PHOTOMORPHOGENESIS

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What is photomorphogenesis……

- Photomorphogenesis is referring to the response of plant to light, which is the central theme in plant development
- Plants can sense light gradients and detect subtle differences in spectral composition
- For this plants have sophisticated photosensory system
    - Light sensitive photoreceptors
    - Signal transduction pathway
- photo receptor reads the information contained in the light by selectively absorbing different wave length of light.
- Absorbtion of light causes conformational change in the pigment or associated protein – causes photochemical oxidation reduction

Photoreceptors

- Most photomorphogenic responses in higher plants appear to be under control of one (or more) of four classes of photoreceptors:

  1. Phytochromes (red and far-red)

  2. Cryptochrome (blue and UV-A): seedling development and flowering

  3. Phototropin (blue and UV-A): differential growth in a light gradient

  4. UV-B receptors: unknown

- The phytochromes absorb red (660 nm) and far red light (735 nm)
- Have a major role in every stage of development from seed germination to flowering.
- Cryptochromes and phototropin detects both blue (400-450nm) and UV –A light (320-400nm).
- Cryptochrome have a major role during seedling development, flowering and resetting the biological clock
- Phototropin mediates phototropic responses

- Phytochrome, cryptochrome and phototropin are all chromoproteins
- chromoproteins containing a light absorbing group or chromophore attached to a protein with catalytic properties – Apoprotien
- Cromophore+Apoprotien = Holoprotien
- Phytochrome is a blue light protein pigment – 125kDa MM
- It can exist in 2 state

  -absorption maximum in the red (R, 665nm)

  -absorption maximum in the far red (Fr, 730nm)

Chemical nature of phytochrome

- It is a dimer composed of 2 polypeptides
- Each of its subunit consist of 2 components

    Light absorbing pigment molecule – chromophore

    A poly peptide chain - apoprotien

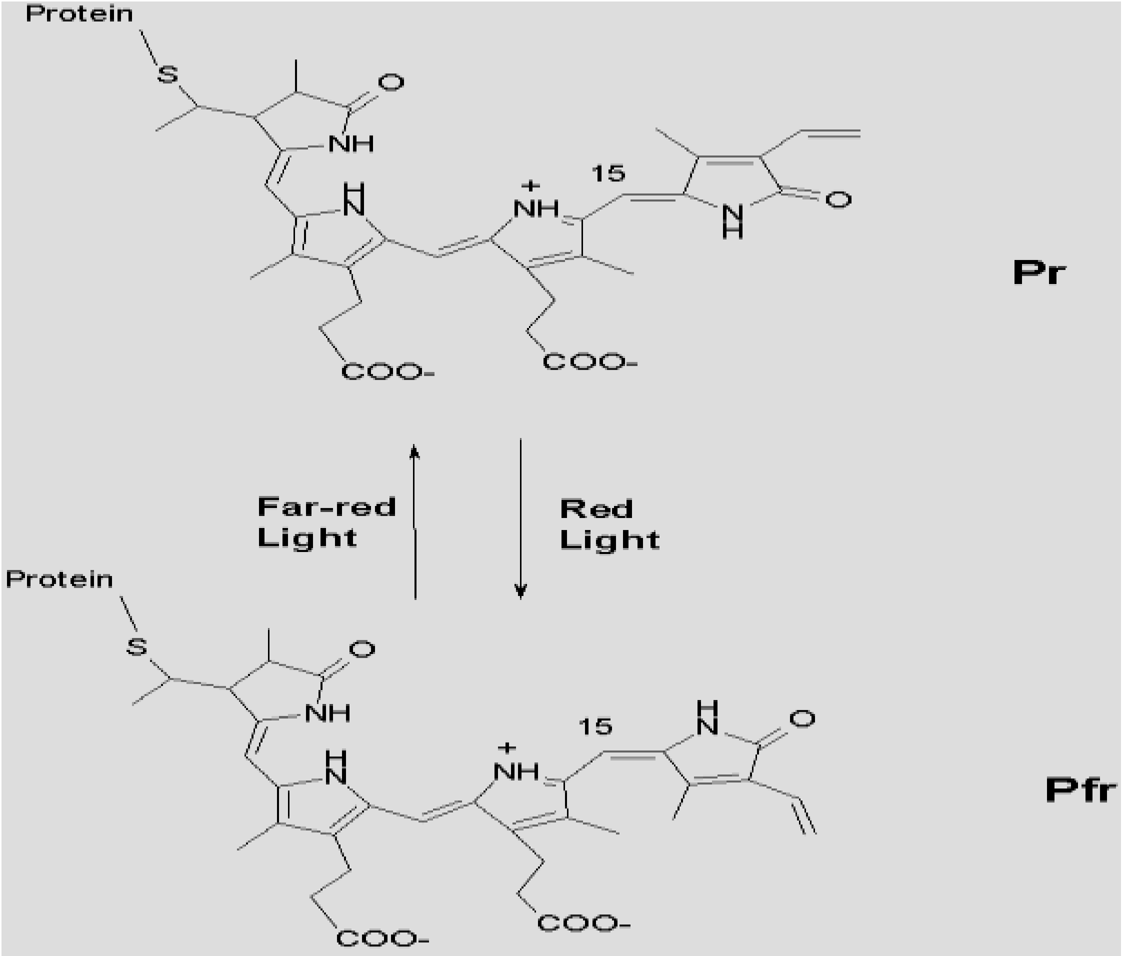
Cromophore+Apoprotien = Holoprotien

- In higher plants chromophore of phytochrome is a linear tetrapyrrole – Phytochromobilin
- Phytochrome apoprotien alone cannot absorb light (R or Fr)
- It can absorb only when the polypeptide is covalently linked with phytochromobilin - holoprotien

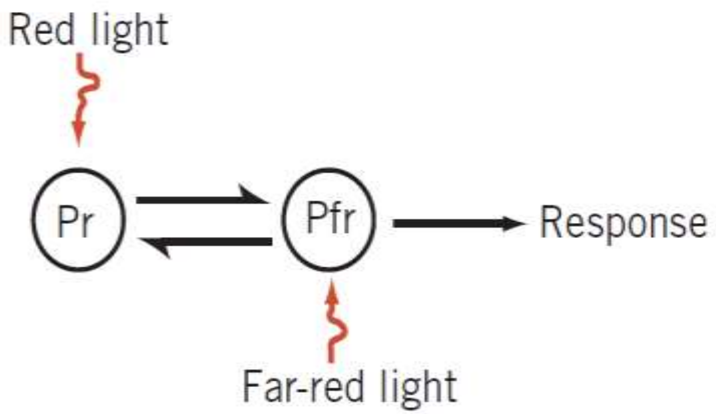
- Phytochromobilin is synthesized in plastids (from 5-amino levulinic acid)
- Plastids $\longrightarrow$ cytosol (passive process)
- Assembly of phytochrome apoprotien and chromophore is autocatalytic

Both chromophore and apoprotien undergoes conformational change.....

