Light Perception and Transduction

13

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Plants utilize light not only for photosynthesis but also as an environmental signal for many developmental processes. Plants are very sensitive to seasonal, daily, and moment-to-moment changes in solar radiation. They are thus capable of perceiving wavelength, intensity, direction, duration, and other attributes of light to bring about appropriate physiological and developmental changes. These light-triggered growth and developmental responses are known as photomorphogenic responses (Box 13.1). Thus, **photomorphogenesis** may be defined as the developmental response of an organism to the information in light, which may be its quantity, quality (i.e., the wavelength), and direction or relative length of day and night (photoperiod). The importance of light in plant development can be most dramatically illustrated in case of early seedling growth. A dark-grown seedling is said to be **etiolated**. In etiolation, embryonic stem (hypocotyl in dicots and epicotyl in monocots) of seedlings exhibits very rapid and extensive elongation of internodes. There is no cotyledon/leaf expansion, and seedlings appear pale as there is no chloroplast development. So, etiolated plants are pale yellow, and the hypocotyl remains "hooked" at the apex. Curving of the hypocotyl is thought to protect the apical meristem from damage during seedling growth through the soil. On exposure to light, hypocotyl elongation slows down; cotyledons and leaves expand and become green. The apical hook straightens. In monocots, the etiolated coleoptile exhibits extended growth which gets decelerated (slows down) in light and developing leaves pierce its tip (Fig. 13.1). In contrast with **scotomorphogenesis**, seedlings grown in light exhibit photomorphogenesis and exhibit relatively shorter embryonic stem, lose apical hook, and develop expanded green cotyledons, and there is rapid initiation of leaf development at the shoot meristem. Upon sensing light, a seedling emerging from the soil switches from scotomorphogenesis to photomorphogenesis. This process is known as **de-etiolation**, and it involves the coaction of red/far-red light-absorbing phytochrome and blue light-sensitive cryptochrome.

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Box 13.1 History of Photobiology

- Joseph Priestley (1772) discovered that green plants utilize light as their source of energy for production of organic substances.
- Julius Sachs (1864) demonstrated that blue region of visible light results in phototropic bending of plants.
- Charles Darwin and his son Francis (1881) examined light signal transduction of phototropism and demonstrated that photoreceptive site (shoot tip) is different from the region showing bending response (subapical region) in monocot seedlings.
- Julien Tournois (1914) discovered that night length rather than day length determines flowering time.
- Wightman Garner and Harry Allard (1920) discovered that most plants could be classified as "short-day" or "long-day" plants and established the concept of "photoperiodism."
- Karl Hamner and James Bonner (1938) made a decisive contribution to photoperiodism by finding that a brief exposure of light in midnight, given under normally inductive conditions for flowering, caused cocklebur, a short-day plant, to remain completely vegetative.
- Harry Borthwick and his colleagues (1952) discovered the red (R) and far-red (FR) photoreversible effect on seed germination in lettuce and night-break of photoperiodic floral induction in cocklebur.
- Warren Butler, Karl Noris, Bill Siegelman, and Sterling Hendricks (1959) showed photoreversible absorption changes at 660 nm and 730 nm upon alternately given R and FR actinic light in etiolated maize tissues and a crude extract of the relevant proteinaceous pigment.
- The term "phytochrome" was half-jokingly used by Butler in his laboratory and then published by Borthwick and Hendricks (1960).
- From the 1960s to 1980s, only phytochrome was the known photoreceptor for photomorphogenesis.
- Hans Mohr and Schäfer (1983) in Freiburg (Germany) extensively investigated the effect of blue and far-red light on photomorphogenesis in terms of sensor pigments, signal amplification, and gene expression and established the concept of "high-energy reaction."
- Margaret Ahmad and Tony Cashmore (1993) isolated an *Arabidopsis* mutant, *hy4*, which was defective in blue light-dependent photomorphogenesis. The protein encoded by Hy4 was a member of the photolyase family and was named cryptochrome (cry).
- Chentao Lin and colleagues (1996) cloned and characterized a second member of the cry family containing a distinct C-terminal sequence, which was named cry 2, and Hy-4 encoded cry was renamed as cry 1.
- Winslow Briggs and colleagues cloned and characterized genes of *nph* (non-phototropic hypocotyl) mutants and showed that the gene product of NPH1 was a blue light receptor.

Huala et al. (1997) cloned and characterized phototropin from *Arabidopsis thaliana*.

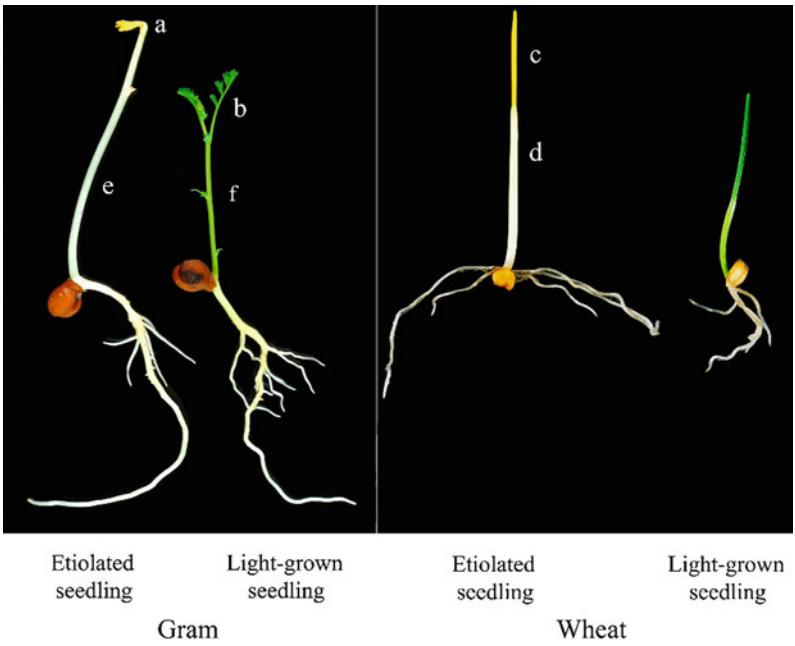


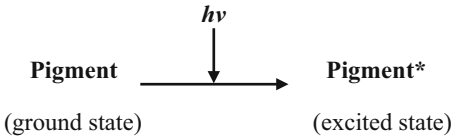
Fig. 13.1 Characteristics of etiolated and light-grown dicot and monocot seedlings. (**a**) Apical hook; (**b**) open, expanded green leaves; (**c**) shoot; (**d**) coleoptile; (**e**) elongated hypocotyl; (**f**) short hypocotyl

Many seeds require light for germination, a process called **photoblasty** [e.g., lettuce (*Lactuca sativa*)]. In some plants, leaves fold up at night (**nyctinasty**) [e.g., lotus (*Lotus japonica*)] and open at dawn (**photonasty**) [e.g., common evening-primrose (*Oenothera biennis*)]. In contrast with directional bending of shoots in response to unilateral or unequally distributed light (**phototropism**), photonastic movements take place in response to nondirectional light. Many plants flower at specific times of the year in response to changing day length, a phenomenon called as **photoperiodism**. In order to understand and appreciate the importance of light to plants, it is necessary to understand the physical nature of light.

13.1 Light Absorption by Pigment Molecules

For light to be effective in inducing a response in any living being, it must first be absorbed. A molecule that can interact with photons from the visible portion of the electromagnetic spectrum is called a **pigment**. Any photobiological process requires

a light-absorbing molecule or pigment. Plants contain a variety of pigments. Since pigments selectively absorb certain parts of the visible wavelength range, they appear colored to the human eye. The absorption of photons by the pigment molecule shifts the pigment molecule from its lowest-energy (ground) state to an excited state.



h = Planck's constant; ν = photon's frequency

This is caused by the shifting of one of the electrons of the pigment from its lower-energy molecular orbital (closer to the nucleus) to a higher-energy orbital. This transition to an excited state is possible when the atom of the pigment absorbs a light quantum with an energy that matches the energy difference between the molecule's non-excited (ground) state (Eg) and excited state (Ee).

$$E_e - E_g = hc/\lambda$$

h = Planck's constant, c = speed of light, λ = wavelength (nm)

13.1.1 Quantitative Requirement for Pigment Excitation

Two types of excited states can exist in light-sensitive molecules. The *singlet state* is relatively short-lived than the *triplet state*, in which electrons take longer to de-excite. An excited pigment molecule has a very short life (around 10^{-9} s), and it must get rid of excess energy and return to ground state. This dissipation of excess energy by the **exciton** (energized electron) is accomplished in several ways (Fig. 13.2):

1. *Thermal deactivation*: It involves loss of energy as heat into the environment and electron comes back to singlet state from ground state.
2. *Fluorescence*: It involves emission of light photons during a relatively slow process of return of exciton from first excited singlet state to ground state. The emitted photon has lower energy than its excited form, and so emission is in longer wavelength range. Thus, for example, chlorophyll molecules emit red fluorescence in solution upon excitation by blue light absorption.

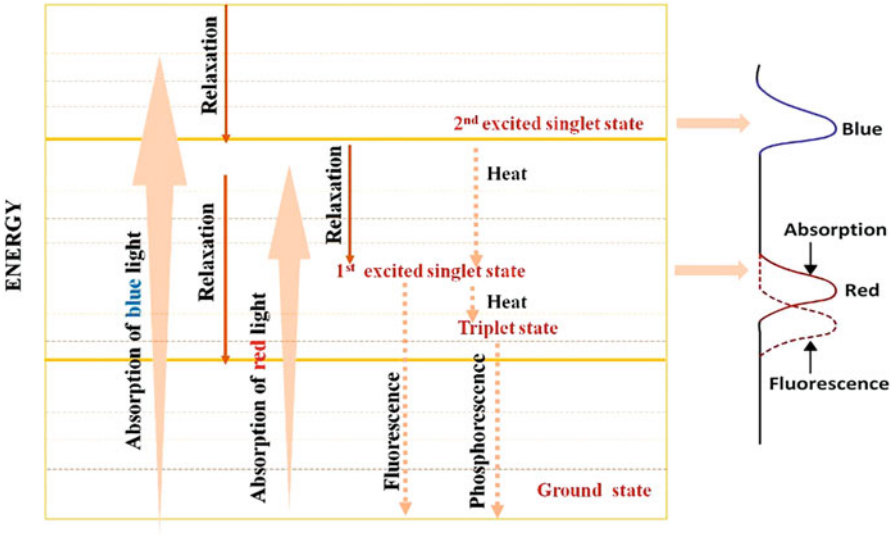


Fig. 13.2 Different energy levels attained by chlorophyll molecules upon absorption of blue or red light

3. *Energy transfer (inductive resonance)*: This involves transfer of energy to another molecule, generally involving an array of pigment molecules. This process accounts for much of the transfer of energy between pigment molecules in chloroplasts.
4. *Charge separation (photochemistry)*: An excited molecule may transfer energy (**hv**) to another molecule, leaving the donor pigment molecule positively charged and the acceptor molecule negatively charged. This phenomenon is central to the process of photosynthesis.

$$\text{Pigment} + \text{acceptor} \xrightarrow{hv} \text{pigment}^* + \text{acceptor} \rightarrow \text{pigment}^+ + \text{acceptor}-$$

5. *Shift to triplet state*: A molecule in singlet state may revert to another excited state, called the **triplet state**. Triplet state is more important than singlet state because longer lifetime of the metastable triplet state (around 10^{-3} s) allows photooxidation of the pigment (donor), and the acceptor molecule is reduced.
6. *Vibrational energy*: Although the energy levels of infrared and other longer wavelengths are very low to facilitate electron jump, their absorption by molecules can create vibrational energy in bonding systems. Thus, absorption

of infrared radiation by "greenhouse gases" (e.g., CO_2 and CH_4) traps atmospheric heat, and this is the cause of global warming and climate change.

