regulatory Sequences Control Light-Regulated Transcription

The *cis*-acting regulatory sequences required to confer light regulation of gene expression have been studied extensively. Most eukaryotic promoters for genes that encode proteins comprise two functionally distinct regions: a short sequence that determines the transcription start site (the **TATA box**, named for its most abundant nucleotides) and upstream sequences, called ***cis*-acting regulatory elements**, that regulate the amount and pattern of transcription (see Chapter 14 on the web site). These regulatory sequences bind specific proteins, called ***trans*-acting factors**, that modulate the activity of the general

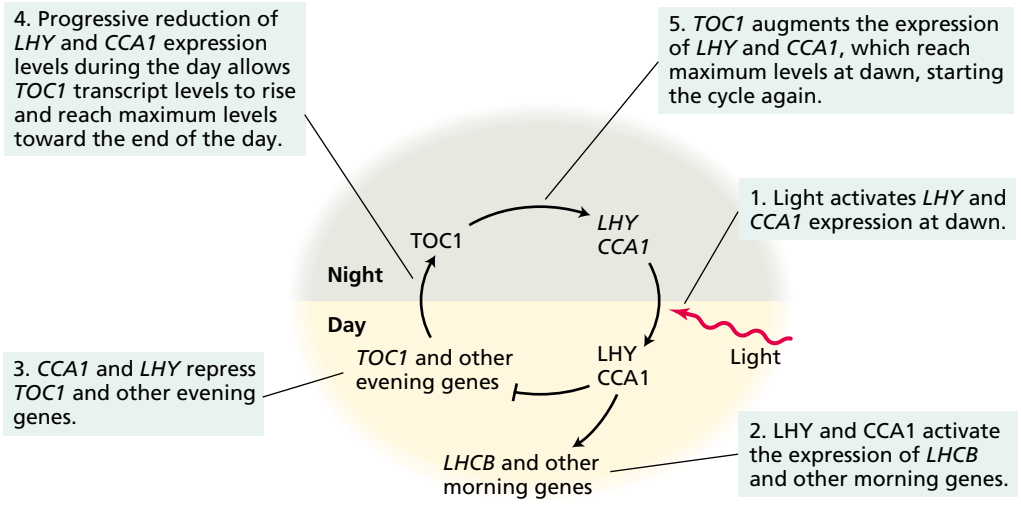


FIGURE 17.18 Circadian oscillator model showing the hypothetical interactions between the *TOC1* and *MYB* genes *LHY* and *CCA1*. Light acts at dawn to increase *LHY* and *CCA1* expression. *LHY* and *CCA1* act to regulate other daytime and evening genes.

transcription factors that assemble around the transcription start site with RNA polymerase II.

Overall, the picture emerging for light-regulated plant promoters is similar to that for other eukaryotic genes: a collection of modular elements, the number, position, flanking sequences, and binding activities of which can lead to a wide range of transcriptional patterns. No single DNA sequence or binding protein is common to all phytochrome-regulated genes.

At first it may appear paradoxical that light-regulated genes have such a range of elements, any combination of which can confer light-regulated expression. However, this array of sequences allows for the differential light- and tissue-specific regulation of many genes through the action of multiple photoreceptors. (For an expanded discussion, see **Web Topic 17.8**.)

Regulatory factors. As might be expected, the diverse range of phytochrome regulatory sequences can bind a wide variety of transcription factors. At least 50 of these regulatory factors have been identified recently by the use of genetic and molecular screens (Tepperman et al. 2001).

Although some of the early-acting signaling pathways are specific to phyA or phyB, it is clear that late-acting signaling pathways common to multiple photoreceptors must be used because different light qualities can trigger the same response (Chory and Wu 2001).

For example, SPA1 is a phyA-specific signaling intermediate that acts as a light-dependent repressor of photomorphogenesis in *Arabidopsis* seedlings (Hoecker and Quail 2001). The SPA1 protein has a coiled-coil protein domain that enables it to interact with another factor, COP1 (*co*nstitutive *p*hotomorphogenesis *1*), that acts downstream of both phyA and phyB. The COP1 protein was identified in the screen for constitutive photomorphogenesis mutants that has yielded several other factors that act downstream of photoreceptors (see **Web Topic 17.9**). COP1 is an E3 ubiquitin ligase that targets other proteins for destruction by the 26S proteasome (see Chapter 14 on the web site).