- Conformational changes in the protein are initiated in chromophore because it absorb light
- By the absorption of light Pr chromophore undergoes *a cis-trans* isomerization of the double bond b/w C 15-16 and rotation of the C 14-15 single bond



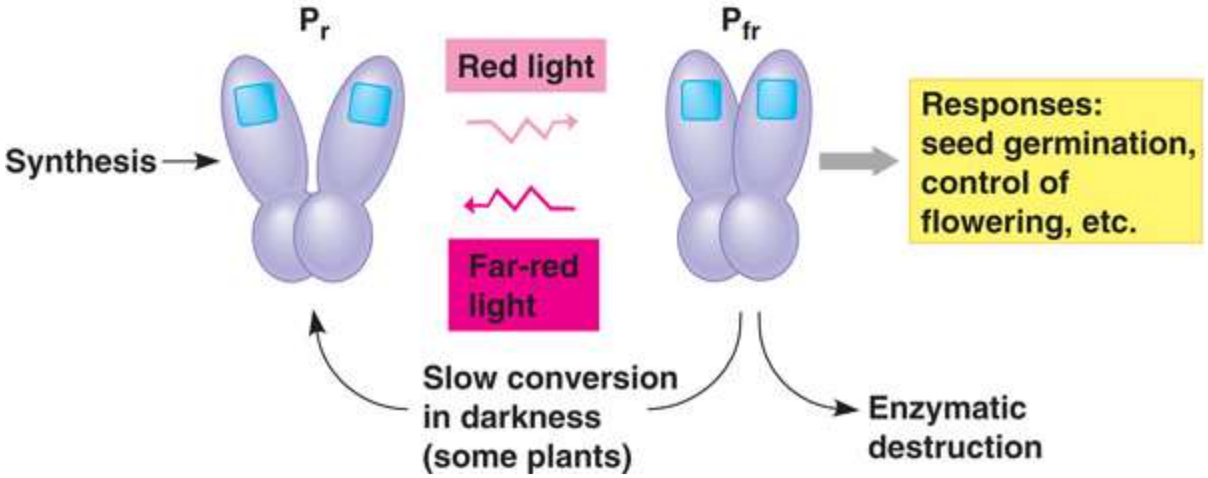
- During Pr toPfr, protein component of holoprotien also undergoes conformational changes
- Due to these conformation change phytochrome can interconvert between Pr and Pfr forms
- In dark grown or etiolated plants phtochrome is present in a red light absorbing form – Pr
- These Pr is then converted in to far red light absorbing form Pfr
- This process is known as Photoreversibility



- In addition to absorbing red light both Pr and Pfr absorb light in the blue light region
- So phytochrome effect can be elicited bt also by blue light
- Blue light response results from one or more blue light receptors

Intermediates in photoconversion

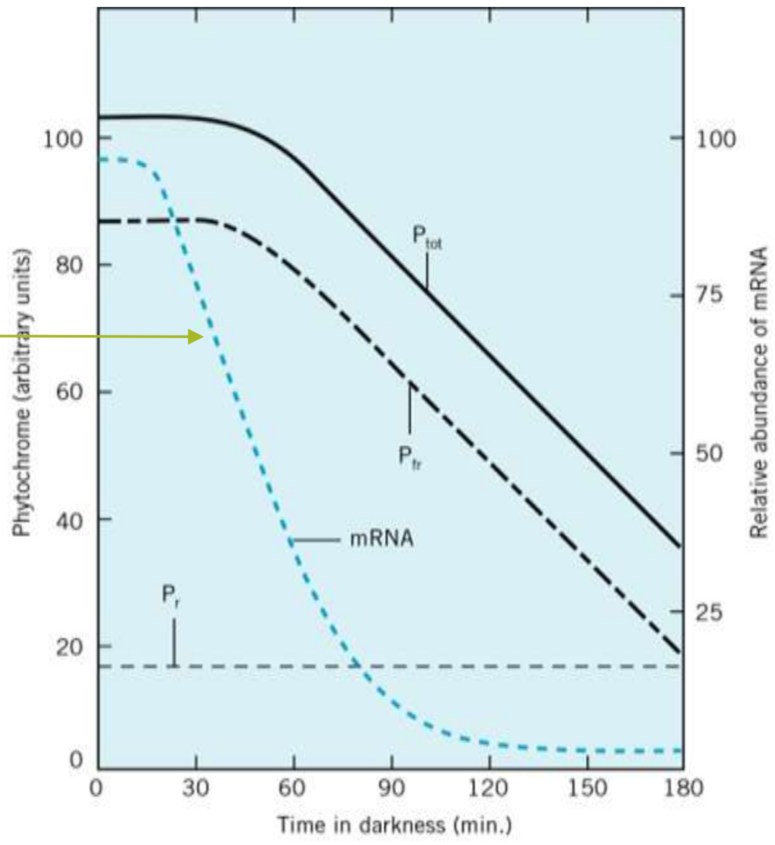
- Conversion of Pr $\rightleftarrows$ Pfr is not a single step process
- During phytochrome cycle intermediate form accumulate and make up significant fraction of total phytochrome
- These intermediate have role in initiating or amplifying phytochrome response under natural sunlight



PHYSIOLOGICALLY ACTIVE FORM OF PHYTOCHROME - Pfr

- **Phytochrome is synthesized as the Pr form, which** accumulates in dark-grown tissue and is generally considered to be physiologically inactive.
- When Pr absorbs red light, it is converted to the Pfr form, which is the physiologically active form of the pigment for most known responses
- Exposure of Pfr to far-red light returns the pigment to the Pr form.
- Both physiological and spectrophotometric experiments have also indicated that some Pfr may revert to Pr by a temperature-dependent process called dark reversion.

- In the presence of this physiologically active form, the transcription rate of phytochrome genes decreases
  - Enough protein is present to do its job, no more is required
  - Pfr does this directly!
- Existing P_{fr} protein is gradually broken down (**proteolyzed**)
- This feedback loop maintains a relatively constant amount of phytochrome in autotrophic cells



A.

B.