Under the above-stated multiple options available for an excited pigment molecule to revert to ground state, the process with fastest rate is followed over others in plant cells. For most pigments found in photosynthetic apparatus, fluorescence occurs in nanoseconds (10^{-9} s), whereas photochemistry occurs more rapidly in picoseconds (10^{-12} s). Thus, because of the availability of 1000-fold more rapid photochemical pathway, fluorescence is hardly observed, and photosynthesis proceeds with high efficiency. It is the singlet state of the chlorophyll molecules which participates in energy transfer and photochemistry. Triplet state of chlorophyll molecules, with relatively long half-life, is not an intermediate in charge separation events in photosynthesis. Under optimum conditions, the **quantum yield (phi; ϕ) of photosynthetic systems** is 1.0, indicating that every absorbed photon is converted into a chemical product. Values less than one would indicate lower photosynthetic efficiency due to other decay pathways. Such losses in other steps of primary photochemical event are associated with providing stability to some of the photochemical reaction products.

$$\text{Quantum yield } (\phi) = \frac{\text{Number of photochemically formed products}}{\text{Number of quanta involved}}$$

$$\text{Maximum value} = 1.0$$

13.2 Nature of Light

By passing white light through a prism, English physicist Sir Isaac Newton (1642–1727) separated light into a spectrum of visible colors, thereby demonstrating that white light consists of different colors, ranging from violet at one end and red at the other end of the spectrum. Separation of light of different colors is possible because they are refracted at different angles while passing through a prism. Subsequently, another British physicist, James Clerk Maxwell (1831–1879), demonstrated that white light is a small component of the vast spectrum of the **electromagnetic radiation**. The radiations in the electromagnetic spectrum travel in waves. The **wavelength** refers to the distance from the crest of one wave to the crest of the next (Fig. 13.3). The wavelengths in the electromagnetic spectrum range from cosmic rays (measured in nanometer; 1 nm = 10^{-14} meter) to those of low-frequency radio waves (measured in kilometers; 1 km = 10^3 meter = 0.6 mile) (Fig. 13.4). Shorter wavelengths have greater energy associated with them. Conversely, longer wavelengths have lower energy. In the visible spectrum of light,

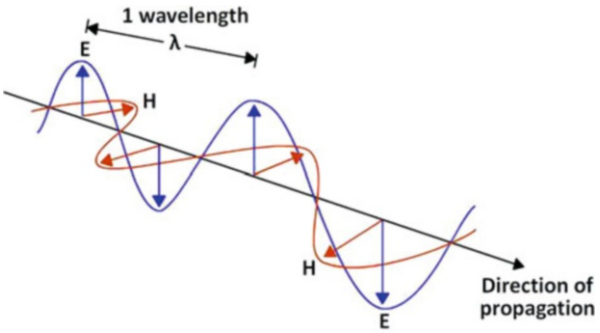


Fig. 13.3 The wave nature of light

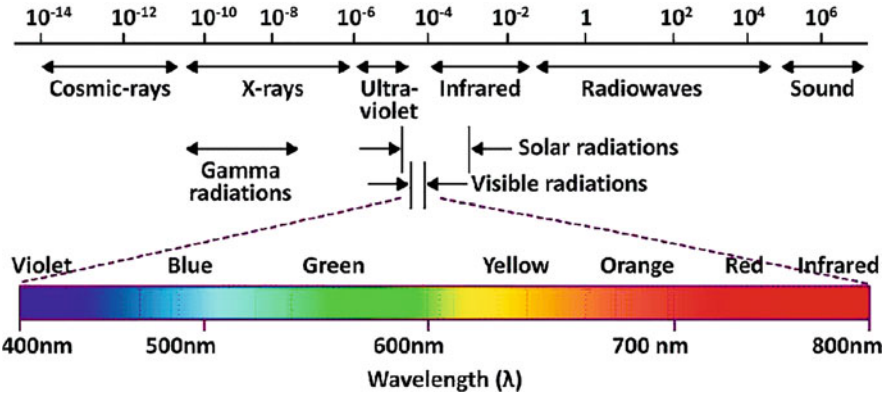


Fig. 13.4 The electromagnetic spectrum. Visible light is only a small part of the electromagnetic spectrum

violet light has the shortest wavelength and has almost twice the energy as compared to of the longest rays of far red (Table 13.1).

According to the **particle model of light** proposed by Albert Einstein in 1905, light is composed of particles of energy, called **photons** or **quantas**. The energy of a photon or quanta of light is inversely proportional to its wavelength, i.e., the longer the wavelength, the lower is the energy associated with it. Both the above-stated models of light (wave model and particle model) are complementary to each other and are required for a full understanding of the properties of light.

Each quantum or photon of light contains energy equal to **Planck's constant**, h [6.626×10^{-34} Joule (s^{-1}) multiplied by the frequency of the radiation, ν, in cycles per second (s^{-1})]

$$E = h\nu$$

Since different wavelengths of light have different energy levels, the energy of photons of a particular wavelength can be described as

Table 13.1 Wavelengths capable of causing biological responses and their respective energy levels

Color	Wavelength range (nm)	Average energy (kJ. mol^{-1} photons)
Ultraviolet	100–400	
UV-C	100–280	471
UV-B	280–320	399
UV-A	320–400	332
Visible	400–740	
Violet	400–425	290
Blue	425–490	274
Green	490–550	230
Yellow	550–585	212
Orange	585–640	196
Red	640–700	181
Far-red	700–740	166
Infrared	Longer than 740	85

$$E = hc/\lambda$$

where c is the velocity of light (3×10^8 $m.sec^{-1}$) and λ is the wavelength (m).

The energy of light is inversely proportional to its wavelength. Thus, blue light (490 nm) has 274 kJ (kilo Joules) of energy per mole of photons in contrast with red light (740 nm), which has only 181 kJ of energy per mole of its photons. Gamma and X-rays are at the short wavelength end of the electromagnetic spectrum and are very energetic in contrast with radio waves of long wavelength. Short wavelength photons are energetic enough to provide sufficient kinetic energy to break electrons free from atoms. Therefore, these radiations are referred as **ionizing radiations**. These radiations (wavelength < 295 nm) are filtered by the atmosphere and do not reach earth, thereby avoiding their hazardous effect on living beings. Ultraviolet (UV) radiation is generally subdivided into UV-A (400–320 nm), UV-B (320–280 nm), and UV-C (280–100 nm). It may be noted that oxygenic photosynthetic organisms use visible light (400–700 nm) for photosynthesis, growing in anoxygenic, whereas many photosynthetic organisms can photosynthesize in near-infrared wavelengths greater than 700 nm.

13.3 Absorption and Action Spectra

Absorption of light by a pigment (e.g., chlorophyll) plotted as a function of wavelength is known as **absorption spectrum**. Such a spectrum provides information about the extent to which principal bands of light spectrum are absorbed by the pigment. The height and/or width of the absorption curve indicates the extent by which light of that wavelength is absorbed. Each light-absorbing molecule has a unique absorption spectrum. Thus, an absorption spectrum is like a fingerprint of the pigment molecule which serves as a key to its identification. Varying forms of

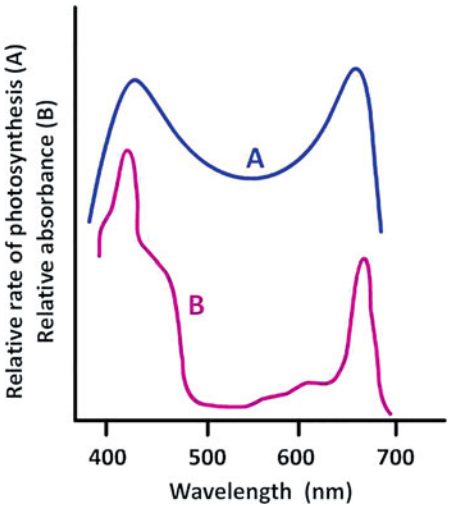


Fig. 13.5 (**A**) Action and (**B**) absorption spectra of chlorophyll pigments. The peaks in red and blue regions of the action spectrum correspond with principal absorption peaks

chlorophyll molecules absorb optimally at specific wavelengths, indicated as peaks of absorbance in the absorption spectrum wavelengths of light. An **action spectrum** is a graph which depicts light action/response. One of the earliest action spectra was generated by T.W. Engelmann in the late 1800s by using a prism to disperse sunlight into a spectrum. These separated wavelengths of visible light were used to illuminate *Spirogyra* filaments which lead to preferential accumulation of oxygen-seeking bacteria in the filament's regions exposed to blue and red light. These regions of light are strongly absorbed by chlorophyll molecules leading to enhanced photosynthesis (and oxygen evolution). Thus, analysis of action spectra provides critical information for the discovery of photosynthesis in oxygen evolving photosynthetic organisms. In modern laboratories, action spectra are also generated by using **spectrographs** fitted with monochromatic sources of light. A comparison of the absorption and action spectra of a pigment can provide useful information on the identity of the pigment responsible for a photosynthetic process. Figure 13.5 shows typical absorption and action spectra of chlorophyll from leaves. It may be noted that the action spectrum has pronounced peaks in red and blue regions of the spectrum which correspond with absorption maxima for chlorophyll, thereby indicating the role of chlorophyll in photosynthesis.

13.4 Light Parameters Which Influence Plant Responses

Light can influence the physiology and development of plants in many ways. Each light source may have different effects on plant development and behavior due to variations in spectral distribution. Three parameters of light play significant role in modulating plant development. These are 1. light intensity, 2. light composition/(quality), and 3. light duration (photoperiod). Light intensity is most commonly

measured in terms of fluence. **Fluence** is defined as the amount of radiant energy falling on a small sphere, divided by the cross section of the sphere. Since light energy is absorbed or emitted as photons, fluence is expressed either as photons or quantas (in moles, mol) or as amount of energy (in Joules, J). The term **photon fluence** (units = $mol.m^{-2}$) refers to total number of photons incident on the sphere. **Energy fluence** (units = $J.m^{-2}$) refers to the total amount of energy incident on the sphere. In terms of rate, the terms are **photon fluence rate** (units = $mol.m^{-2}.s^{-1}$) and **energy fluence rate** (units = $J.m^{-2}.s^{-1}$ or $W.m^{-2}$). **Irradiance** ($W.m^{-2}$), which is often interchangeably used for energy fluence rate, refers to the flux of energy on a flat surface (rather than a sphere). It may be noted that time (seconds, s) is contained within the term watts 1 W = 1 Joule $(J)s^{-1}$. Another term, **quantum flux** or **photon flux density (PED)**, refers to the number of incident quantas of light striking the leaf. It is expressed in $mol.m^{-2}.s^{-1}$, where moles refers to the number of photons (1 mol of light = 6.02×10^{23} photons; Avogadro number). When considering photosynthesis and light, it is preferred to express light intensity as **photosynthetic photon flux density (PPFD)**, i.e., the flux of light ($\mu mol.m^{-2}.s^{-1}$) in the photosynthetically active radiation (PAR) range (i.e., 400–700 nm). On a clear sunny day, plants usually experience a PPFD of about 200 $\mu mol.m^{2-}.s^{-1}$ at the top of the forest canopy. It may be as little as 10 $\mu mol.m^{-2}.s^{-1}$ at the bottom of the canopy.

13.5 Light Absorption Depends on Leaf Anatomy and Canopy Structure

On an average, each square meter of earth surface receives about 340 W of solar energy every day. But a major fraction of solar radiation (50%) is either of too short range or too long range to be absorbed by the photosynthetic pigments. Furthermore, only a small percentage of photosynthetically active radiation (PAR, 400–700 nm) incident on a leaf is surface transmitted through the leaf, and the rest (15%) is reflected or transmitted from the leaf surface (Fig. 13.6). Since chlorophylls absorb blue and red wavelengths of light most strongly, green wavelengths are maximally transmitted and/or reflected, giving green color to the vegetation. A fraction of PAR absorbed by the leaf is also lost through metabolism (20%) and as heat (10%). Thus, at the end, about 5% of the sun's total radiant energy falling on leaf surface is utilized in photosynthesis to produce carbohydrates (Fig. 13.7).

Leaf anatomy is well adapted for maximum utilization of light. The epidermal cells are generally convex so that they focus light for enhanced capture by the chloroplasts. Epidermal focusing of light is common among herbaceous plants and in tropical plants growing on the forest floor where light levels are very low. The palisade cells below the epidermis are shaped like pillars arranged in parallel columns which provide sieve effect and channeling of light. The **sieve effect** is caused by nonuniform distribution of chlorophyll molecules within the chloroplasts which results in shaded areas in chloroplasts which do not receive light. Consequently, much less light is absorbed in palisade cells than would be expected if chlorophyll molecules are uniformly distributed. Presence of large vacuoles and air

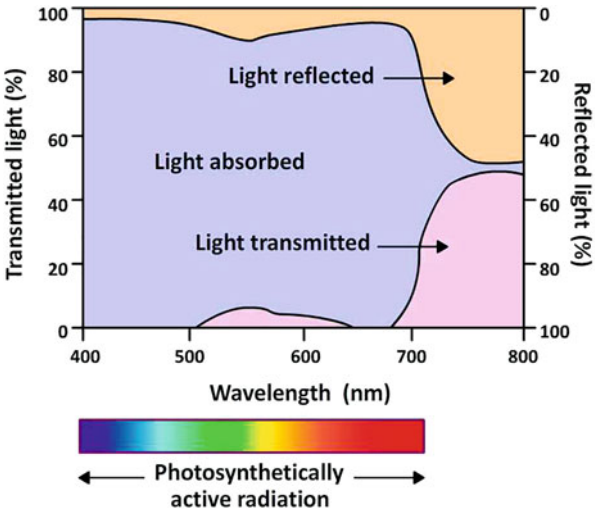


Fig. 13.6 Relative proportions of visible light absorption reflected and transmitted by green plants as a function of wavelength. The transmitted and reflected green light (500–600 nm) gives leaves their color

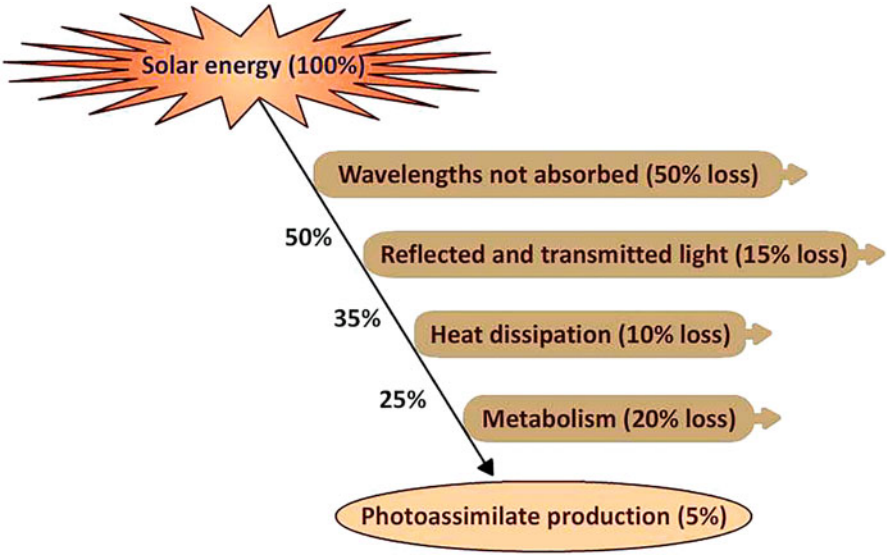


Fig. 13.7 Utilization of solar energy in metabolism and photoassimilate (carbohydrate) production in leaves

spaces in the zone of palisade cells causes **light channeling** into deeper regions of the leaf. The large air spaces in the cluster of irregular-shaped spongy mesophyll cells reflect and refract light, thereby causing light scattering. Thus, sieving effect of palisade cells and scattering effect of spongy cells together contribute in an efficient absorption of light throughout the leaf. Desert plants exposed to excess light develop

hairs, salt glands, and epicuticular wax to enhance light reflection from leaf surface, thereby reducing overheating due to absorption of excess amount of solar energy.