The functions of many of these factors are probably modulated through the action of HY5, a protein first identified through the long-hypocotyl screen, discussed earlier in the chapter. HY5 is a basic leucine zipper–type transcription factor that is always located in the nucleus (see Chapter 14 on the web site). HY5 binds to the G-box motif of multiple light-inducible promoters and is necessary for optimal expression of the corresponding genes. In the dark, HY5 is ubiquitinated by COP1 and degraded by the 26S proteasome complex.

Phytochrome Moves to the Nucleus

It has long been a mystery as to how phytochrome could act in the nucleus when it is apparently localized in the cytosol. Recent exciting work has finally opened up the black box between phytochrome and gene expression. The most surprising finding is that in some cases phytochrome itself moves to the nucleus in a light-dependent manner.

Detection of this movement relied on the ability to fuse phytochrome to a visible marker, **green fluorescent protein** (**GFP**), that can be activated by light of an appropriate wavelength being shone on plant cells. A big advantage of GFP fusions is that they can be visualized in living cells, making it possible to follow dynamic processes within the cell under the microscope.

Both phyA–GFP and phyB–GFP show light-activated import into the nucleus (Figure 17.19) (Sakamoto and Nagatani 1996; Sharma 2001). The phyB fusion moves to the nucleus in the Pfr form only, and transport is slow, taking several hours for full mobilization. In contrast, phyA–GFP can move in the Pfr or the Pr form, provided that it has cycled through Pfr first. Movement of phyA–GFP is much more rapid than that of phyB–GFP, taking only about 15 minutes.

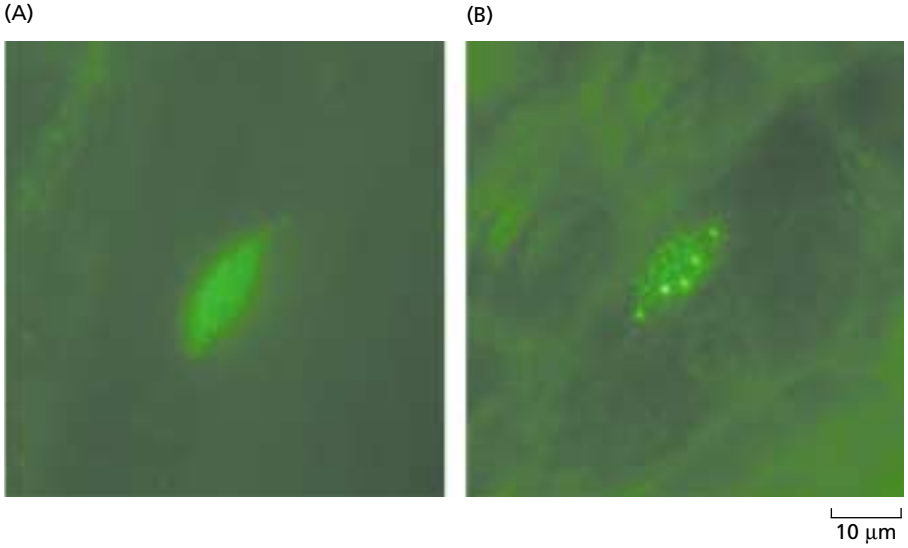


FIGURE 17.19 Nuclear localization of phy–GFP fusion proteins in epidermal cells of *Arabidopsis* hypocotyls. Transgenic *Arabidopsis* expressing phyA–GFP (left) or phyB–GFP (right) was observed under a fluorescence microscope. Only nuclei are visible. The plants were placed either under continuous far-red light (left) or white light (right) to induce the nuclear accumulation. The smaller bright green dots inside the nucleus are called "speckles." The significance of speckles is unknown. (From Yamaguchi et al. 1999, courtesy of A. Nagatani).

Most satisfying is the observation that phyB–GFP transport is promoted by red light and inhibited by far-red light, while transport of phyA–GFP is maximal under continuous far-red light. Furthermore, nuclear translocation of phyB is under circadian control, as would be expected, since phyB regulates the expression of clock-regulated genes. These light conditions are the ones known to be responsible for activation of phyA and phyB and would be consistent with their activity in the nucleus.