Types of phytochromes

- Phytochrome is most abundant in seedlings than green tissue
- 2 different classes of phytochromes , with distinct properties
    1. Type – 1
    2. Type – 2
- Type 1 is 9 times more abundant than Type 2 in dark grown pea seadling but equal in light grown seedling
- These two phytochromes have been shown to be distinct protiens
- Research has shown that there are two different classesof phytochrome with distinct properties

CHARACTERISTICS OF PHYTOCHROMEINDUCED WHOLE-PLANT RESPONSES

- phytochrome-induced responses maybe logically grouped into two types:
- 1. Rapid biochemical events
- 2. Slower morphological changes, including movements
- and growth
- Some of the early biochemical reactions affect laterdevelopmental responses

Phytochrome Responses Vary in Lag Time and Escape Time

- Morphological responses to the photo activation of phytochrome may be observed visually after a *lag time*—the time between a stimulation and an observed response.
- lag time may be as brief as a few minutes or as long as several weeks
- Eg - Red-light inhibition of the stem elongation rate of light grown pigweed (*Chenopodium album*) is observed within 8minutes after its relative level of Pfr is increased.
- studies using *Arabidopsis* have confirmed this observation and further shown that phyA acts within minutes after exposure to red light
- Information about lag time help researchers to find out type of biochemical reaction during the response

- shorter the lag time, the more limited therange of biochemical events that could have been involved
- **photoreversibility** Variety in phytochrome responses can also be seen in the phenomenon
- Red light–induced events are reversible by far-red light for only a limited period of time, after which the response is said to have "escaped" from reversal control by light
- phytochrome-controlled morphological responses are the result of a step-by-step sequence of linked biochemical reactionsin the responding cells
- escape time for different responses ranges from less than a minute to, remarkably, hours.

Phytochrome Responses Can Be Distinguished by the Amount of Light Required

- phytochrome responses can be distinguished by the amount of light required to induce them
- The amount of light is referred to as the **fluence** - number of photons impinging on a unit surface area (quanta per square meter)
- In addition to the fluence, some phytochrome responses are sensitive to the **irradiance** or *fluence rate* of light (quanta per square meter per second)
- Each phytochrome response has a characteristic range of light fluences over which the magnitude of the response is proportional to the fluence

There are 3 categories of plant responses to phytochrome

The categories are light-level dependent

1. **VLFRs** (very low fluence responses)
   - < 10^{-3} μmol photons/m^2 (converts 0.01% of phytochrome)
2. **HIR** (high irradiance responses)
   - >1000 μmol photons/m^2, continuous irradiation, dependent on actual fluence
3. **LFRs** (low fluence responses)
   - 1-1000 μmol photons/m^2, FR light reversible

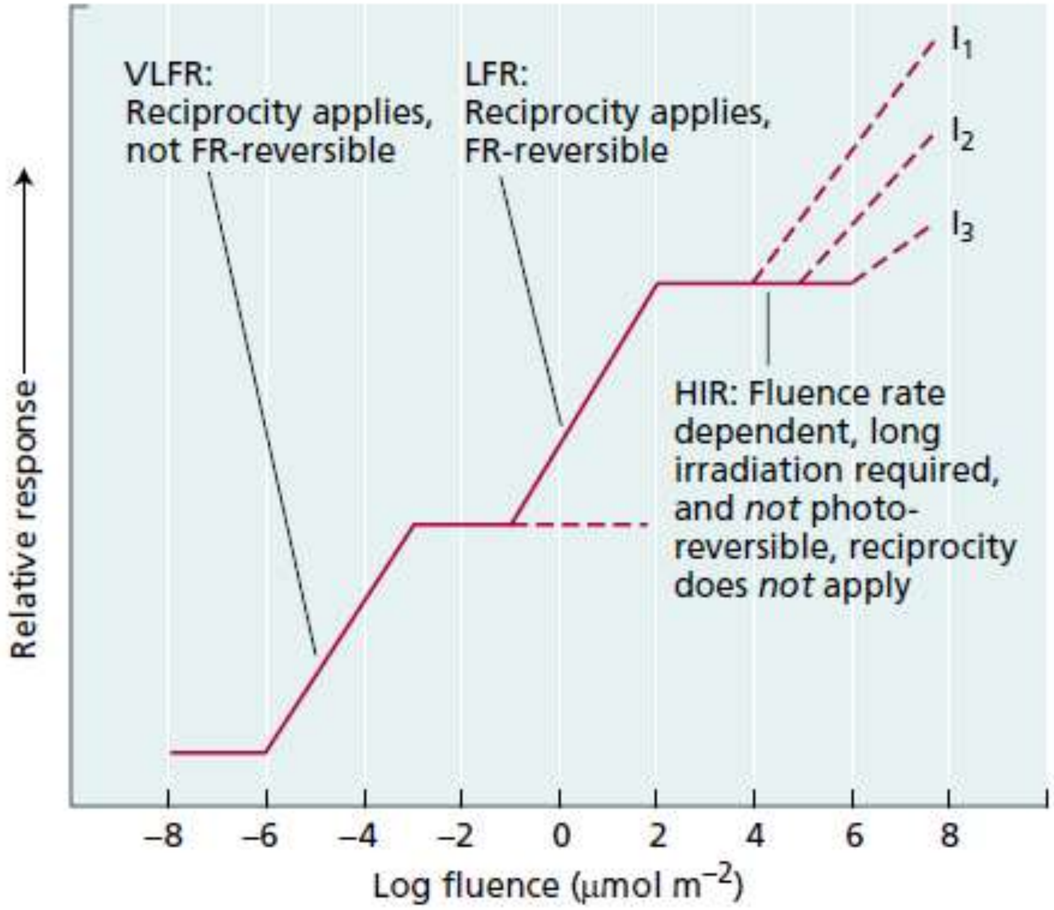


FIGURE 17.7 Three types of phytochrome responses, based on their sensitivities to fluence. The relative magnitudes of representative responses are plotted against increasing fluences of red light. Short light pulses activate VLFRs and LFRs. Because HIRs are also proportional to the irradiance, the effects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