Leaves covered by other leaves (shaded leaves) capture lesser light and light of different quality. They have lower photosynthetic efficiency than the leaves fully exposed to sunlight. **Sunflecks** (patches of sunlight passing through small gaps in leaf canopy in dense forests) play important role in rapid and short-term light capture for plants growing on forest bed and in densely planted crops where lower leaves are shaded by upper leaves. Under natural conditions, leaves at the top of the canopy tend to receive less than the maximum light available, by having a steep leaf angle. This also facilitates light to penetrate more into the canopy. On the other hand, some leaves maximize leaf absorption by **solar tracking (heliotropism)** by continuously adjusting the orientation of leaf lamina such that they are perpendicular to the leaf surface. For example, leaves of cotton, alfalfa, soybean, and lupine show the phenomenon of solar tracking. Leaves which maximize light capture by heliotropism are referred as **diaheliotropic**. Some leaves employ their heliotropic movement to avoid or reduce light capture. They are referred as **paraheliotropic**. Some plants (e.g., soybean) display diaheliotropic leaf movement when they are well watered and paraheliotropic movement when they are under water stress.

Light quality or **spectral energy distribution (SED)** can vary depending on the nature of light source. In case of natural light, it can vary depending on the cloud cover, quality of atmosphere, and time of the day. SED of fluorescent lamps is rich in blue part of the spectrum, whereas incandescent lamps have high emissions in far red and infrared. The fluence rate and spectral quality of visible light are constantly changing throughout the day. Diffuse skylight in the early morning hours is rich in blue wavelength because shorter wavelengths are preferentially scattered by moisture droplets and dust of the atmosphere. Hence, normal daylight is enriched with blue sky (hence, the blue sky!). At twilight with solar elevation of 10° or less from the horizon, light scattering and refraction at low angles enrich light with longer wavelengths of red and far-red region. Generally, the path traveled by sunlight at twilight is up to 50 times longer than from the overhead sun. In such a situation (twilight), just before sunset much of the violet and blue light are scattered, leaving red and orange light to the observer's sight. Cloud cover reduces irradiance, and the proportion of scattered (blue) light is increased. Likewise, air pollutants cause both light scattering and absorption.

13.6 Photoreceptors

Plant pigments, such as chlorophyll and accessory pigments of photosynthesis, absorb visible light at specific wavelengths and reflect or transmit non-absorbed wavelengths, which are perceived as colors. **Photoreceptors**, unlike plant pigments (such as chlorophyll), are chromophore-containing biomolecules which absorb photons of a given wavelength and use the energy derived from it as a signal to initiate a photoresponse. All photoreceptors contain a protein (**chromoprotein**) attached to a light-absorbing, non-protein, prosthetic group called as **chromophore**.

The protein part of the chromoprotein is called **apoprotein.** The complete molecule, or **holochrome**, consists of chromophore plus the protein. Other common features of all photoreceptors are their sensitivity to light quantity (number of photons), quality (wavelength), and duration of exposure. All photoreceptors perceive light and initiate cellular signals leading to a response. In the following pages, a detailed account of plant photoreceptors is being presented.

13.7 Protochlorophyllide

Etioplasts are plastids whose development from proplastids to chloroplasts has been arrested due to the absence of light. They lack chlorophyll but produce **protochlorophyllide—a precursor of chlorophyll a**. Etioplasts contain a prominent structure called **prolamellar body** which contains membrane lipids, protochlorophyllide, and the light-requiring enzyme—**protochlorophyllide oxidoreductase (POR)**. Prolamellar bodies are formed when membrane lipid synthesis continues in the absence of corresponding amounts of thylakoid protein synthesis, which requires light. The high concentration of lipids in the prolamellar bodies (~ 75%) results in the formation of lipid tubes which branch in three dimensions to form a semicrystalline lattice. When etioplasts are illuminated, they begin to develop into chloroplasts. Light triggers chlorophyll biosynthesis from protochlorophyllide. It also results in the assembly of stable chlorophyll-protein complexes, resulting in the outgrowth of thylakoid membranes from the prolamellar body. Protochlorophyllide is structurally similar to chlorophyll a, except that it has a double bond between carbon C17 and C18 in D ring (Fig. 13.8). Protochlorophyllide is not green and cannot absorb light for photosynthesis. It is a photoreceptor with an absorption maximum at 650 nm. Light absorption by protochlorophyllide takes place in the presence of NADPH-protochlorophyllide oxidoreductase (POR), resulting in the addition of protons to the double bond between C17 and 18 in ring D, and protochlorophyllide gets converted into chlorophyll a. It is now established that aerobic photosynthetic bacteria, liverworts, and gymnosperms contain a light-independent form of POR. At some point during evolution, angiosperms lost this enzyme, and they have only light-dependent form of POR. Thus, angiosperms have an absolute requirement of light for chlorophyll a biosynthesis in chloroplasts. Light-

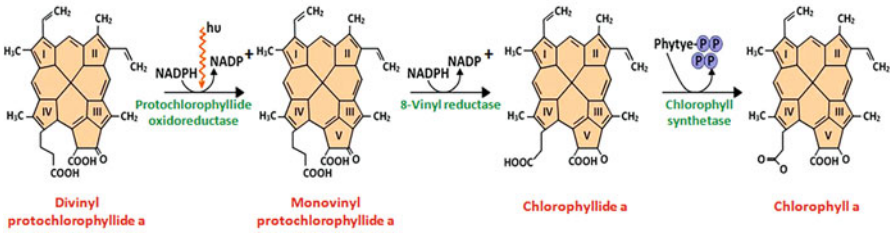


Fig. 13.8 Conversion of protochlorophyllide a into chlorophyll a in response to light

dependent POR is a monomeric enzyme of 35–38 kDa. Barley has two POR genes, while *Arabidopsis* has three. In *Arabidopsis*, POR A is specifically expressed in dark and is downregulated by phytochrome. POR B is constitutively expressed, and POR C is induced by light. The lattice-like structure of prolamellar bodies in etioplasts is largely composed of a complex of protochlorophyllide, NADP and POR. Upon illumination, POR rapidly converts protochlorophyllide into chlorophyllide, and the internal structure of etioplasts is reorganized into a typical chloroplast. Subsequently, POR is released and degraded by proteolysis. Thus, newly developed chloroplasts do not contain POR protein.

$$\text{Protochlorophyllide} + \text{NADPH} + \text{H}^{+} \xrightarrow[\text{Light}]{\text{POR}} \text{Chlorophyllide a} + \text{NADP}$$

The photoreceptor properties of POR are comparable with those of phytochrome in the following ways:

1. POR possesses a tetrapyrrole chromophore which is responsible for triggering photomorphogenic changes (chloroplast development).
2. The form and abundance of POR are regulated by light.

Most of the chlorophyll molecules in chloroplasts are conjugated with thylakoid proteins. The absorption spectra of pigment-protein complexes are markedly different from that of free pigment in solution (Fig. 13.9). This chlorophyll-protein conjugation helps to maintain the pigment molecules in the precise relationship for efficient light absorption and energy transfer. It also provides a unique environment to each pigment molecule, resulting in their specific absorption maxima. These slight differences in absorbance maxima facilitate an orderly transfer of energy through various pigments to the reaction center for photochemical reactions. Lastly, chlorophyll-protein interaction also reduces photosensitization of plants which

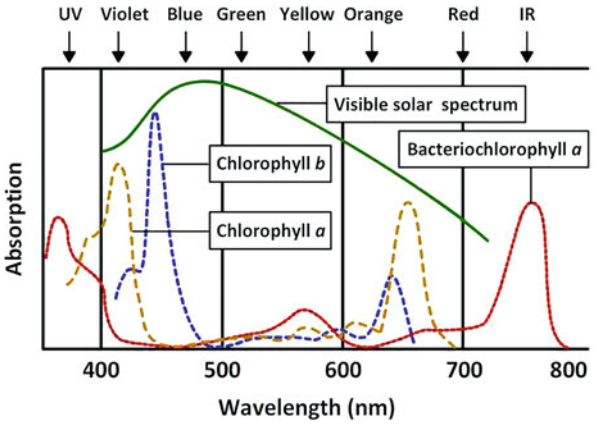


Fig. 13.9 Absorption spectra of chlorophyll a, b and bacteriochlorophyll in solution. Note that the spectra of these pigments exhibit substantial shifts in absorbance in vivo where they are associated with specific proteins

would lead to destruction of chloroplasts. Free form of chlorophyll is less efficient in photosynthetic energy transfer, but it reacts more readily with oxygen to produce reactive oxygen species (ROS) such as oxygen free radicals, hydroxyl radicals, and singlet oxygen, which can disrupt chloroplasts.

13.8 Phycobilins

Phycobilins are open-chain tetrapyrrole pigment molecules present in red algae and cyanobacteria (Fig. 13.10). Of the four phycobilins, three (phycoerythrin, phycocyanin, and allophycocyanin) are involved in photosynthesis, and the fourth one (phytochromobilin) is an important photoreceptor (phytochrome) that regulates various aspects of growth and development. In addition to their open-chain tetrapyrrole structure, the phycobilin pigments differ from chlorophylls by the fact that the tetrapyrrole group is covalently linked with a protein, and these phycobilins are organized into macromolecular complexes called **phycobilisomes**. Phycocyanin, phycoerythrin, and allophycocyanin are exclusively found in cyanobacteria and red algae where they function for light harvesting during photosynthesis. In addition to other wavelengths, these pigments (particularly phycoerythrin) also absorb energy in green region of the visible spectrum where chlorophylls do not absorb. The red algae appear almost black because chlorophyll and phycoerythrin together absorb almost all wavelengths of the visible spectrum (Fig. 13.11). The fourth phycobilin

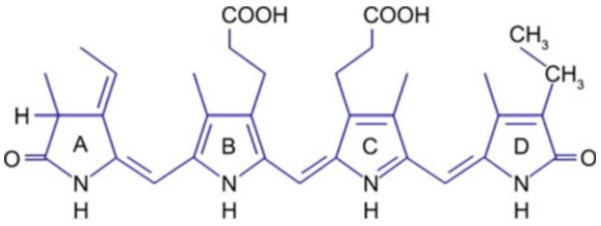


Fig. 13.10 Structure of phycocyanin chromophore—an open-chain tetrapyrrole molecule

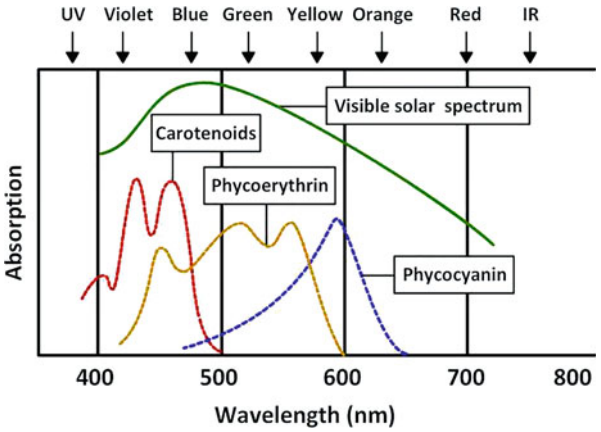


Fig. 13.11 Absorption spectra of accessory photosynthetic pigments

(phytochromobilin) is **phytochrome** which is a receptor in higher plants and plays important roles in many photomorphogenesis phenomena.

13.9 Phytochromes

Phytochromes were first identified among flowering plants as photoreceptors for photomorphogenesis in response to red light and far-red light. They strongly absorb red (620–700 nm) and far-red (700–800 nm) wavelengths but can also absorb blue light (350–500 nm) and UV-A radiation (320–400 nm). Phytochromes were discovered through experiments on **red/far-red reversibility** of seed germination in lettuce. It is now known that phytochromes are members of a gene family present in all land plants, streptophyte algae, cyanobacteria, other bacteria, fungi, and diatoms. Since red or far-red light cannot penetrate deep water, phytochromes in deep water aquatic organisms, mainly algae, have been reported to sense orange, green, or even blue light indicating the potential of phytochromes to be separately tuned to absorb different wavelengths during natural selection. Table 13.2 highlights some of the established photoreversible roles of phytochrome in a variety of organisms.