What happens when Pfr moves to the nucleus? Two nuclear proteins that interact with phytochrome have been identified to date, although there are probably additional targets. The first, ***p*hytochrome *i*nteracting *f*actor *3*** **(PIF3)**, reacts with the C-terminal end of phyA or phyB. However, it reacts preferentially with the full-length phyB protein in a light-dependent manner, and it is thought to be a functional primary reaction partner for this phytochrome.

Although its precise function is not yet known, PIF3 resembles transcription factors that bind to a particular element in plant promoters, the G-box motif, that confers light regulation to genes. It is also known that phyB in the Pfr form can form a complex with PIF3 bound to its target DNA. A picture is therefore emerging in which some phytochrome-regulated genes are activated directly by movement of phyB to the nucleus in the Pfr form. Once in the nucleus, phyB interacts with transcription factors such as PIF3. A model for the direct activation of gene expression by phyB in the nucleus is shown in Figure 17.20.

Phytochome Acts through Multiple Signal Transduction Pathways

Using biochemical approaches, researchers have shown that signaling involves several different mechanisms, including G-proteins, Ca^{2+}, and phosphorylation. We will consider the evidence for each of these in turn.

G-proteins and calcium. Well-characterized signaling pathways in other systems (e.g., mating in yeasts) often include **G-proteins** (which are reviewed in Chapter 14 on the web site). These protein complexes are normally membrane associated, have three different subunits, and bind GTP or GDP on one subunit. The hydrolysis of GTP to GDP is required for regulation of G-protein function. Sequences that encode G-protein subunits have been cloned from plants, indicating that this type of system is present. One way that the function of G-proteins can be tested is to treat cells with chemicals that activate or inhibit the ability of the complex to bind or break down GTP.