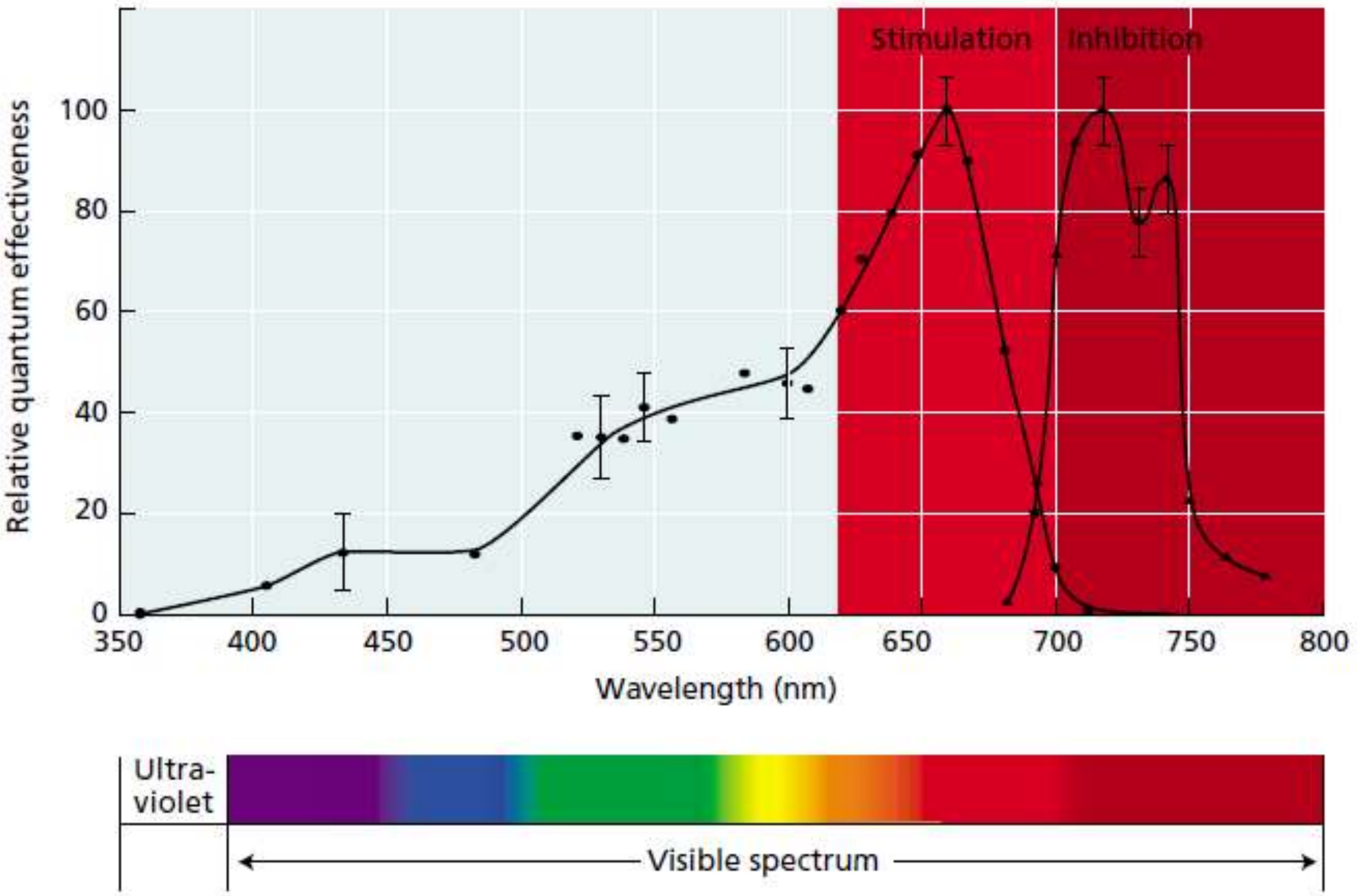
Very-Low-Fluence Responses Are Nonphotoreversible

- Some phytochrome responses can be initiated by fluences as low as 0.0001 µmol m–2 (one-tenth of the amount of light emitted from a firefly in a single flash)
- Saturate (i.e., reach a maximum) at about 0.05 µmol m-2.
- *Arabidopsis* seeds can be induced to germinate with red light in the range of 0.001 to 0.1 µmol m–2
- These remarkable effects of vanishingly low levels of illumination are called **very-low-fluence responses (VLFRs)**

- The minute amount of light needed to induce VLFRs converts less than 0.02% of the total phytochrome to Pfr
- far-red light cannot reverse VLFRs
- The VLFR action spectrum matches the absorption spectrum of Pr, supporting the view that Pfr is the active form for these responses

Low-Fluence Responses Are Photoreversible

- phytochrome responses cannot be initiated until the fluence reaches 1.0 μmol m^{-2}
- Saturated at 1000 μmol m^{-2} - **low-fluence responses (LFRs)**
- include most of the red/far-red photoreversible responses, such as the promotion of lettuce seed germination and the regulation of leaf movements
- LFR spectra include a main peak for stimulation in the red region (660 nm), and a major peak for inhibition in the far-red region (720 nm).


Stimulation
Inhibition
Relative quantum effectiveness
100
80
60
40
20
0
350
400
450
500
550
600
650
700
750
800
Wavelength (nm)
Ultra-
violet
Visible spectrum

- Both VLFRs and LFRs can be induced by brief pulses of light
- The total fluence is a function of two factors

  1. fluence rate
  2. irradiation time

- brief pulse of red light will induce a response, provided that the light is sufficiently bright, and conversely, very dim light will work if the irradiation time is long enough.
- This reciprocal relationship between fluence rate and time is known as the **law of reciprocity** (R. W. Bunsen and H. E. Roscoe in 1850)
- VLFRs and LFRs both obey the law of reciprocity.

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

- 3rd type of Phytochrome responses – highirradiance responses (HIRs)
- HIRs require prolonged or continuous exposure to light of relatively high irradiance
- HIR is proportional to irradiance (loosely speaking, the brightness of the light) rather than to fluence - reason that these responses are called high-irradiance responses rather than high-fluence responses
- HIRs saturate at much higher fluences than LFRs—at least 100 times higher—and are not photoreversible
- Because neither continuous exposure to dim light nor transient exposure to bright light can induce HIRs
- HIRs do not obey the law of reciprocity.

Genetics of phytochromes

- Phytochrome Is Encoded by a Multigene Family
- cloning of phytochrome genes – helps in the detailed comparison of the amino acid sequences of the related proteins
- The first phytochrome sequences cloned were from monocots
- phytochromes are soluble proteins
- phytochrome gene family is named *PHY*
- its five individual members are *PHYA*, *PHYB*, *PHYC*, *PHYD*, and *PHYE*

- The apoprotein by itself (without the chromophore) is designated PHY
- holoprotein (with the chromophore) is designated phy
- Different phytochrpmes are expressed in different tissues at different times in development and mediate different light responses
- PHY A and PHY B mediates red and far red responses
- Phytochromes also interact with each other and also with other receptors and devolopmentala stimuli

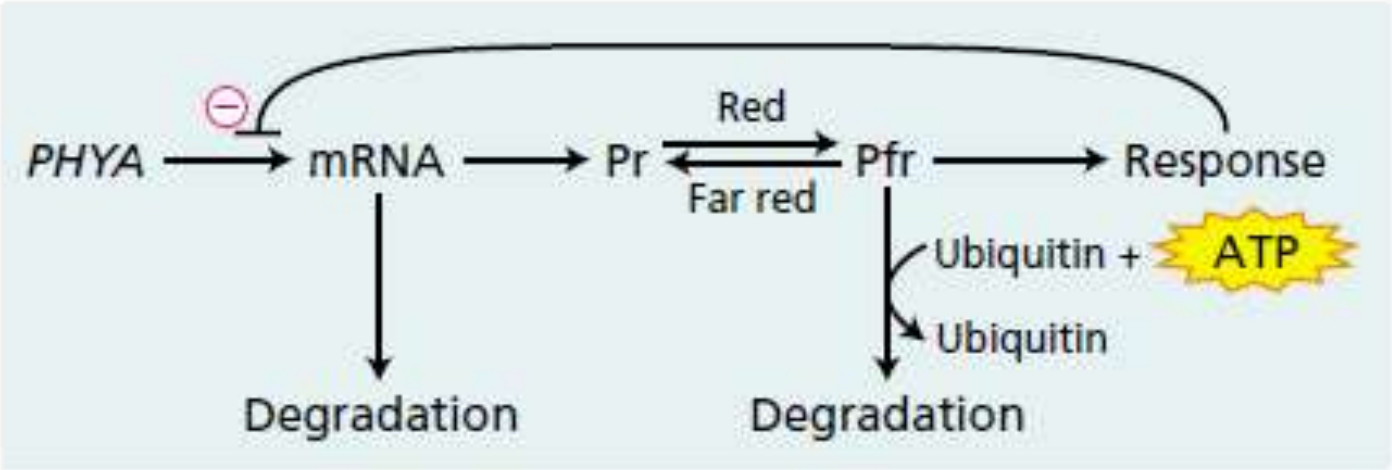
- Understanding their complex signal transduction pathway is one of the most challenging areas of study
- Some of the *hy* mutants have turned out to be selectively deficient in specific phytochromes.
- Eg - *hy3* is deficient in phyB, and *hy1* and *hy2* are deficient in chromophore
- These and other *phy* mutants have been useful in determining the physiological functions of the different phytochromes