Table 13.2 Photoreversible responses induced by phytochrome in a variety of organisms

Group	Genus	Stage of development	Effect of red light
Angiosperms	Lettuce	Seeds	Promotes germination
	Oat	Seedlings (etiolated)	Promotes de-etiolation (e.g., leaf unrolling)
	Mustard	Seedlings	Promotes formation of leaf primordial, development of primary leaves, and production of anthocyanin
	Pea	Adult plant	Inhibits internode elongation
	Cocklebur	Adult plant	Inhibits flowering (photoperiodic response)
Gymnosperm	Pine	Seedlings	Enhances rate of chlorophyll accumulation
Pteridophytes	*Onoclea* (sensitive fern)	Young gametophytes	Promotes growth
Bryophytes	*Polytrichum* (moss)	Germling	Promotes replication of plastids
Algae	*Mougeotia*	Mature gametophytes	Promotes orientation of chloroplasts to directional dim light
Fungi	*Aspergillus nidulans*	Sexual development	Represses sexual development

13.9.1 Photoreversibility of Phytochrome-Modulated Responses and Its Significance

Photoreversibility (**photoconversion**; also referred as **photochromism**) is a defining feature of phytochromes. Phytochromes are synthesized as an inactive red light-absorbing isomer, called P_r. Red light absorption by P_r form converts it into an active, far-red-absorbing isomer called P_{fr}. Absorption of far-red light by P_{fr} form converts it back to P_r form. Thus, red light promotes phytochrome responses by converting inactive P_r into active P_{fr} form. Far-red light inhibits the red light-induced response by converting P_{fr} back to P_r spontaneously as well. Such a spontaneous reversion of P_{fr} to P_r occurs both in light and dark. Since it is measured by a decrease in P_{fr} concentration, it is referred as **dark reversion**. Phytochrome pool is never fully converted to P_{fr} or P_r form because the absorption spectra of P_{fr} and P_r forms overlap (Fig. 13.12). When P_r molecules are exposed to red light, most of it is converted to P_{fr}, but some of P_{fr} is spontaneously converted back to P_r. The proportion of phytochrome in P_{fr} form after providing saturating red light condition is about 88%. Likewise, a very small amount of far-red light absorbed by P_{fr} form leads to incomplete conversion of P_{fr} to P_r. Thus, an equilibrium of 98% P_r and 2% P_{fr} is established. This state of equilibrium is termed as **photostationary state**. Photoconversion of phytochrome provides assessment of the available red-to-far-red light ratio in the plant's environment. Low R:FR ratio has a variety of development effects on various species. It inhibits seed germination in *Arabidopsis* and induces shade escape responses during plant growth. Plants growing under a canopy use phytochrome to sense R:FR ratio so as to regulate shade avoidance, seed germination, etc. In natural conditions, plants are exposed to a broad spectrum of light. R:FR ratio is strongly affected by plant canopy because chlorophyll absorbs red light but not far-red light, thereby affecting R:FR ratio for plants growing below the canopy.

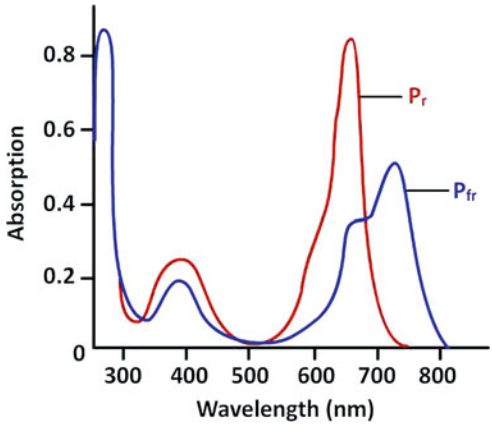


Fig. 13.12 Absorption spectra of two forms of phytochrome (P_r and P_{fr})

13.9.2 Chemical Nature of Phytochrome Chromophore

Phytochrome is a biliprotein consisting of 120 kDa apoprotein with a photosensitive prosthetic group (**chromophore**). The chromophore is a straight chain tetrapyrrole, a bilin called as **(3E)-phytochromobilin** or PϕB, where 3E refers to the isomeric form of the photoactive molecule. Phytochromobilin is synthesized inside the plastids and shares its biosynthetic origin with cyclic tetrapyrrole molecules, such as heme and chlorophyll. Phytochromobilin is exported from plastids into the cytosol where it gets attached to the apoprotein through a thioether linkage (ethers in which oxygen is replaced by sulfur in a cysteine residue of the protein). The four pyrrole rings are referred as A, B, C, and D. Phytochromobilin exists in two molecular forms: $P\phi B_r$ and $P\phi B_{fr}$. The red wavelength-absorbing form of phytochromobilin ($P\phi B_r$) has a peak absorbance at 660 nm and gets converted into $P\phi B_{fr}$, which has a peak absorbance at 730 nm in the far-red region. $P\phi B_{fr}$ gets converted into $P\phi B_r$ form upon exposure to far-red light (Fig. 13.13). The phytochrome biliprotein, in association with $P\phi B_r$ chromophore, is the **red-absorbing form** of the phytochrome and is referred as P_r. The **far-red-absorbing form** is P_{fr} with chromophore in $P\phi B_{fr}$ configuration. Red light exposure brings about a cis to trans change in configuration in the methane bridge between C and D pyrrole rings. *Far-red light drives it back into cis form*. $P\phi B_{fr}$ also gets converted to $P\phi B_r$ in dark through a slow process (dark reversion). *It is the $P\phi B_{fr}$ form which triggers photomorphogenesis*. The biological output of phytochrome cycle is estimated by the ratio of $P\phi B_r$ and $P\phi B_{fr}$ forms of the chromophore. This, in turn, gives an indication of the relative levels of red and far-red light in the plant's environment.

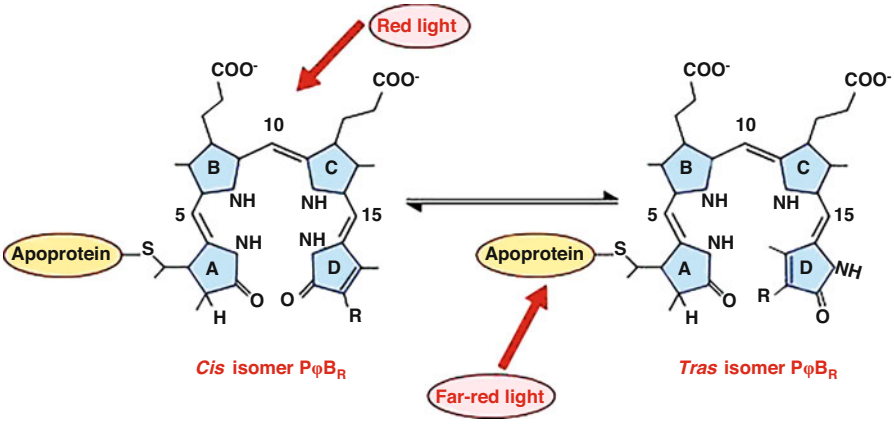


Fig. 13.13 Red/far-red light-induced *cis-trans* isomerization of phytochrome chromophore brought about by a change in the configuration at the double bond between carbon atoms 15 and 16 between C and D ring

13.9.3 The Multidomain Structure of Phytochrome Protein

Phytochromes are soluble proteins and exist as dimers. Each subunit of the dimer is bound to a phytochromobilin molecule. Light absorption by phytochromobilin leads to its isomerization which results in changes in its absorption spectrum and conformation of the phytochrome protein. The phytochrome protein consists of an N-terminal **photosensory region** and a C-terminal **regulatory domain**. The two are attached with a **hinge** between them (Fig. 13.14). The photosensory part has four domains (P1 to P4). P1 and P4 function in the inhibition of dark reversion of P_{fr} to P_r. P_2 (a PAS type of domain) is involved in sensing of signals, and P_3 is the binding site for the phytochromobilin molecule. The regulatory region of phytochrome protein consists of two PAS-type domains. PAS *(Per-ARNT-Sim) refers to some (α/β) conserved structural domains in a wide range of prokaryotic and eukaryotic proteins involved in sensing of signals*. C-terminal domain of the regulatory region exhibits kinase activity. It may be noted that higher plant phytochrome lacks a functional histidine kinase (HK) domain which is present in bacteria. They (higher plants) contain histidine kinase-related domain (HKRD) in place of functional histidine kinase of prokaryotes. HKRDs are 13–17% identical to HKs of the bacterial two component system.

In the red-absorbing form of the holoprotein (P_r), both subunits of the phytochrome dimer are folded at the hinge so that N-terminal photosensory and C-terminal regulatory regions are brought into contact with each other. Red light exposure opens up the structure in the far-red-absorbing form of phytochrome (Pfr), making its surface accessible to **phytochrome-interacting factors (PIFs)** in the signal transduction pathway. To sum up, phytochrome is activated in the cytoplasm by the absorption of light and enters the nucleus as P_{fr}, and P_{fr} interact with PIFs to alter gene transcription. The opened-up structure of phytochrome protein allows **light-dependent phosphorylation** of exposed serine and threonine residues, possibly

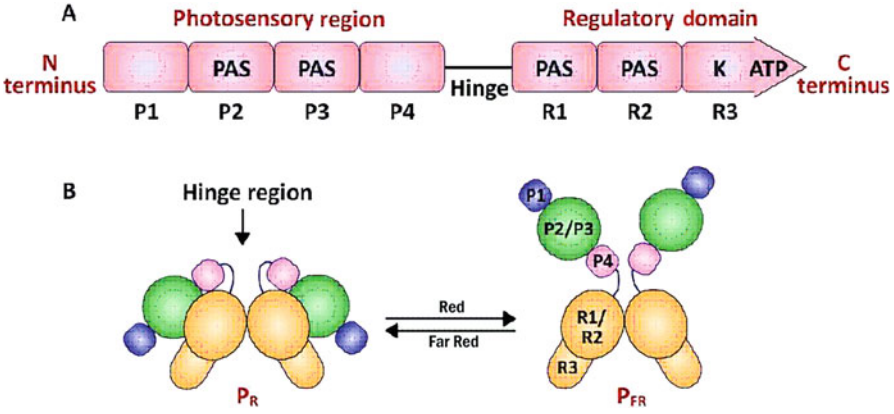


Fig. 13.14 Structure of phytochrome apoprotein and modulation of the folding pattern of its dimer by red/far-red light

triggered by autophosphorylation by R3 kinase at the C-terminal. A P_{fr}-specific **phosphatase** removes phosphate group from the amino acids in the hinge region, thereby enhancing the affinity of phytochrome protein for interaction with PIFs. Phytochrome probably also acts as a signal in cytoplasm in addition to its action through nuclear gene transcription. Such cytoplasmic signaling might occur via phosphorylation of substrate proteins by phytochrome. Conformational differences between P_r and P_{fr} are also responsible for differences in their relative stability. *Thus, the half-life of P_r is about 1 week, whereas that of P_{fr} is 2 h.* This also reflects the difference in the compactness of the protein. Thus, phytochrome action is under a fine control of red/far-red modulated phosphorylation/dephosphorylation events.

13.9.4 Forms of Biologically Active Phytochrome

Different forms of phytochrome are produced by plants. Five genes encode for phytochrome in *Arabidopsis* (PHY A–E). Rice has three genes (PHY A–C). Most conifers have four genes, cycads have three, ferns have two, and lycopods have one gene encoding phytochrome. Phytochromes produced by these genes have different molecular properties. Five different phytochromes (phy A–E) produced in *Arabidopsis* possess identical chromophores, showing the same absorption spectra, but have different apoproteins displaying distinct functions. Only phy A is light labile, and all others (phy B-E) are light stable. The light-labile form of phytochrome (phy A) is predominant in etiolated seedlings which strongly express PHY A gene, resulting in high accumulation of P_rA. Prolonged light exposure causes phy A concentration to drop by almost 100-fold. Light also inhibits PHY A transcription. Though less abundant, phy A still functions in light-grown plants. In contrast, phy B–E (light-stable phytochromes) have the similar abundance in both light- and dark-grown plants and are majorly found in mature plants. In *Arabidopsis*, only phy A forms **homodimers**, whereas other phytochromes can form **heterodimers** with each other. The physiological significance of such dimerization is not clear.

13.9.5 Phytochrome-Mediated Responses

Phytochrome-mediated responses can be grouped into **high irradiance responses (HIRs)** or **low fluence response (LFRs)**. A unique feature of LFRs is that they obey the principle of reciprocity; i.e., they may be caused by long exposure to dim light or short exposure to high irradiance. LFRs are further grouped into two categories: 1. low fluence red/far-red reversible responses (LFRs) and 2. very low fluence response (VLFR). Table 13.3 summarizes the characteristics of phytochrome responses at different fluences.