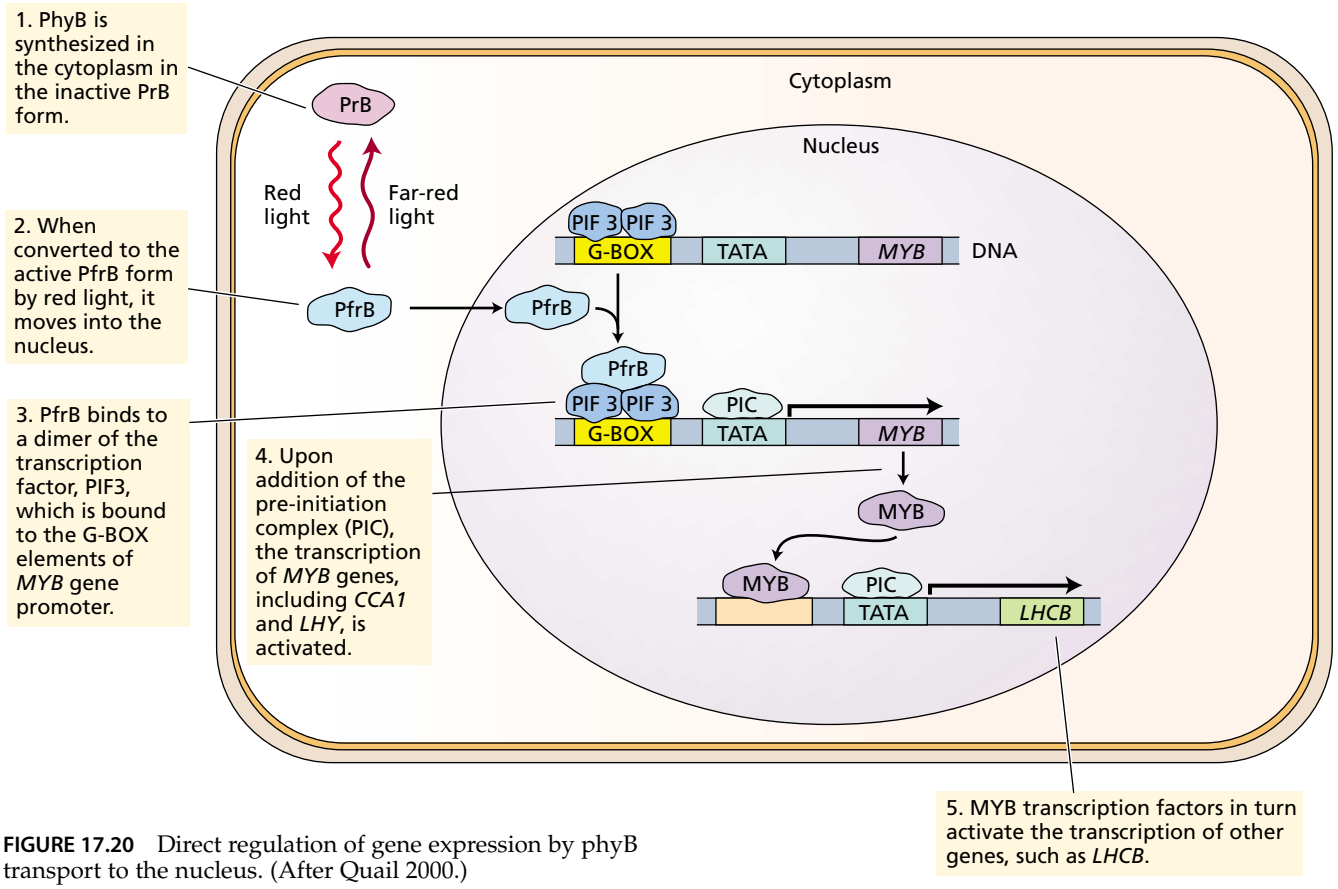
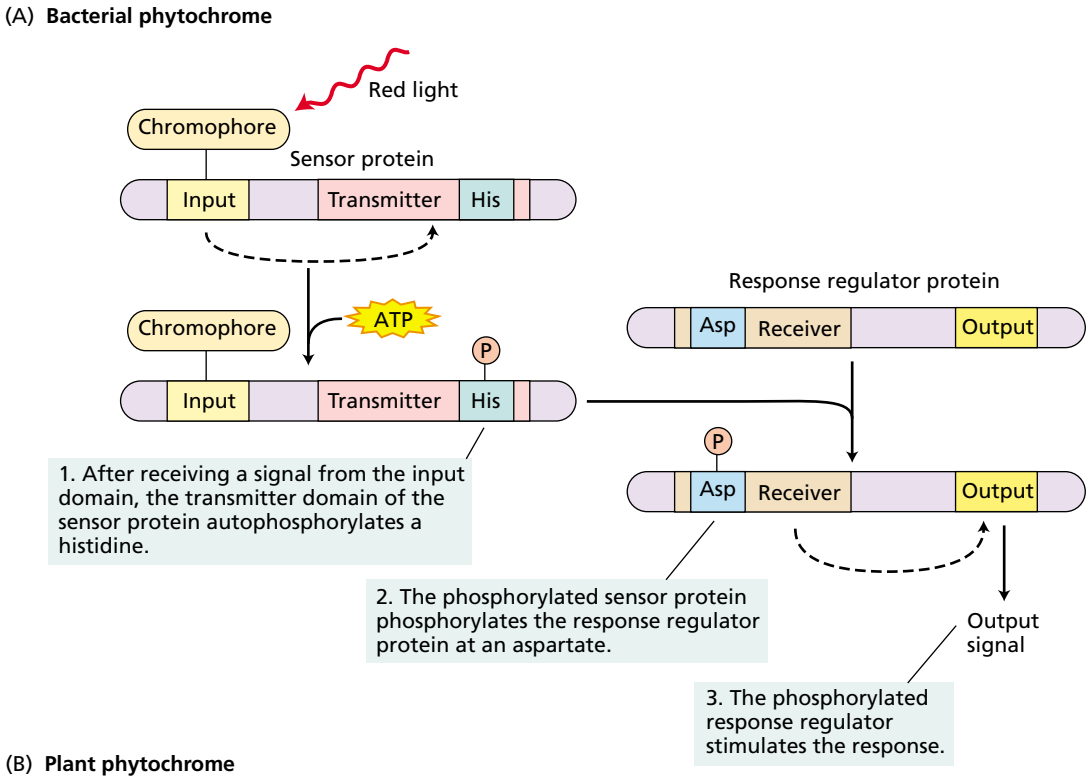


FIGURE 17.20 Direct regulation of gene expression by phyB transport to the nucleus. (After Quail 2000.)

Microinjection experiments (see **Web Topic 17.10**) indicate that phytochrome signaling can occur in single cells and does not require light after activation of phytochrome. At least one G-protein may function downstream of phytochrome. After the G-protein step, there are at least two branching pathways. One of these pathways—gene expression and chloroplast development—requires Ca^{2+} and calmodulin; the other—anthocyanin synthesis—is Ca^{2+} independent.