PHY Genes Encode Two Types of Phytochrome

- On the basis of their expression patterns, the products of members of the *PHY* gene family can be classified as
  - Type I
  - Type II phytochromes
- *PHYA* is the only gene that encodes a Type I phytochrome.
- Additional studies of plants that contain mutated
- forms of the *PHYA* gene (termed *phyA* alleles) have confirmed this conclusion and have given some clues about the role of this phytochrome in whole plants

- In dark-grown oat, treatment with red light reduces phytochrome synthesis because the Pfr form of phytochrome inhibits the expression of its own gene.
- *PHYA* mRNA is unstable, so once etiolated oat seedlings are transferred to the light, *PHYA* mRNA rapidly disappears
- inhibitory effect of light on *PHYA* transcription is less dramatic in dicots
- The amount of phyA in the cell is also regulated by protein destruction
- The Pfr form of the protein encoded by the *PHYA* gene, called **PfrA**, is unstable

- There is evidence that PfrA may become marked or tagged for destruction by the ubiquitin system (*ubiquitin* is a small polypeptide thatbinds covalently to proteins and serves as a recognition sitefor a large proteolytic complex, the *proteasome*)



- In dicots, phyA levels also decline in the light as a result of proteolysis, but not as dramatically.
- The remaining *PHY* genes (*PHYB* through *PHYE*) encode the Type II phytochromes
- these phytochromes are also present in etiolated plants

    the expression of their mRNAs is not significantly changed by light, and the encoded phyB through phyE proteins are more stable in the Pfr form than is PfrA.

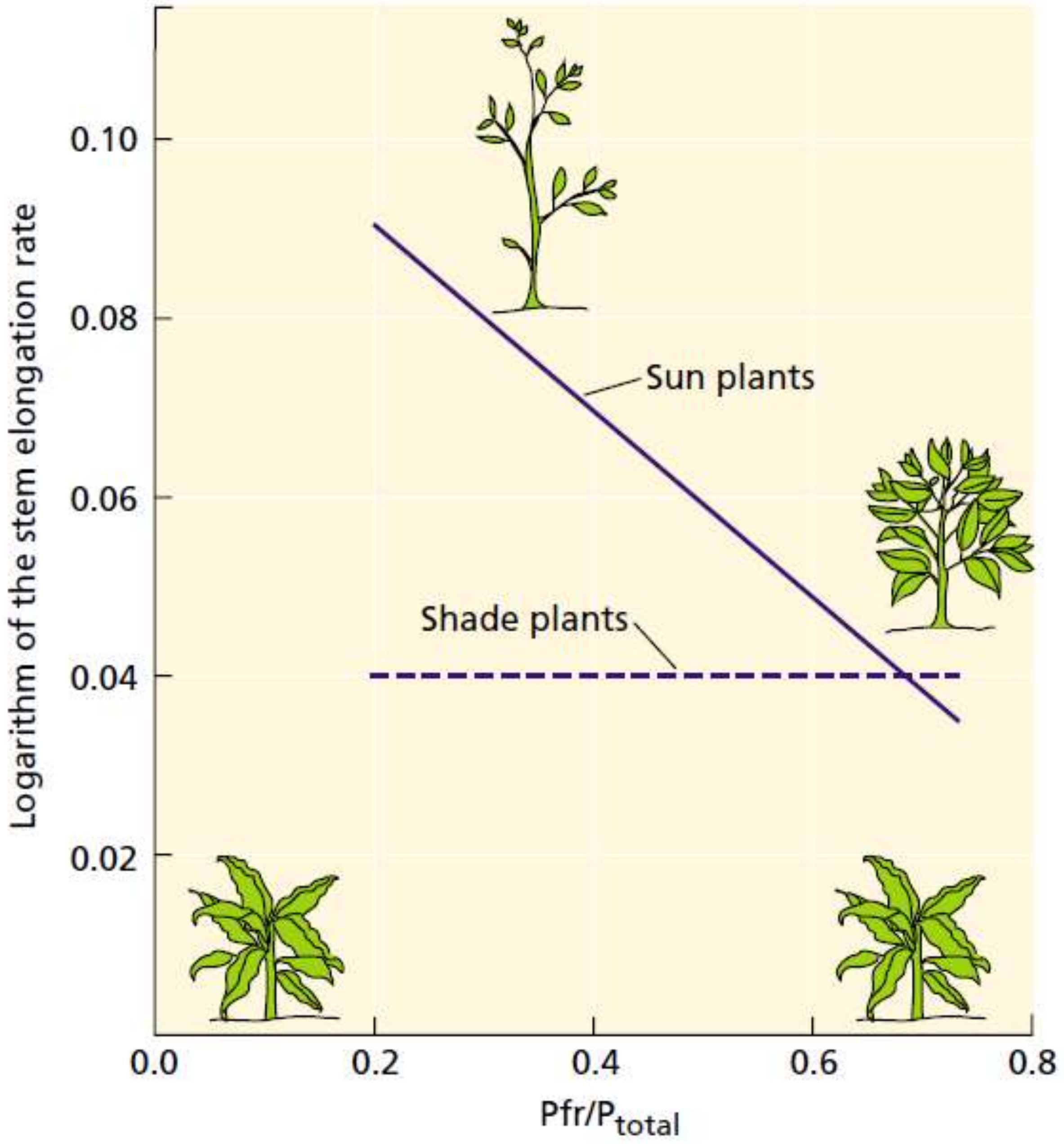
Physiological effects of phytochromes

1) Help to adapt with changing light conditions

- Presence of red/far red reversible pigments in plants helps plants to adjust to their environment
- ratio of R to Pfr varies considerably in different environment
- R/FR =

$$\frac{\text{Photon fluence ratein 10 nm band centered on 660 nm}}{\text{Photon fluence ratein 10 nm band centered on 730 nm}}$$