Phytochrome-induced responses may be further grouped into two types: 1. **rapid biochemical changes** and 2. **slow morphological changes**, including movement and growth. Rapid biochemical changes induced by phytochrome often affect a number of developmental responses taking place later through a series of signal

Table 13.3 Characteristics of phytochrome responses at different fluences

Very low fluence response (VLFR)	Low fluence response (LFR)	High irradiance response (HIR)
Fluence range		
0.0001–0.1 μmol m^{-2}	1–1000 μmol m^{-2}	>1000 μmol m^{-2}
R-FR reversibility		
Not FR reversible	FR reversible	Not FR reversible
Fluence-time reciprocity		
Dependent on product of fluence rate and time of irradiation	Dependent on product of fluence rate and time of irradiation	Requires long irradiation times, dependent on fluence rate (e.g., I1 > I2 > I3) but not on rate X time
Examples		
Red light stimulation of coleoptile and inhibition of mesocotyl growth in etiolated seedlings	Promotion of lettuce seed germination, regulation of leaf movements	Anthocyanin biosynthesis, ethylene production, plumular hook opening in lettuce

FR far red, *R* red

transduction events. The morphological responses due to photoactivation of phytochrome generally exhibit a **lag period**, the time between phytochrome activation and the biological response. The lag period may vary from a few minutes to several weeks, depending on the response. Noteworthy among the **rapid responses** are phytochrome-induced reversible movement of organelles and cell volume changes (swelling or shrinking). Even some growth responses are very fast. Thus, for example, inhibition of stem elongation in *Arabidopsis* and *Chenopodium album* occurs within minutes after red light exposure. In contrast, lag period of several weeks is required for phytochrome-mediated floral induction in *Arabidopsis* and some other species. A number of phytochrome responses also exhibit the phenomenon of **escape from photoreversibility**. In these events, red light-induced response is reversed by far-red light in a limited time after red light exposure, after which the response is said to have "escaped" from reversible control by far-red light. This is due to the multistep sequence of linked biochemical reactions leading to the response. Early stages in the sequence of biochemical reactions may be reversible by removing P_{fr}, and beyond some point, the reactions proceed irreversibly in the forward directions, leading to response. The escape period for different phytochrome-mediated responses ranges from less than a minute to few hours.

13.9.6 Phytochrome Action Involves Its Partitioning Between Cytosol and Nucleus

Seedlings raised in dark synthesize P_r (inactive) form of phytochrome. Under these conditions, phytochrome is exclusively cytosolic. Conversion of P_r to P_{fr} upon exposure to red light causes its movement from the cytosol to the nucleus. Since

phytochromes are too large to exhibit passive diffusion across nuclear pores, they are transported by active means across the nuclear membrane. The process differs between phy A and phy B. Nuclear import of P_{fr} A is extremely rapid and occurs within minutes of red light exposure. Since dark-grown seedlings contain high concentration of phy A, sufficient P_{fr} A gets transported into the nucleus. Red light exposure to phy B exposes the nuclear localization sequence associated with it during conformational changes from P_r B to P_{fr} B. This facilitates binding of nuclear import proteins leading to transport of phy B across the nuclear membrane. Compared to nuclear migration of phy A, transport of phy B by the above-stated process is slow and occurs in a few hours after red light exposure (Fig. 13.15).

Both phy A and phy B accumulate in the nucleus as a cluster of small particles, called "**speckles**." Together these clusters of speckles are also referred as "**nuclear bodies**" *(NBs).* The number and size of these speckles are correlated with light responsiveness. The nuclear bodies associated with P_{fr} B disappear upon exposure to far-red light, accompanying the conversion of P_{fr} to P_r. Accumulation of nuclear bodies also exhibits circadian pattern. Thus, NBs disappear at night and reappear shortly before dawn, indicating an integration between light and clock signaling. Nuclear P_r formed as a result of far-red light exposure is exported from the nucleus to the cytosol. Keeping in view the fact that the rate of phytochrome photoconversion (P_r-P_{fr}) is more rapid than the rate of phytochrome migration (cytosol to nucleus and reverse), both forms of phytochrome are present in both cytosol and nucleus in light-grown plants.

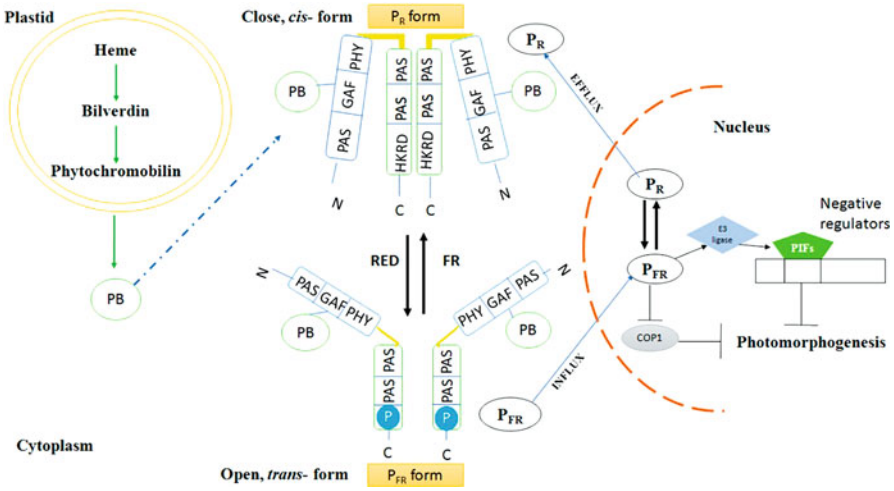


Fig. 13.15 Photoconversion of phytochrome and its intracellular migration between the cytoplasm and nucleus. *HRKD* histidine kinase-related domain, *PIF_3* phytochrome integrating factor 3, *PAS* Per-ARNT-Sim domain, *PB* phytochromobilin, *GAF* cGMP-specific phosphodiesterase, adenyl cyclase, and FhlA domain

13.9.7 Phytochrome Signaling Mechanisms

Light sensing by phytochrome chromophore modulates the interaction of phytochrome protein with other cellular components which ultimately leads to changes in growth, development, or position of the organ. Phytochrome action either involves **changes in ion fluxes** (leading to rapid turgor responses) or brings about alteration of **gene expression** (resulting in slower, long-term responses).

1. *Regulation of membrane potential and ion fluxes*: Rapid changes in membrane properties can be brought about by phytochrome on sensing light. Thus, membrane potential of roots and oat coleoptiles changes within few seconds of red light exposure. This leads to ion flux changes across the plasma membrane suggesting that some of the phytochrome-induced rapid responses are initiated at or near the plasma membrane.
2. *Regulation of gene expression*: Long-term alterations in metabolism brought about by changes in gene expression are responsible for **de-etiolation** of seedlings. As a result, etiolated seedlings with elongated stem, folded cotyledons, and lack of chlorophyll exhibit slowing down of stem growth, opening of cotyledons, and chlorophyll formation in light. The stimulation and repression of transcription can be very rapid, and lag time can be as short as 5 min. Nuclear import of phytochrome proteins (phy A and phy B) as a response to light quality represents a major control point in phytochrome signaling. A number of transcription factors are upregulated following a shift of seedlings from dark to light, and they subsequently activate the expression of several genes. Such genes which encode rapidly upregulating proteins are called **primary response genes**. Expression of primary response genes is dependent on signal transduction pathways and is independent of protein synthesis. The expression of later or **secondary response genes** requires new protein synthesis. Phytochrome signaling brings about changes in gene expression largely by affecting the stability of transcription factors. It promotes the degradation of transcription factors that act as negative regulators of light response, whereas it stabilizes the transcription factors which act as positive regulators.
3. *Phytochrome-interacting factors (PIFs)*: PIFs are proteins which primarily act as regulators of photomorphogenic responses. They are basic helix-loop-helix transcription factors and consist of 15 proteins in *Arabidopsis*. PIF_3 was the first PIF identified. PIFs specifically bind with P_{fr}. They regulate phytochrome-mediated seed germination, shade avoidance, and hypocotyl elongation. PIFs promote **skotomorphogenesis** (etiolated development in dark) by acting as transcriptional activators of dark-induced genes and also by repressing some light-induced genes. Upon light exposure, PIF_3 co-localizes with phytochrome in nuclear bodies and is subsequently degraded, resulting in the inhibition of etiolation and promotion of photomorphogenesis.

Degradation of PIF3 correlates with its phosphorylation by the phytochrome and is further dependent on its ubiquitination followed by targeting to 26S proteasome.

In contrast with promotion of ubiquitination of PIF3, phytochrome signaling inhibits ubiquitination of transcription factors HY5, LAF1, and HFR1, which promote expression of light-induced genes. These transcription factors are ubiquitinated by the E3 enzyme-COP1 in dark, leading to degradation by 26S proteasome. COP1-dependent ubiquitination also leads to phy A degradation in light. Inhibition of COP1 activity is caused by phytochrome binding to COP1, causing exclusion of COP1 from the nucleus. Consequently HY5, LAF1, and HFR accumulate and induce light-responsive genes. Lastly, phytochrome activity is also modulated by its autophosphorylation, more so in P_{fr} than in P_r form. Phosphorylation of P_{fr} affects its stability and affinity for interacting partners, such as PIF_3. Phosphorylated phytochrome can again be dephosphorylated by a phosphatase (type 5 protein phosphatase; PAPP5). In this way, the amplitude of phytochrome signal can be modulated through a balance of its phosphorylation and dephosphorylation status.

13.10 Blue Light-Mediated Responses and Photoreceptors

Blue light (320–500 nm) affects a wide variety of growth responses in higher plants, algae, ferns, fungi, and prokaryotes. The major responses involving a role of blue light include:

- Activation of gene expression (e.g., for chalcone synthase, chlorophyll biosynthesis, smaller subunit of Rubisco, chlorophyll binding protein, D-II subunit of PS-II reaction center)
- Membrane depolarization due to opening of anion channels
- Chloroplast movement
- Inhibition of hypocotyl elongation
- Stomatal opening
- Phototropism
- Phototaxis—movement of motile unicellular organisms toward or away from light

All blue light-mediated responses exhibit three unique features:

- A typical "three-finger" action spectrum with λ_{max} at around 420, 450, and 475 nm (Fig. 13.16)
- A significant lag period between the sensing of light signal and the response
- Persistence of the response after light has been switched off

Whereas photosynthesis response is fully activated immediately after sensing light and ceases as soon as light is switched off, blue light-mediated responses exhibit a lag period of variable duration after sensing light stimulus. The persistence of blue light response is explained on the basis of photochemical cycle in which a blue light receptor activated by blue light is slowly converted to its inactive form after

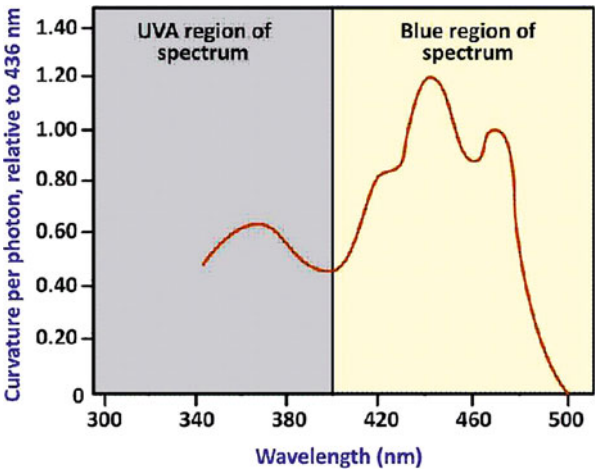


Fig. 13.16 Typical "three-finger" action spectrum of blue light-mediated responses in plants

switching off blue light. This reversal of blue light-mediated responses appears to involve four main processes: receptor dephosphorylation by protein phosphatases, breaking of covalent carbon-sulfur bond, dissociation of the receptor from its target molecules, and dark reversion of blue light-driven conformational changes in the receptor. Thus, the rate of response reversal depends on the time taken by the photoreceptor to convert back to its inactive form from the active state. Plants contain three types of UV-A and blue light-sensitive photoreceptors. These are cryptochromes (CRY 1 and 2), phototropins (PHOT 1 and 2), and zeitlupe (ZTL). All of them contain flavins as chromophores. Table 13.4 summarizes the various physiological responses mediated by these photoreceptors.

13.11 Cryptochromes

Cryptochromes are blue light-sensitive photoreceptors which are responsible for cotyledon expansion, suppression of hypocotyl elongation, membrane depolarization, anthocyanin production, and circadian clock function (Box 13.2). Cryptochromes are known to exist in cyanobacteria, ferns, and algae. Three types of cryptochromes have been reported so far from the analysis of *Arabidopsis* mutants. These are CRY1, CRY2, and CRY3. CRY1 was initially isolated by detecting *Arabidopsis* seedlings with abnormally long hypocotyls when grown in blue or UV-A (320–400 nm) light but exhibiting normal hypocotyl length in R or FR light. Analysis of this mutant showed that long hypocotyl phenotype of the said mutant (*hy4*) was specific to blue light inhibition of hypocotyl elongation. It is now established that cryptochromes are responsible for long-term blue light-induced inhibition of hypocotyl elongation, whereas phototropins trigger rapid inhibitory responses (Fig. 13.17).