The branching pathways can be distinguished further by the *cis*-acting regulatory elements targeted and the signaling intermediate employed. For many years, it has been known that both cyclic AMP (cAMP) and cyclic GMP (cGMP) are important intermediates in hormone- and light-



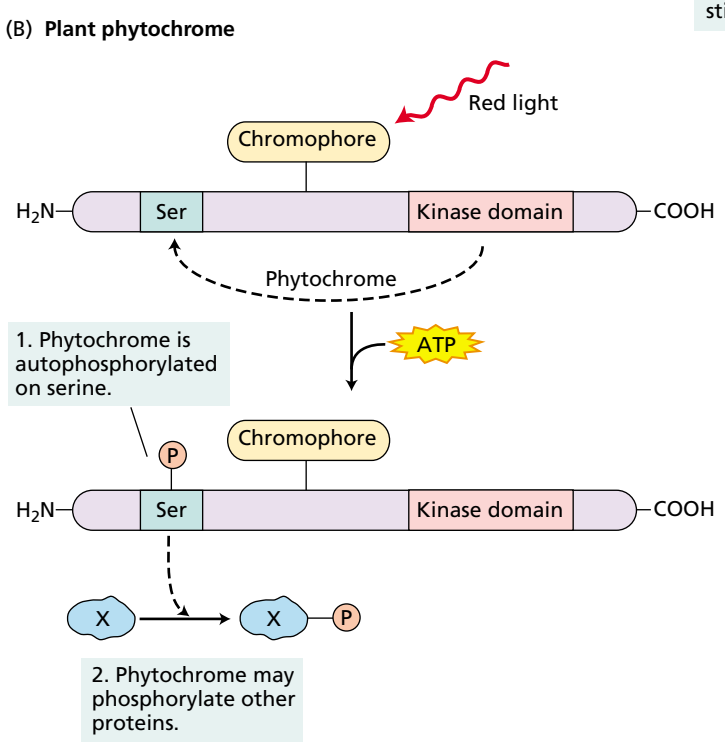


FIGURE 17.21 Phytochrome is an autophosphorylating protein kinase. (A) Bacterial phytochrome is an example of a two-component signaling system, in which phytochrome functions as a sensor protein that phosphorylates a response regulator (see Chapter 14 on the web site). (B) Plant phytochrome is an autophosphorylating serine/threonine kinase that may phosphorylate other proteins (X).

induced signaling pathways in animals (see Chapter 14 on the web site). Although the presence of cAMP has been difficult to demonstrate in plants, the presence of cGMP in plant tissues is well established. Indeed, recent studies have shown that cGMP may serve as a second messenger in phytochrome action.

However, the role of the G-protein cascade in plants is still controversial. Some key genes (e.g., guanylylate cyclase) have not yet been identified in plant genomes, and cGMP levels are vanishingly small in plants. On the other hand, studies with inhibitors have implicated cGMP as a second messenger for the hormones gibberellin (see Chapter 20) and abscisic acid (see Chapter 23). Thus a role for cGMP in phytochrome signaling, although controversial, remains a possibility.

Phosphorylation. The evidence for a potential role of phosphorylation in phytochrome action first came from red-light regulation of protein phosphorylation and phosphorylation-dependent binding of transcription factors to the promoters of phytochrome-regulated genes. Some highly purified preparations of phytochrome were also reported to have kinase activity.

Kinases are enzymes that have the capacity to transfer phosphate groups from ATP to amino acids such as serine or tyrosine, either on themselves or on other proteins. Kinases are often found in signal transduction pathways in which the addition or removal of phosphate groups regulates enzyme activity.

Phytochrome is now known to be a protein kinase. The evolutionary origin of phytochrome is very ancient, predating the appearance of eukaryotes. Bacterial phytochromes are light-dependent histidine kinases that function as **sensor proteins** that phosphorylate corresponding **response regulator** proteins (Figure 17.21A). (See also Chapter 14 on the web site and **Web Topic 17.11**)

However, although higher-plant phytochromes have some homology with the kinase domains, they do not function as histidine kinases. Instead, they are serine/threonine kinases. In addition, recombinant versions of higher-plant and algal phytochromes have been shown to be light- and chromophore-modulated kinases that can phosphorylate themselves, as well as other proteins (Figure 17.21B) (Sharma 2001).