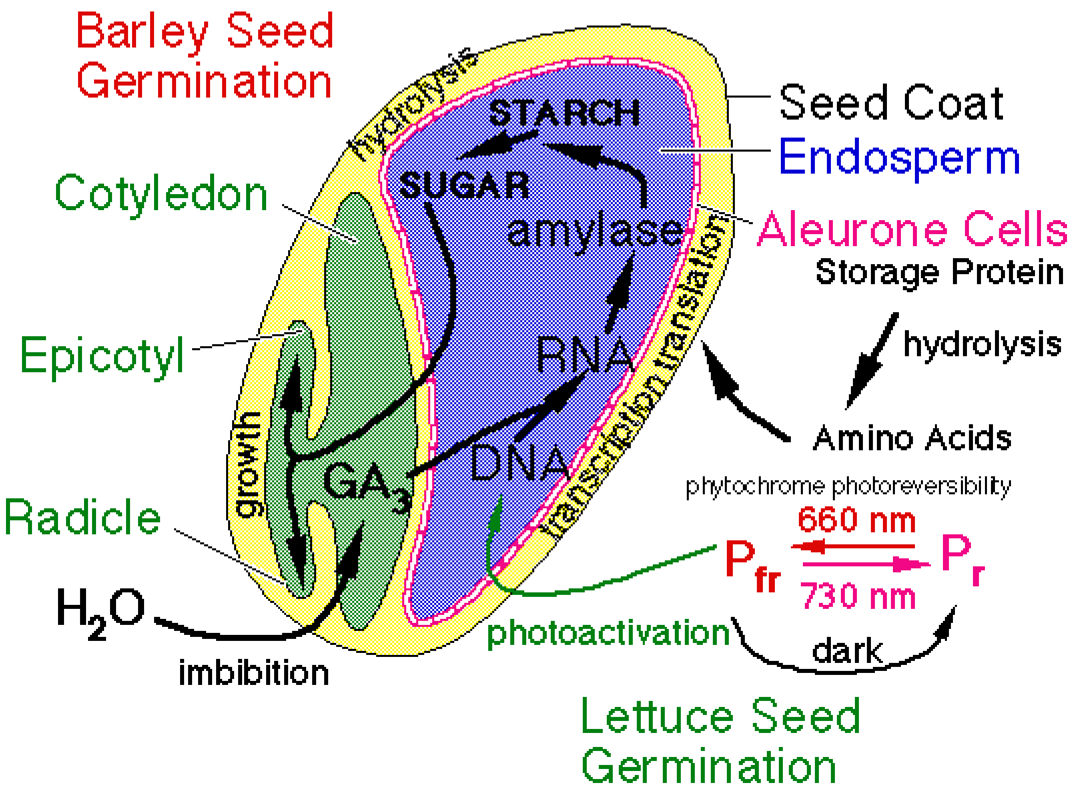
- ***R:FR ratio and shading***
- phytochrome enables plants to sense shading by other plants
- Plants that increase stem extension in response to shading are said to exhibit a **shade avoidance response**
- As shading increases, the R:FR ratio decreases
- The greater proportion of far-red light converts more Pfr to Pr, and the ratio of Pfr to total phytochrome (Pfr/Ptotal) decreases
- sun plants (plants that normally grow in an open-field habitat), the higher the far-red content (i.e., the lower the Pfr:Ptotal ratio), the higher the rate of stem extension


0.10
0.08
0.06
0.04
0.02
Logarithm of the stem elongation rate
Sun plants
Shade plants
0.0
0.2
0.4
0.6
0.8
Pfr/Ptotal

Role of phytochrome in seed germination

- The germination of many seeds is influenced by light
- large-seeded species, with ample food reserves to sustain prolonged seedling growth in darkness
- Some seeds are stimulated to germinate by light - **positivelyphotoblastic seeds- (tomato), Pfr inhibits germination**
- The germination of others is inhibited by light – **negatively photoblastic seeds (lettuce), Pfr promotes germination**
- Many seeds, such as lettuce, may require only brief
- exposure to light, measured in seconds or minutes, while others may require as much as several hours or even days of constant or intermittent light

Lettuce is positively photoblastic



Role of phytochrome in regulating **CIRCADIAN RHYTHMS**

- **circadian rhythms** is considered to be **endogenous**

Phytochrome Regulates the Sleep Movements of Leaves

- The sleep movements of leaves, referred to as **nyctinasty**
- In nyctinasty, leaves and/or leaflets extend horizontally (open) to face the light during the day and fold together vertically (close) at night
- The change in leaf or leaflet angle is caused by rhythmic turgor changes in the cells of the **pulvinus** **-** a specialized structure at the base of the petiole