Table 13.4 Blue light-mediated physiological responses and corresponding photoreceptors

	Cryptochromes		Phototropins		Zeitlupe
Responses	CRY1	CRY2	PHOT1	PHOT2	(ZLT/ADO)
Gross effects on gene expression	+	+			
Increased proton efflux	+		+	+	
Increase in intracellular [Ca^{2+}]	+		+	+	
Chloroplast movement, accumulation			+	+	
Stimulation of chlorophyll biosynthesis	+	+			
Inhibition of hypocotyl/ stem elongation	High fluence rates	Low fluence rates	Rapid response		+
Cotyledon/leaf expansion	+		+	+	
Phototropism			Low intensity	High intensity	
Circadian clock entrainment	+	+			
Flowering time	+	+			+

Box 13.2 Effects of Green Light on Plant Development

Cryptochromes and phytochromes are known to absorb green light (500–550 nm) to initiate photomorphogenic responses. It is now evident that green light exerts specific and frequently antagonistic functions in directing light responses in two ways:

1. Those that antagonize normal light-mediated responses
2. Those that function to forward normal developmental processes

With reference to the first category of responses, Fritz Went (1957) described that green light retards growth of tomato seedlings by opposing the effects of red and blue light. Later, Klein (1964) observed that green light inhibition of crown gall callus was correlated with fluence rate. Green light was also found to inhibit root gravitropism which could be reversed with an orange/red treatment. Green light induces phototropic curvature in *Arabidopsis* and lettuce seedlings with characteristics distinct from blue light-mediated phototropism. These observations suggest a separate green light-sensing pigment.

There is substantial evidence that green light also modulates stomatal aperture. A blue light pulse leads to an increase in stomatal aperture. If the blue pulse is immediately followed by a green light pulse or if blue and green

(continued)

Box 13.2 (continued)
pulses are delivered simultaneously, stomatal opening does not occur. The action spectrum for reversal of stomatal opening has a peak at 540 nm. The absence of response in **npq1** mutants (containing a lesion in zeaxanthin de-epoxidase activity and therefore no zeaxanthin production) suggests that blue and green light-induced changes in stomatal aperture may be regulated through trans-cis isomerization of bulk zeaxanthin in chloroplasts. Narrow-bandwidth green light also causes hypocotyls of dark-grown seedlings to be slightly longer than dark-grown seedlings, in a phytochrome-independent manner.

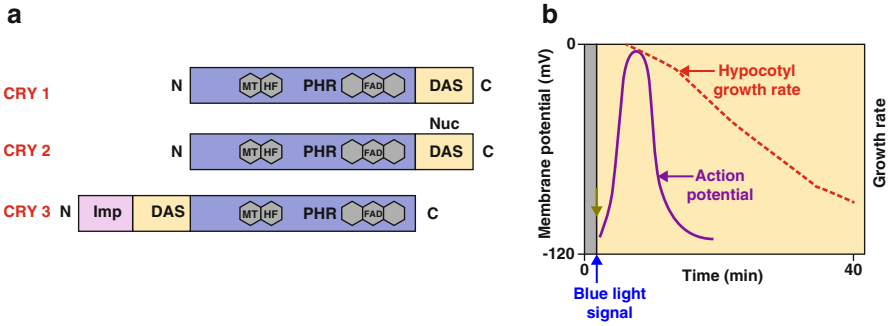


Fig. 13.17 (**a**) Domain structures of three cryptochromes (CRY1, CRY2, and CRY3). (**b**) Blue light-triggered generation of action potential followed by reduction in hypocotyl elongation rate

The term "cryptochrome" was initially coined for blue light photoreceptors as a reflection of the "cryptic" (mysterious) nature of these chromoproteins. Subsequently, CRY genes were found to encode 70–80 kDa proteins which form dimers in active state. CRY1 and CRY2 exhibit 58% homology in their amino acid sequence at the amino-terminal region but only 14% homology at the C-terminal region. The sequence significantly matches with microbial **photolyase**, which is a blue light-activated enzyme responsible for the repair of pyrimidine dimers in DNA damaged by exposure to UV radiation. CRY1 and 2 proteins, however, do not exhibit any photolyase activity. The function of CRY3 is not yet fully known, but it does not exhibit photolyase activity for single-stranded DNA lesions. Cryptochromes contain **two cofactor chromophores—flavin adenine nucleotide (FAD)** and **pterin (methenyltetrahydrofolate, MTHF)**. The cofactor-binding region of the protein is referred as PHR (photolyase-related) domain. This domain of the protein is structurally similar to photolyase, and, in addition to serving as the binding site for the chromophore cofactors, it also mediates dimerization of photoprotein (Fig. 13.18). All three cryptochrome (CRY1, 2, and 3) proteins contain a highly conserved motif called DAS (DQXVP-acidic-STAES). In CRY1 and

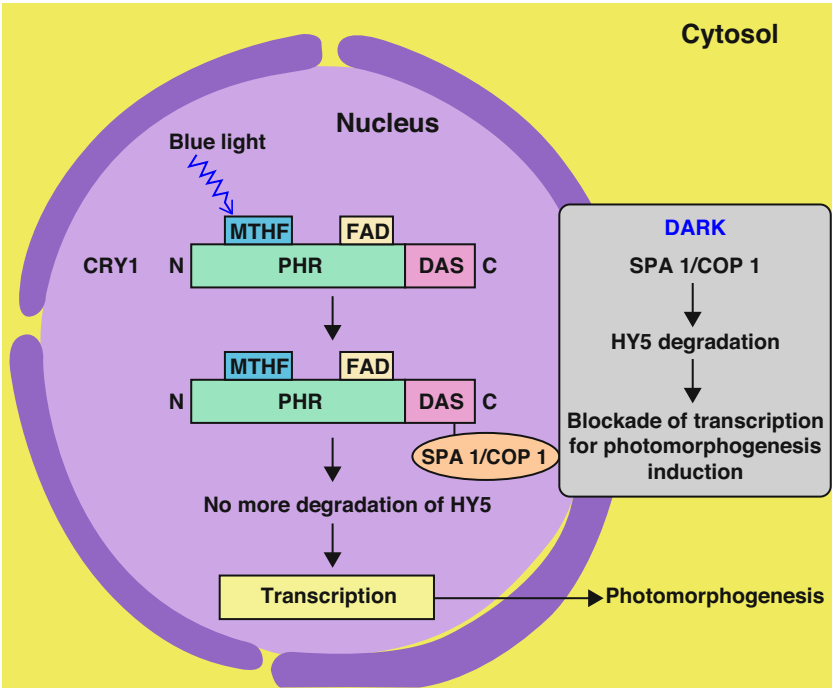


Fig. 13.18 Mechanism of CRY1 action in the nucleus. This blue light-mediated response of CRY1 plays a primary role in inhibition of hypocotyl elongation and anthocyanin production

CRY2, DAS is localized at the C-terminal. It also contains a nuclear localization signal (*Nuc*), whereas CRY3 contains an N-terminal extension required for the import of protein into chloroplasts and mitochondria (**Imp**). Another major difference between CRY1 and 2 is that CRY1 protein is much more stable in light than CRY2 which gets preferentially degraded under blue light. Overexpression of CRY1 protein in transgenic *Arabidopsis* or tobacco plants brings about stronger blue light-induced inhibition of hypocotyl elongation and anthocyanin production, whereas similar overexpression of CRY2 protein brings about only a small enhancement of the inhibition of hypocotyl elongation in wild plants. These overexpression studies indicate that CRY1 plays a primary role in the inhibition of hypocotyl elongation and anthocyanin production. Both CRY1 and CRY2 are present in the nucleus and cytoplasm, whereas CRY3 is localized in chloroplasts and mitochondria. Absorption of blue light by the chromophore FAD brings about conformational changes at the C-terminus, leading to its (cry protein) physical interactions with other signaling factors. Dimerization of photoprotein is essential for it to undertake subsequent signaling activities. It has been further observed that nuclear and cytoplasmic pools of CRY1 have distinct biological functions. One of the fastest blue light-mediated responses, i.e., changes in membrane depolarization, is regulated by nuclear (rather than cytoplasmic) localized CRY1. There is no evidence that

cryptochrome moves from the cytosol to the nucleus in response to light. COP1 is one of the signaling factors known to mediate blue light-induced morphogenic/biochemical changes through cryptochromes. COP1, along with another signaling factor, SPA, is known to degrade transcription factors such as HY5 in dark, which induces gene expression required for photomorphogenesis. Blue light triggers binding of C-terminus of CRY1 in the nucleus with SPA1 and COP1, thereby preventing the degradation of HY5, and other transcription factors, leading to promotion of blue light-mediated photomorphogenesis. Blue light-induced phosphorylation could also be contributing to maintain C-terminus of CRY1 in an active form (Fig. 13.18).

13.12 Phototropins: Molecular Nature and Associated Phototropic Bending Response

Isolation of several non-phototropic hypocotyl (*nph*) mutants of *Arabidopsis* leads to identification of the phototropin genes (*phot 1* and *phot 2*) with partially overlapping functions. In addition to their effect on phototropic bending response to blue light, phototropin protein receptors also modulate stomatal opening, ion transport, chloroplast movement, and cotyledon and hypocotyl growth (Table 13.5). The term "**phototropin**" should not be confused with "**phytotropin**" which refers to noncompetitive inhibitors of polar transport of auxins in plants. Some commonly used phytotropins are triiodobenzoic acid (TIBA), morphactin, and naphthylphthalamic acid (NPA). Unlike cryptochromes, phototropin receptors are associated with plasma membrane and function as light-activated serine-threonine kinases. The N-terminal of phototropin protein has the photosensory domain, and it is connected by a hinge with C-terminal serine-threonine kinase domain. The photosensory region has two similar motifs of 110 amino acids each. They are called LOV 1 and LOV 2 because of their sensitivity to light, oxygen, and voltage and their ability to bind the light-sensing cofactor-FMN (flavin mononucleotide). In dark, these two LOV domains are appressed and kinase at the C-terminal is inactive. Blue light absorption by the LOV domains leads to covalent binding of LOV domains with FMN via a conserved cysteine residue. LOV 2 interacts with a conserved helical region called Jα-helix located on the C-terminal side of the photosensory domain. The N-terminal photosensory region controls the activity of the C-terminal of the phototropin, which contains a serine-threonine kinase domain. In dark, the N-terminal region and the

Table 13.5 Unique and overlapping functions of PHOT 1 and PHOT 2

Functions	PHOT 1	PHOT 2
Phototropism	✓ (Low and high fluence)	✓ (High fluence)
Cotyledon growth	✓	
Hypocotyl growth	✓	
Chloroplast movement	✓	✓
Stomatal opening	✓	✓
Ion transport	✓	✓

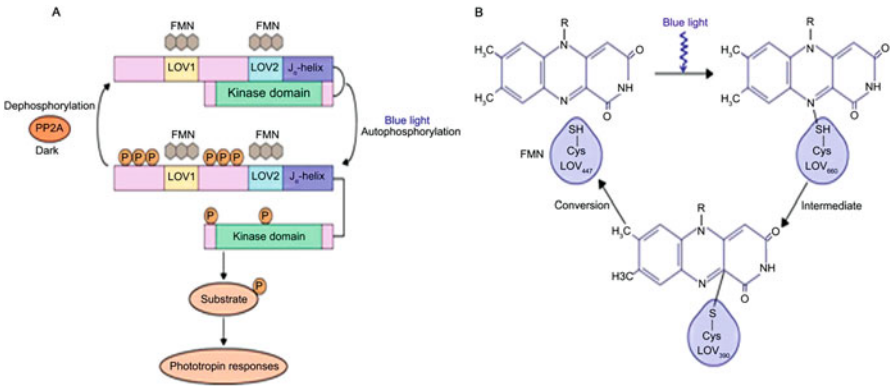


Fig. 13.19 Model for blue light-mediated autophosphorylation of phototropin. PHOT often leads to generation of auxin concentration gradient leading to bending response toward light

LOV domains "cage" the C-terminal and inhibit kinase activity. Blue light absorption by LOV domains leads to the uncaging of kinase domain and its activation by the unfolding of Jα-helix. The activated C-terminal kinase domain brings about autophosphorylation of the phototropin molecule on serine residues. Phototropin autophosphorylation seems to be critical for all phototropin-mediated morphogenic responses. A protein phosphatase action on the autophosphorylated phototropin results in its inactivation in dark. With reference to phototropic bending response of grass seedlings, it is now established that unilateral blue light induces a gradient of PHOT 1 autophosphorylation, which leads to generation of auxin concentration gradient (Fig. 13.19). Higher auxin concentration on the darker side of the shoot results in greater growth on that side causing its bending response toward light.