At least one potential target is a cytosolic protein termed ***p*hytochrome *k*inase *s*ubstrate *1***, or **PKS1**, that can accept a phosphate from phyA. Phosphorylation occurs on serines, and to a lesser extent on threonines. The PKS1 phosphorylation is regulated by phytochrome both in the test tube and in the plant, with Pfr having a twofold higher level of activity than Pr. Overexpression of PKS1 in transgenic plants suggests that it may function to negatively regulate phyB-mediated events (Fankhauser et al. 1999).

Another protein kinase associated with phytochrome is ***n*ucleoside *dip*hosphate *k*inase 2** (**NDPK2**). Phytochrome A has been found to interact with this protein, and its kinase activity is increased about twofold when phyA is bound in the Pfr form. Because the NDPK2 protein is found in both the nucleus and the cytosol, the location of its primary site of action is unclear.

A summary of the possible signaling and regulatory pathways of phytochrome is shown in Figure 17.22.

Phytochrome Action Can Be Modulated by the Action of Other Photoreceptors

The recent isolation of the genes encoding the cryptochrome and phototropin photoreceptors (see Chapter 18) mediating blue light–regulated responses has made it possible to analyze whether these photoreceptors have overlapping functions (Chory and Wu 2001). This possibility was suspected because mutations in the cryptochrome *CRY2* gene led to delayed flowering under continuous white light, and flowering time was also known to be under phytochrome control.

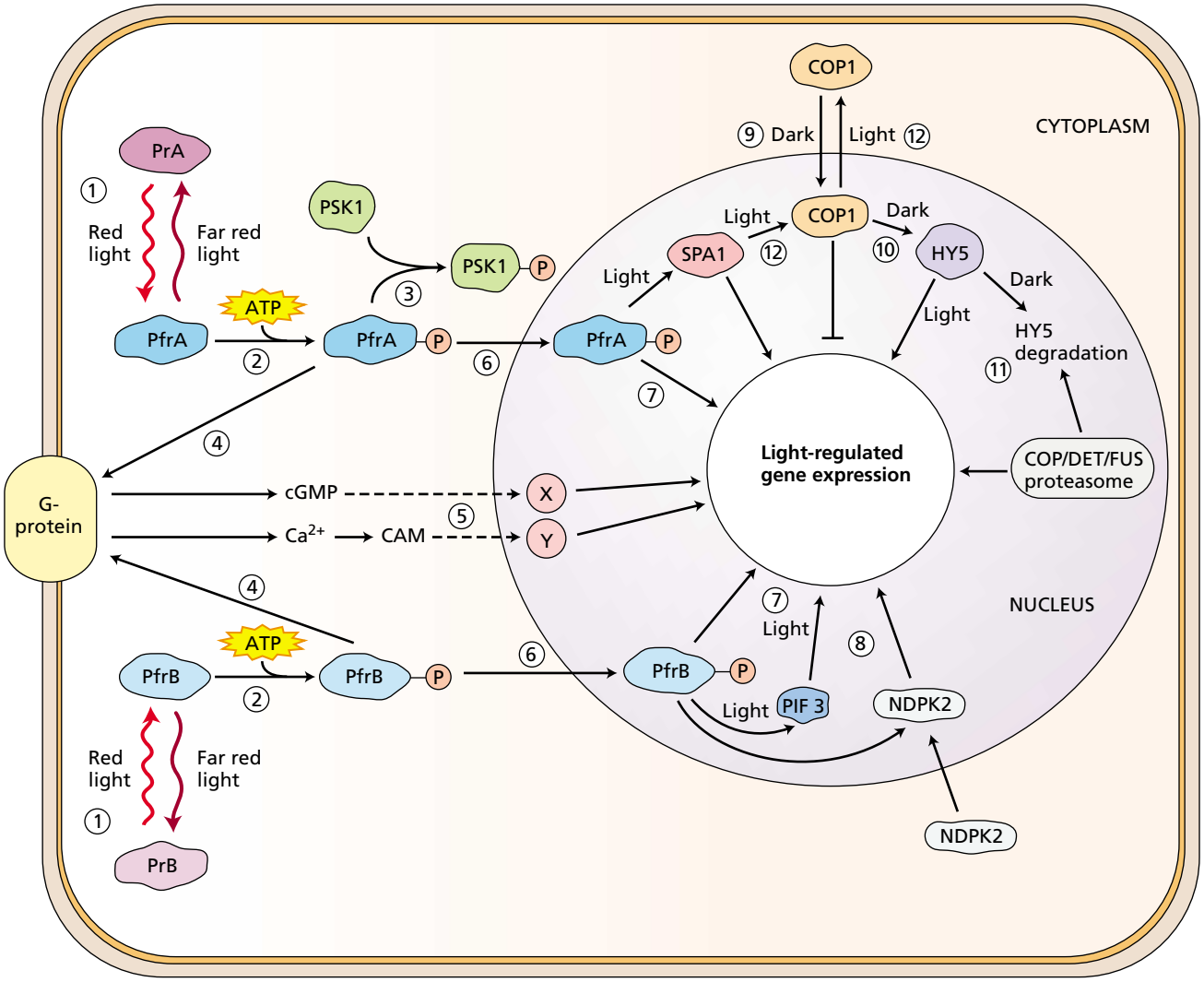
In *Arabidopsis*, continuous blue or far-red light treatment leads to promotion of flowering, and red light inhibits flowering. Far-red light acts through phyA, and the antagonistic effect of red light is through the action of phyB. One might expect the *cry2* mutant to be delayed in flowering, since blue light promotes flowering. However, *cry2* mutants flower at the same time as the wild type under either continuous blue or continuous red light. Delay is observed only if both blue and red light are given together. Therefore, cry2 probably functions to promote flowering in blue light by repressing phyB function.