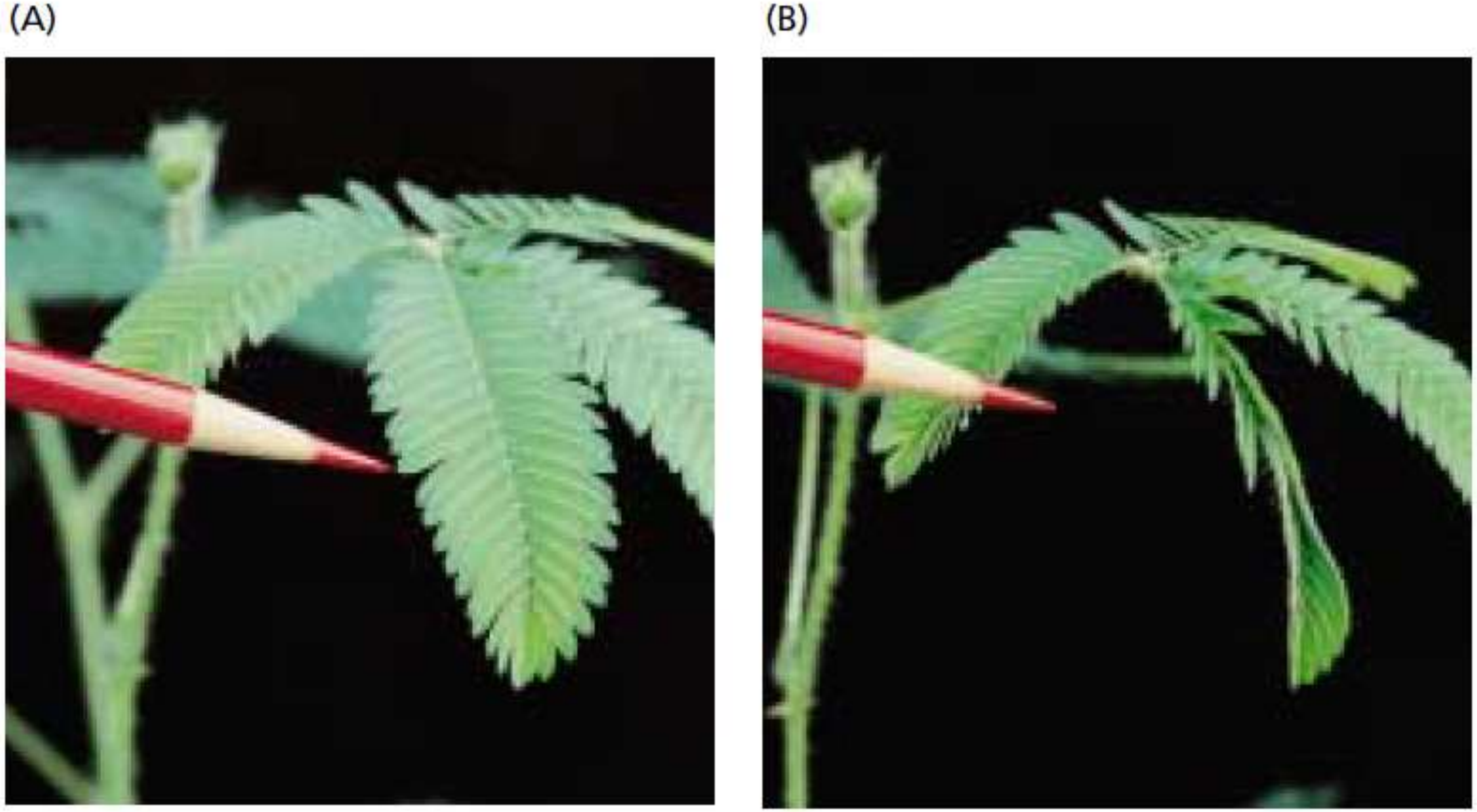


FIGURE 17.12 Nyctinastic leaf movements of *Mimosa pudica*. (A) Leaflets open. (B) Leaflets closed. (Photos © David Sieren/Visuals Unlimited.)

- Light also directly affects movement
  - Blue light stimulatesclosed leaflets to open
  - red light followed by darkness causes open leaflets to close
- The physiological mechanism of leaf movement is well understood
- It results from turgor changes in cells located on opposite sides of the pulvinus, called **ventral motor cells** and **dorsal motor cells**
- These changes in turgor pressure depend on K^+ and Cl^- fluxes across the plasma membranes of the dorsal and ventral motor cells
- Leaflets open when the dorsal motor cells accumulate K^+ and Cl^-, causing them to swell, while the ventral motor cells release K^+ and Cl^-, causing them to shrink
- Reversal of this process results in leaflet closure.

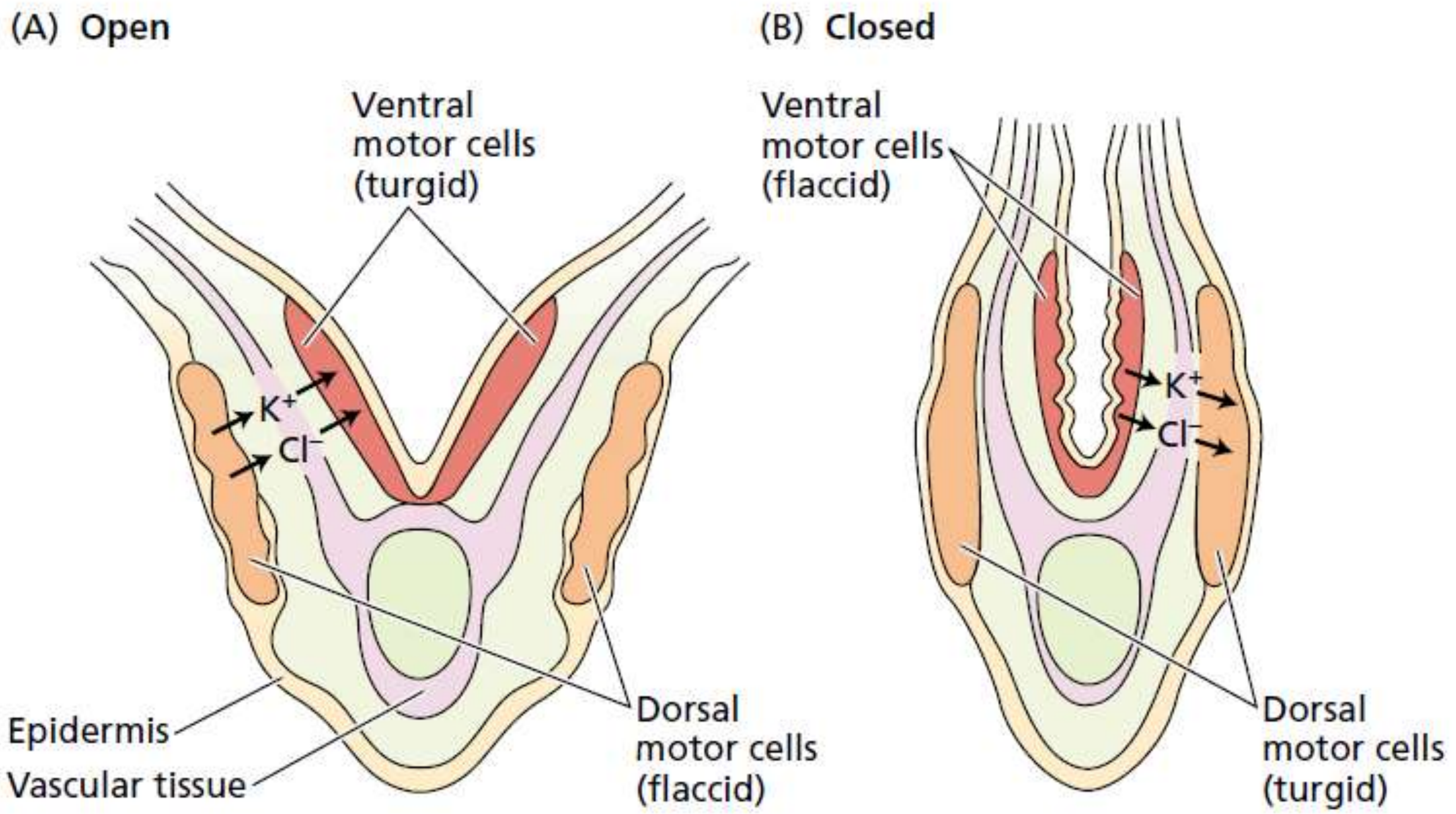


FIGURE 17.14 Ion fluxes between the dorsal and ventral motor cells of *Albizia pulvini* regulate leaflet opening and closing. (After Galston 1994.)

Gene expression and circadian rhythms.