13.13 Phototropin-Modulated Chloroplast Movement

Light absorption and photodamage in green plants are effectively modulated by changes in the intracellular distribution of chloroplasts in leaves in response to changing light conditions. Chloroplasts gather near the upper and lower walls of palisade cells under weak illumination, thereby maximizing light absorption. On the other hand, strong illumination leads to chloroplast movement to the lateral walls so as to minimize light absorption and avoid photodamage. At night, chloroplasts move to the bottom of the cell. The physiological significance of such a migration is, however, not yet clear (Fig. 13.20). Analysis of chloroplast movement pattern in low and bright light in *phot 1*, *phot 2*, and *phot 1*/*phot 2* double mutants has demonstrated the specific and common roles of PHOT 1 and PHOT 2 in the avoidance and accumulation responses. Thus, *phot 1* mutants have a normal avoidance response and a poor accumulation response. *phot 2* mutants lack the avoidance response but retain normal accumulation response. Cells from the double mutant (*phot 1*/*phot 2*) lack both avoidance and accumulation responses. *phot 2* mutant plants are unable to survive under bright sunlight due to photooxidative damage. Based on the molecular

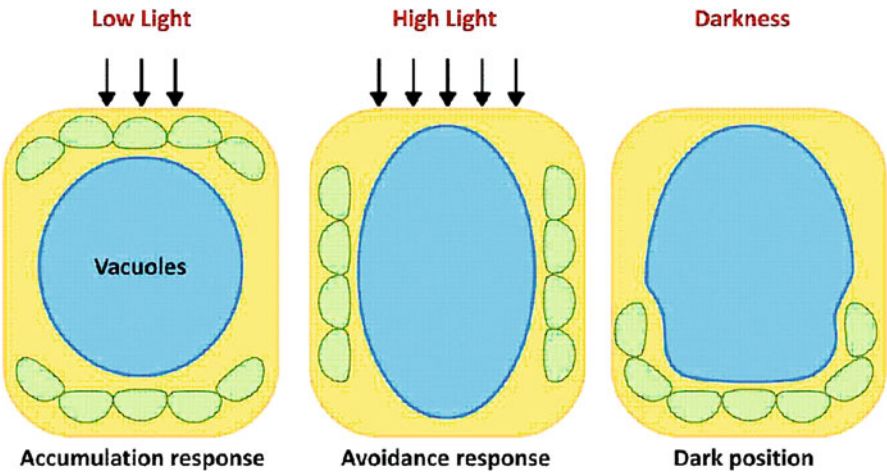


Fig. 13.20 Changes in chloroplast distribution pattern in leaf cells in response to varying light intensity and in dark

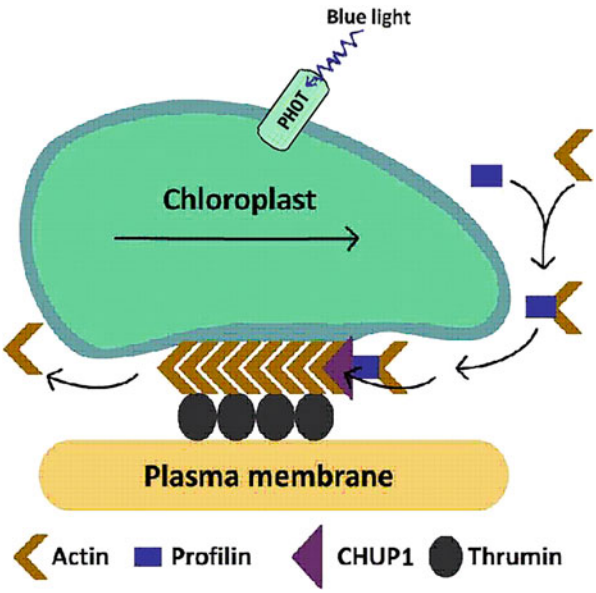


Fig. 13.21 Mechanism of phototropin-mediated chloroplast movement. Chloroplast movement in cytoplasm in response to light is aided by cytoskeletal elements majorly actin (G-actin and F-actin), profilin, and thrumin. F-actin binding protein CHUP1 (chloroplast unusual positioning 1) brings about change in movement

analysis of these mutants, it is now evident that both PHOT 1 and PHOT 2 are localized on the plasma membrane, whereas PHOT 2 is also localized on the chloroplast membrane. Chloroplast movement is brought about by changes in the cytoskeleton. These changes are brought about by a novel, F-actin binding protein called as **chloroplast unusual positioning 1 (CHUP 1)**. In bright light, CHUP 1 localizes itself on the plasma membrane via protein interactions and binds to the chloroplast envelop. CHUP 1 recruits G-actin and profilin (actin-polymerizing protein) to extend an existing F-actin filament which pushes the chloroplast from its earlier position (Fig. 13.21).

13.14 Phototropin Signaling-Dependent Light-Induced Stomatal Opening

Stomatal opening is governed by a combination of endogenous and environmental factors, such as light, CO_2, humidity, hormones, temperature, and plant's internal clock and water status. The cellulose microfibrils in guard cell wall cause an elongation and outward bending of guard cells due to an increase in turgor. This leads to opening of stomatal aperture. Conversely, a decrease in turgor shrinks the guard cells and closes stomata. In most plants, stomata open in response to blue light component of light. On absorbing blue light, the phototropins localized on the guard cell membrane get autophosphorylated and activate a membrane-associated kinase called **blue light signaling** *1* (BLUS 1) through its phosphorylation. Phosphorylated BLUS 1 regulates the activity of a protein phosphatase called PP1c. PP1c regulates the activity of an unknown protein kinase (PK) which promotes the binding of a 14-3-3 protein to the plasma membrane H^+-ATPase, leading to its activation. Proton-pumping activity of H^+-ATPase leads to membrane hyperpolarization. This causes K^+ uptake through K^+ channels, and the resulting decrease in water potential drives water uptake and stomatal opening (Fig. 13.22).

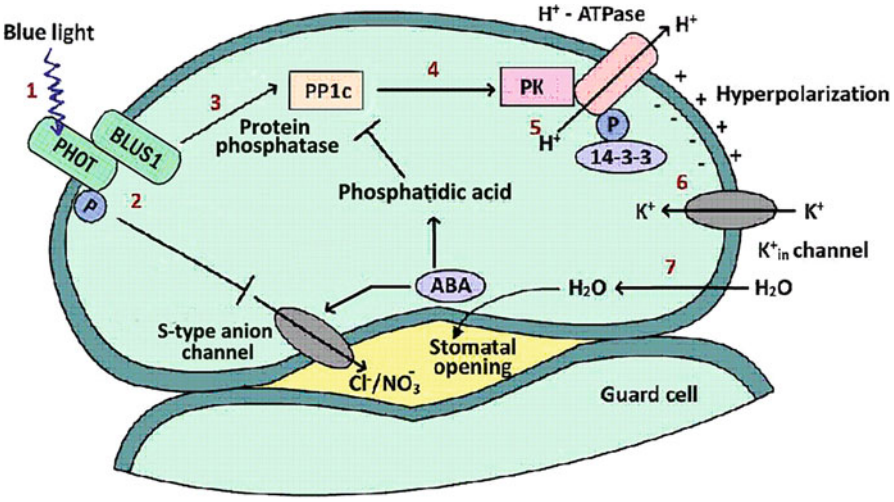


Fig. 13.22 Phototropin-mediated stomatal opening mechanism. The opening and closing are turgor mediated. On blue light absorption, phototropin on guard cell membrane undergoes autophosphorylation and activates BLUS. BLUS further regulates protein phosphatase PP1c, which, in turn, regulates protein kinase, leading to activation of H^+-ATPase. This causes hyperpolarization of the membrane. Subsequent K^+ uptake results in decreased water potential and eventually stomatal opening

13.15 UVR 8: A Photoreceptor for UV-B-Mediated Photomorphogenic Responses

UV-B (280–315 nm) radiation is known to affect gene regulation, flavonoid biosynthesis, hypocotyl growth suppression, epidermal cell expansion, stomatal density, entrainment of circadian clock, and increase in photosynthetic efficiency. The photoreceptor for UV-B-mediated photomorphogenic responses is a seven-bladed β-propeller protein called as **UVR 8**. UVR8 lacks a prosthetic group and exists as a homodimer in its inactive form. The two subunits are linked by a network of salt bridges formed between tryptophan residues, which serve as sensors of UV-B radiation. UV-B absorption by tryptophan residues leads to breaking of salt bridges leading to separation of active monomers of UVR8. An interaction of UVR8 monomers with COP1-SPA complex brings about gene expression. Contrary to the role of COP1-SPA as a negative regulator for transcription factors for degradation during phytochrome and cryptochrome responses, it acts as a positive regulator during UV-B signaling by interacting with UVR8 in the nucleus. UVR8-COP1-SPA complex activates the transcription of the transcription factor HY5, thereby regulating the expression of genes induced by UV-B (Fig. 13.23). RUP

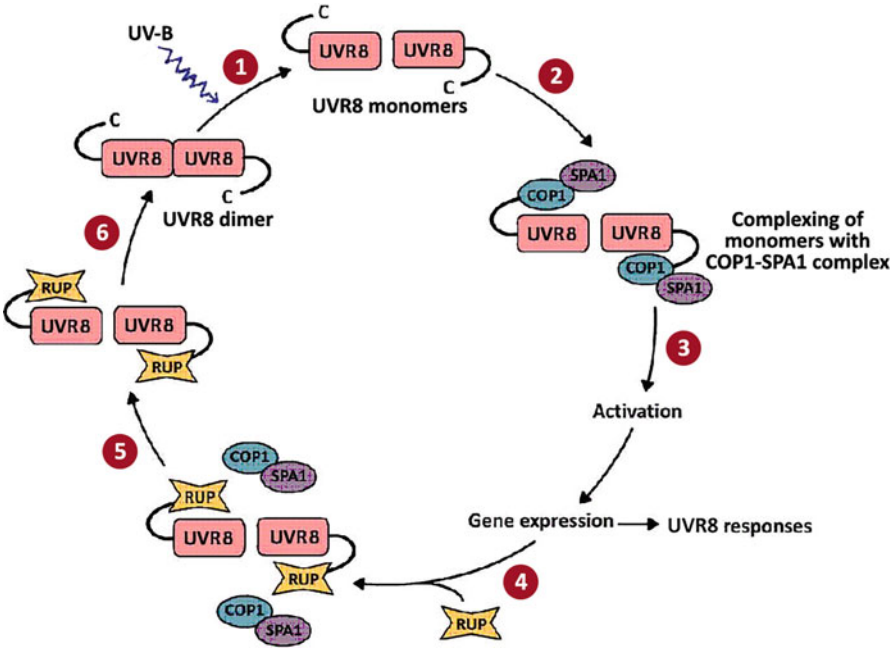


Fig. 13.23 UVR8-mediated signaling mechanism via action of RUP protein

(REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 and 2) is a negative regulator of UVR8 receptor. It facilitates the dimerization of the UVR8 receptor.

13.16 Other LOV Domain-Containing Photoreceptors in Plants

Zeitlupe (ZTL/ADO) [German word for "slow motion"] photoreceptors reported from *Arabidopsis*, rice, poplar, maize, and pine are involved in the targeted proteolysis of signaling components which control flowering time and circadian clock. Like phototropins, ZTL/ADO photoreceptors resemble PHOTs but have a photoactive FMN-binding LOV region along with a F-box motif of about 50 amino acids for protein-protein interaction. **Neochrome** is another plant photoreceptor with LOV domain, found in ferns and algae. Neochrome has a phototropin-like protein sequence fused with a phytochrome chromophore-binding domain. Therefore, it acts as dual red/blue light photoreceptor. *Mougeotia*, a filamentous green alga, has a large single, ribbon-like chloroplast in each cell. It displays a striking light-avoidance response through neochrome and phototropin-mediated movement of its chloroplast. In dim light, the flat side of the chloroplast faces light, and in bright light, the edges face the light (Fig. 13.24).

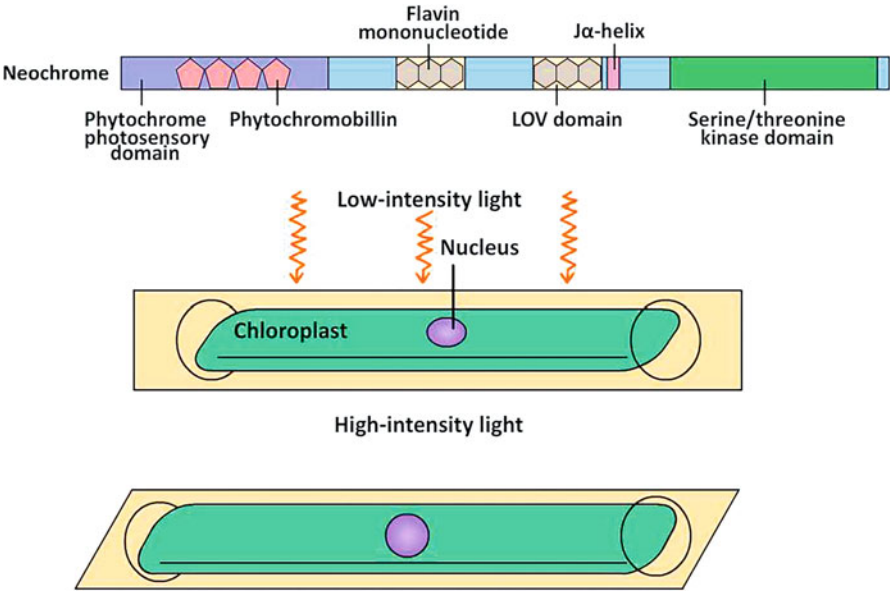


Fig. 13.24 Chloroplast movement in *Mougeotia* in response to light of low or high fluence, as influenced by neochrome

13.17 Rhodopsin-Like Photoreceptors

Movement of *Chlamydomonas reinhardtii*—a unicellular alga—toward or away from the directional light (**phototaxis**) is controlled by two rhodopsin-like molecules called as channel rhodopsins (ChR1 and ChR2) located in the eye spot. These photoreceptors are light-gated proton channels which regulate photoaxis response to blue-green light (λ_{max} 500 nm).