Additional experiments have confirmed that the other cryptochrome, cry1, also interacts with phytochromes. Both cry1 and cry2 interact with phyA in vitro and can be phosphorylated in a phyA-dependent manner. Phosphorylation of cry1 has also been demonstrated to occur in vivo in a red light–dependent manner. Indeed, the importance of cryptochromes as developmental regulators has been underscored by their subsequent discovery in animal systems, such as mouse and human.

SUMMARY

The term *photomorphogenesis* refers to the dramatic effects of light on plant development and cellular metabolism. Red light exerts the strongest influence, and the effects of red light are often reversible by far-red light.

Phytochrome is the pigment involved in most photomorphogenic phenomena. Phytochrome exists in two forms: a red light–absorbing form (Pr) and a far-red light–absorbing form (Pfr). Phytochrome is synthesized in the dark in the Pr form. Absorption of red light by Pr converts it to Pfr, and absorption of far-red light by Pfr con-



1. Red light converts PrA and PrB to their Pfr forms.
2. The Pfr forms of phyA and phyB phytochrome can autophosphorylate.
3. Activated PfrA phosphorylates phytochrome kinase substrate 1 (PKS1).
4. Activated PfrA and PfrB may interact with G-proteins.
5. cGMP, calmodulin (CAM), and calcium (Ca^{2+}) may activate transcription factors (X and Y).
6. Activated PfrA and PfrB enter the nucleus.
7. PfrA and PfrB may regulate transcription directly or through interaction with phytochrome interacting factor 3 (PIF3).
8. Nucleoside diphosphate kinase 2 (NDPK2) is activated by PfrB.
9. In the dark, COP1 enters the nucleus and suppresses light-regulated genes.
10. In the dark, COP1, an E3 ligase, ubiquitinates HY5.
11. In the dark, HY5 is degraded with the assistance of the COP/DET/FUS proteasome complex.
12. In the light, COP1 interacts directly with SPA1 and is exported to the cytoplasm.

FIGURE 17.22 Summary diagram of the known factors involved in phytochrome-regulated gene expression. It is likely that additional shared and phytochrome-specific pathways will be uncovered as more signaling intermediates are identified. (After Sharma 2001.)

verts it to Pr. However, the absorption spectra of the two forms overlap in the red region of the spectrum, leading to an equilibrium between the two forms called a photostationary state.

Pfr is considered to be the active form that gives rise to the physiological response; however, Pr, particularly cycled Pr, plays a role in phyA-mediated responses. Other factors in addition to light regulate the steady-state level of Pfr, including the expression level of the protein and its stability in the Pfr form.

Phytochrome is a large dimeric protein made up of two equivalent subunits. The monomer has a molecular mass of about 125 kDa and is covalently bound to a chromophore molecule, an open-chain tetrapyrrole called phytochromobilin.

Phytochrome is encoded by a family of divergent genes that give rise to two types of proteins: Type I and Type II. Type I, which is encoded by the *PHYA* gene, is abundant in etiolated tissue. However, Type I phytochrome is present at low levels in light-grown plants because of its instability in the Pfr form, the phyA-mediated suppression of transcription of its own gene, and the instability of its mRNA. Type II phytochrome (encoded by the *PHYB*, *PHYC*, *PHYD*, and *PHYE* genes) is present at low levels in both light-grown and dark-grown plants because its genes are constitutively expressed at low levels and the protein is stable in the Pfr form.

Spectrophotometric and immunological studies indicate that the phytochromes are concentrated in meristematic regions. PhyA and phyB move to the nucleus upon conversion to the Pfr forms.

Phytochrome responses have been classified into very-low-fluence, low-fluence, and high-irradiance responses (VLFRs, LFRs, and HIRs). These three types of responses differ not only in their fluence requirements but also in other parameters, such as their escape times, action spectra, and photoreversibility. Phytochrome B plays an important role in the detection of shade in plants adapted to high levels of sunlight; phytochrome A has a more limited role, mediating the far-red HIR during early greening. Phytochromes C, D, and E also have specific roles during limited phases of development, and these roles are partially redundant with those of phyA and phyB.

Phytochrome is known to regulate the transcription of numerous genes. Many of the genes involved in greening, such as the nuclear-encoded genes for the small subunit of rubisco and the chlorophyll *a/b*–binding protein of the light-harvesting complex, are transcriptionally regulated by phytochrome (both phyA and phyB).

Phytochrome also represses the transcription of various genes, including *PHYA*. Activation or repression of these genes is thought to be mediated by general transcription factors that bind to *cis*-acting regulatory elements within the promoter regions of these genes in a combinatorial fashion. In some cases, phytochrome in the Pfr form interacts directly with these factors. These transcription factors, in turn, are linked to phytochrome action by a complex series of signal transduction pathways involving COP and DET proteins, kinases, cyclic GMP, trimeric G-proteins, Ca^{2+}, and calmodulin.

The discovery and characterization of bacterial phytochrome suggest that flowering-plant phytochrome evolved from a bacterial histidine kinase that participates in two-component signaling pathways.

In addition to the long-term effects involving changes in gene expression, phytochrome induces a variety of rapid responses, including chloroplast rotation in the alga *Mougeotia*, leaf closure during nyctinasty, and alterations in membrane potential. These responses involve rapid changes in membrane properties. The current view is that even these rapid effects of phytochrome involve signal transduction pathways.

Web Material

Web Topics

17.1 The Structure of Phytochromes
The purification and characterization of phytochrome as a homodimer are described.

17.2 *Mougeotia*: A Chloroplast with a Twist
Microbeam irradiation experiments have been used to localize phytochrome in this filamentous green alga.

17.3 Phytochrome and High-Irradiance Responses
Dual-wavelength experiments helped demonstrate the role of phytochrome in HIRs.

17.4 Phytochrome Interactions during Germination
The interactions between phyA and phyB during germination are described.

17.5 Phytochrome Functional Domains
Phytochrome overexpression has been used to characterize the functional domains of phytochrome.

17.6 Phytochrome Effects on Ion Fluxes
Phytochrome regulates ion fluxes across membranes by altering the activities of ion channels and the plasma membrane proton pump.

17.7 Phytochrome Regulation of Gene Expression
Evidence shows that phytochrome regulates gene expression at the level of transcription.

17.8 Regulation of Transcription by *Cis*-Acting Sequences
Phytochrome response elements are described briefly.

17.9 Genes That Suppress Photomorphogenesis

Further information is provided about genes like *COP* and *DET* that negatively regulate photomorphogenesis.

17.10 The Roles of G-Proteins and Calcium in Phytochrome Responses

Evidence suggests that G-proteins and calcium participate in phytochrome action.

17.11 The Origins of Phytochrome as a Bacterial Two-Component Receptor

The discovery of bacterial phytochrome led to the identification of phytochrome as a protein kinase.

Web Essay

17.1 Awakened by a flash of sunlight

When placed in the proper soil environment, seeds acquire extraordinary sensitivity to light so that germination can be stimulated by less than a second of exposure to sunlight during soil cultivations.

17.2 Know thy neighbor through phytochrome

Plants can detect the proximity of neighbors through phytochrome perception of the R:FR of reflected light and produce adaptive morphological changes before being shaded by potential competitors.

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