13.18 Summary

- Photomorphogenesis may be defined as the developmental response of an organism to the information in light, which may be its quantity, quality (i.e., the wavelength), and direction or relative length of day and night (photoperiod). A dark-grown seedling is said to be etiolated. In etiolation, embryonic stem (hypocotyl in dicots and epicotyl in monocots) of seedlings exhibits very rapid and extensive elongation of internodes. There is no cotyledon/leaf expansion, and seedlings appear pale as there is no chloroplast development. So, etiolated plants are pale yellow, and the hypocotyl remains "hooked" at the apex. Upon sensing light, a seedling emerging from the soil switches from skotomorphogenesis to photomorphogenesis. This process is known as **de-etiolation**, and it involves the coaction of red/far-red light-absorbing phytochrome and blue light-sensitive cryptochrome.
- Any photobiological process requires a light-absorbing molecule or pigment. Plants contain a variety of pigments. Absorption of light by a pigment (e.g., chlorophyll) plotted as a function of wavelength is known as absorption spectrum which depicts light. In modern laboratories, action spectra are also generated by using **spectrographs** fitted with monochromatic sources of light. Three parameters of light play significant role in modulating plant development. These are 1. light intensity, 2. light composition/(quality), and 3. light duration (photoperiod).
- Only a small percentage of photosynthetically active radiation (PAR, 400–700 nm) incident on a leaf is surface transmitted through the leaf, and the rest (15%) is reflected or transmitted from the leaf surface. Since chlorophylls absorb blue and red wavelengths of light most strongly, green wavelengths are maximally transmitted and/or reflected, giving green color to the vegetation. The palisade cells below the epidermis are shaped like pillars arranged in parallel columns which provide sieve effect and channeling of light. The **sieve effect** is caused by nonuniform distribution of chlorophyll molecules within the chloroplasts which results in shaded areas in chloroplasts which do not receive light. Desert plants exposed to excess light develop hairs, salt glands, and epicuticular wax to enhance light reflection from leaf surface, thereby reducing overheating due to absorption of excess amount of solar energy. **Sunflecks** (patches of sunlight passing through small gaps in leaf canopy in dense forests) play important role in rapid and short-term light capture for plants growing on

forest bed and in densely planted crops where lower leaves are shaded by upper leaves. Some leaves maximize leaf absorption by **solar tracking (heliotropism)** by continuously adjusting the orientation of leaf lamina such that they are perpendicular to the leaf surface.

- *Photoreceptors*, unlike plant pigments (such as chlorophyll), are chromophore-containing biomolecules which absorb photons of a given wavelength and use the energy derived from it as a signal to initiate a photo response. All photoreceptors contain a protein (**chromoprotein**) attached to a light-absorbing, non-protein, prosthetic group called as **chromophore**.
- Etioplasts contain a prominent structure called **prolamellar body** which contains membrane lipids, protochlorophyllide and the light-requiring enzyme—**protochlorophyllide oxidoreductase (POR)**. Prolamellar bodies are formed when membrane lipid synthesis continues in the absence of corresponding amounts of thylakoid protein synthesis, which requires light. Light triggers chlorophyll biosynthesis from protochlorophyllide. Protochlorophyllide is not green and cannot absorb light for photosynthesis. It is a photoreceptor with an absorption maximum at 650 nm. Light absorption by protochlorophyllide takes place in the presence of NADPH-protochlorophyllide oxidoreductase (POR). Upon illumination, POR rapidly converts protochlorophyllide into chlorophyllide, and the internal structure of etioplasts is reorganized into a typical chloroplast. Subsequently, POR is released and degraded by proteolysis.
- Photoreversibility of phytochrome-modulated responses and its significance: Photoreversibility (photoconversion; also referred as photochromism) is a defining feature of phytochromes. Phytochromes are synthesized as an inactive red light-absorbing isomer, called P_r. Red light absorption by P_r form converts it into an active, far-red-absorbing isomer called P_{fr}. Absorption of far-red light by P_{fr} form converts it back to P_r form. Phytochromes were discovered through experiments on red/far-red reversibility of seed germination in lettuce. Phytochromes are soluble proteins and exist as dimers. The phytochrome protein consists of an N-terminal photosensory region and a C-terminal regulatory domain. The two are attached with a hinge between them. Red light exposure opens up the structure in the far-red-absorbing form of phytochrome (Pfr), making its surface accessible to phytochrome-interacting factors (PIFs) in the signal transduction pathway. The opened-up structure of phytochrome protein allows light-dependent phosphorylation of exposed serine and threonine residues, possibly triggered by autophosphorylation by R3 kinase at the C-terminal. The light-labile form of phytochrome (phy A) is predominant in etiolated seedlings which strongly express PHY A gene, resulting in high accumulation of P_rA. Prolonged light exposure causes phy A concentration to drop by almost 100-fold. Light also inhibits PHY A transcription. Though less abundant, phy A still functions in light-grown plants. Phytochrome-mediated responses can be grouped into high irradiance responses (HIRs) or low fluence response (LFRs). Phytochrome-induced responses may be further grouped into two types: 1. rapid

biochemical changes and 2. slow morphological changes, including movement and growth. Conversion of P_r to P_{fr} upon exposure to red light causes its movement from the cytosol to the nucleus. Compared to nuclear migration of phy A, transport of phy B by the above-stated process is slow and occurs in a few hours after red light exposure. They are transported by active means across the nuclear membrane. Phytochrome action either involves changes in ion fluxes (leading to rapid turgor responses) or brings about alteration of gene expression (resulting in slower, long-term responses).

- Both CRY1 and CRY2 are present in the nucleus and cytoplasm, whereas CRY3 is localized in chloroplasts and mitochondria. Absorption of blue light by the chromophore FAD brings about conformational changes at the C-terminus, leading to its (cry protein) physical interactions with other signaling factors. One of the fastest blue light-mediated responses, i.e., changes in membrane depolarization, is regulated by nuclear (rather than cytoplasmic) localized CRY1. Unlike cryptochromes, phototropin receptors are associated with plasma membrane and function as light-activated serine-threonine kinases. The N-terminal of phototropin protein has the photosensory domain, and it is connected by a hinge with C-terminal serine-threonine kinase domain. The photosensory region has two similar motifs of 110 amino acids each. They are called LOV 1 and LOV 2 because of their sensitivity to light, oxygen, and voltage and their ability to bind the light-sensing cofactor-FMN (flavin mononucleotide).
- On absorbing blue light, the phototropins localized on the guard cell membrane get autophosphorylated and activate a membrane-associated kinase called **blue light signaling 1** (BLUS 1) through its phosphorylation. Phosphorylated BLUS 1 regulates the activity of a protein phosphatase called PP1c. PP1c regulates the activity of an unknown protein kinase (PK) which promotes the binding of a 14-3-3 protein to the plasma membrane H^+-ATPase, leading to its activation. Proton-pumping activity of H^+-ATPase leads to membrane hyperpolarization. This causes K^+ uptake through K^+ channels and the resulting decrease in water potential drives water uptake and stomatal opening.
- The photoreceptor for UV-B-mediated photomorphogenic responses is a seven-bladed β-propeller protein called as **UVR 8**. UVR8 lacks a prosthetic group and exists as a homodimer in its inactive form. The two subunits are linked by a network of salt bridges formed between tryptophan residues, which serve as sensors of UV-B radiation. UV-B absorption by tryptophan residues leads to breaking of salt bridges leading to separation of active monomers of UVR8. UVR8-COP1-SPA complex activates the transcription of the transcription factor HY5, thereby regulating the expression of genes induced by UV-B.
- Neochrome has a phototropin-like protein sequence fused with a phytochrome chromophore-binding domain. Therefore, it acts as dual red/blue light photoreceptor. *Mougeotia*, a filamentous green alga, has a large single, ribbon-like chloroplast in each cell. It displays a striking light-avoidance response through neochrome and phototropin-mediated movement of its chloroplast.

Multiple-Choice Questions

1. Match the correct option

1. Requirement of light for germination	(i). Nyctinasty
2. Folding of leaves at night	(ii). Photonasty
3. Opening of leaves at dawn	(iii). Phototropism
4. Directional bending of shoots to light	(iv). Photoblasty

Chose the correct option

(a) 1, (iv); 2, (iii); 3, (ii); 4, (i)
(b) 1, (i); 2, (iv); 3, (iii); 4, (ii)
(c) 1, (iv); 2, (i); 3, (ii); 4, (iii)
(d) 1, (ii); 2, (iii); 3, (ii); 4, (iv)

2. The process of transfer of energy from one molecule to another molecule is known as:
   (a) Fluorescence
   (b) Inductive resonance
   (c) Vibrational energy
   (d) Quantum yield
3. "Spectrographs" fitted with monochromatic source of light are used for the generation of:
   (a) Action spectra
   (b) Absorption spectra
   (c) Both (a) and (b)
   (d) None of these
4. "Epidermal focusing" for enhanced capture of light by chloroplasts is seen in:
   (a) Grasses
   (b) Deciduous plants
   (c) Conifers
   (d) Tropical plants
5. Patches of sunlight passing through small gaps in leaf canopy is called:
   (a) Para-heliotropism
   (b) Sieve effect
   (c) Sunflecks
   (d) Light channeling
6. Protochlorophyllide and prolamellar bodies are found in:
   (a) Chloroplast
   (b) Amyloplast
   (c) Etioplast
   (d) Leucoplast

7. Protochlorophyllide:
   (i) Is synthesized using chlorophyll b
   (ii) Has an unsaturated double bond between carbon C17 and C18
   (iii) Has a saturated bond between carbon C17 and C18
   (iv) Is synthesized using chlorophyll a
   (v) Absorbs light in the presence of NADH-dependent POR
   (vi) Absorbs light in the presence of NADPH-dependent POR
   Choose the correct combination:
   (a) i, iii, vi
   (b) ii, iv, vi
   (c) i, ii, v
   (d) iii, iv, vi
8. Out of the four phycobilins listed below, which one is known to regulate various aspects of growth and development?
   (a) Phycoerythrin
   (b) Phycocyanin
   (c) Phytochromobilin
   (d) Allophycocyanin
9. Presence of which pigment(s) is responsible for absorption of all wavelengths along the visible spectrum in red algae, giving it almost black appearance:
   (a) Chlorophyll a and d
   (b) Phycocyanin, allophycocyanin
   (c) Phycocyanin, phycoerythrin
   (d) Phycoerythrin, chlorophyll
10. Phytochromes are synthesized as _____, _____ light-absorbing isomer called Pr. Light absorption converts it into an _____ isomer, Pfr. Absorption of _____ light of wavelength _____nm converts Pr back to Pfr.
    (a) Inactive, far-red, active, red, 650 nm
    (b) Active, red, inactive, far-red, 700 nm
    (c) Inactive, red, active, far-red, 700 nm
    (d) Inactive, red, active, far-red, 650 nm
11. Which of the phytochrome-mediated responses follow the "principle of reciprocity"?
    (a) High irradiance responses (HIRs)
    (b) Low fluence responses (LFRs)
    (c) Very low fluence responses (VLFRs)
    (d) Both (a) and (b)
12. "Speckles" or "nuclear bodies" are clusters or aggregates of small particles which accumulate:
    (a) Phycocyanin and allophycocyanin
    (b) Sodium and potassium ions
    (c) phy A and phy B
    (d) Cryptochromes

13. Phytochrome-interacting factors regulate:
    (a) Photomorphogenesis, positively
    (b) Skotomorphogenesis, positively
    (c) Photomorphogenesis, negatively
    (d) Both (b) and (c)
14. Which of these is NOT a unique feature of blue light-mediated response?
    (a) Three-finger action spectra
    (b) Significant lag between sensing the light signal and the response
    (c) Persistence of response after light has been switched OFF
    (d) Appearance of nuclear bodies or speckles
15. Phytotropin is involved in:
    (a) Modulation of stomatal opening
    (b) Phototropic bending in response to blue light
    (c) Noncompetitive inhibition of polar transport of auxin
    (d) Chloroplast movement and ion transport
16. Determine the correct order of phototropin-dependent light-induced stomatal signaling.
    (i) K+ uptake and decrease in water potential
    (ii) Autophosphorylation of phototropin localized on the guard cell membrane and activation of BLUS through phosphorylation
    (iii) Membrane hyperpolarization
    (iv) Binding of 14-3-3 protein to plasma membrane H+ ATPase
    (v) Regulation of protein phosphatase PP1c
        (a) ii, v, iv, iii, i
        (b) i, iii, iv, iii, v
        (c) v, iv, ii, iii, i
        (d) iv, i, iii, ii, i
17. Neochrome, a dual red/blue light photoreceptor, is found in:
    (a) Filamentous green algae, *Mougeotia*
    (b) *Arabidopsis* rhizogenes
    (c) *Arabidopsis thaliana*
    (d) Unicellular algae, *Chlamydomonas reinhardtii*

Answers

1. c	2. b	3. a	4. d	5. c	6. a	7. b
8. c	9. d	10. c	11. b	12. c	13. d	
14. d	15. c	16. a	17. a			

Suggested Further Reading

Ulm R, Jenkins GI (2016) Q&A: how do plants sense and respond to UV-B radiation? BMC Biol 13:45