- Phytochrome can also interact with circadian rhythms at the level of gene expression
- The expression of genes in the *LHCB* family (encoding the light harvesting chlorophyll *a/b*–binding proteins of photosystem II) is regulated at the transcriptional level by both circadian rhythms and phytochrome
- In leaves of pea and wheat, the level of *LHCB* mRNA has been found to oscillate during daily light–dark cycles, rising in the morning and falling in the evening

- Since the rhythm persists even in continuous darkness, it appears to be a circadian rhythm.
- But phytochrome can perturb this cyclical pattern of expression
- When wheat plants are transferred from a cycle of 12 hours light and 12 hours dark to continuous darkness, the rhythm persists for a while, but it slowly *damps out*
- If plants are given a pulse of red light before they are transferred to continuous darkness, no damping occurs

DE-ETIOLATION

- Plants grown in darkness exhibit a distinct morphology
  - The leaves themselves undergo limited development and remain small and clasping, as they were in the embryo.
  - Chlorophyll is absent and the seedlings appear white or yellow in color
  - In monocot cereal grains the first inter node, or mesocotyl, elongates excessively in the dark and the coleoptile,which is a modified leaf, grows slowly.
  - The primary leaves remain within the coleoptile and stay tightly rolled around their midvein.
- This general condition exhibited by dark-grown seedlings is called **etiolation**
- When exposed to light, etiolated seedlings undergo **de-etiolation**, a process under control of both phytochromes and cryptochromes

PHYTOCHROME AND POLLEN GERMINATION

- In some plants short exposure to red light caused early tube germination and its enhanced elongation
- This effect was annulled by far red light exposure

DNA AND PROTIEN SYNTHESIS

- Red light also shown to be induced DNA and protein synthesi in the cells of etiolated pea stem apices
- In dwarf plants red light induced protein which were complexed with GA_3 and suggestively these complex prevents the growth of normal plants

PHYTOCHROME SPECIALIZATION

- Phytochrome is encoded by a multigene family: *PHYA* through *PHYE*.
- Despite the great similarity in their structures, each of these phytochromes performs distinct roles in the life of the plant.

Phytochrome B Mediates Responses to Continuous Red or White Light

- Phytochrome B mediates shade avoidance by regulating hypocotyl length in response to continuous light
- Phytochrome B also appears to regulate photoreversible seed germination, the phenomenon that originally led to the discovery of phytochrome

- **Phytochrome A Is Required for the Response to Continuous Far-Red Light**
- Phytochrome A also appears to be involved in the germination VLFR of *Arabidopsis* seeds
- mutants lacking phyA cannot germinate in response to red light in the verylow- fluence range, but they show a normal response to red light in the low-fluence range
- This result demonstrates that phyA functions as the primary photoreceptor for this VLFR
- recent evidence suggests that phyE is required for this component of seed germination

- **Developmental Roles for Phytochromes C,D, and E Are Also Emerging**
- phyC, phyD, and phyE appear to play roles that are for the most part redundant with those of phyA and phyB
- phyB appears to be involved in regulating all stages of development, the functions of the other phytochromes are restricted to specific developmental steps or responses.

Phytochrome Interactions Are Important Early in Germination

- constant red and far-red
- light absor bed separately by the phyA and phyB systems
- Continuous red light absorbed by phyB stimulates de-etiolation by maintaining high levels of PfrB
- Continuous farred light absorbed by PfrB prevents this stimulation by reducing the amount of PfrB
- The stimulation of de-etiolation by phyA depends on the photostationary state of phytochrome(indicated by the circular arrows)

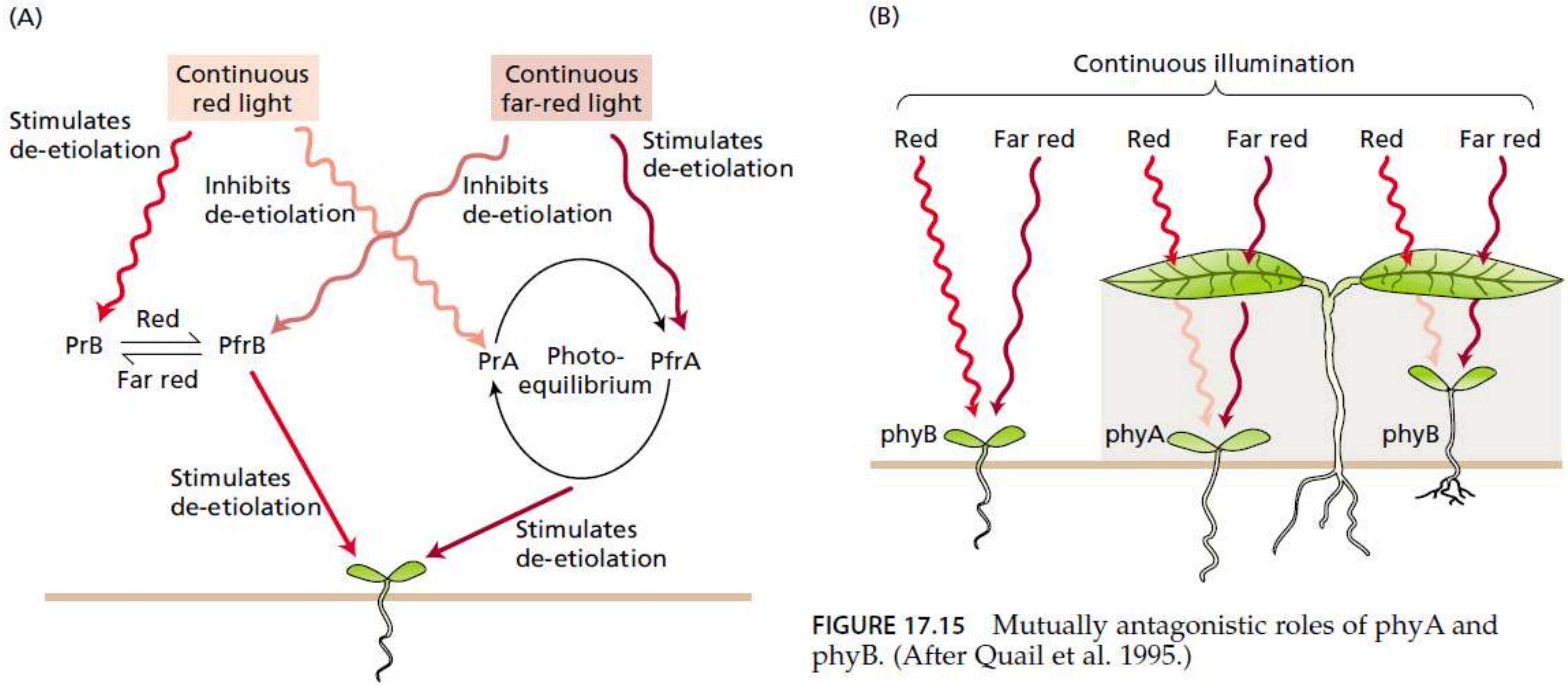
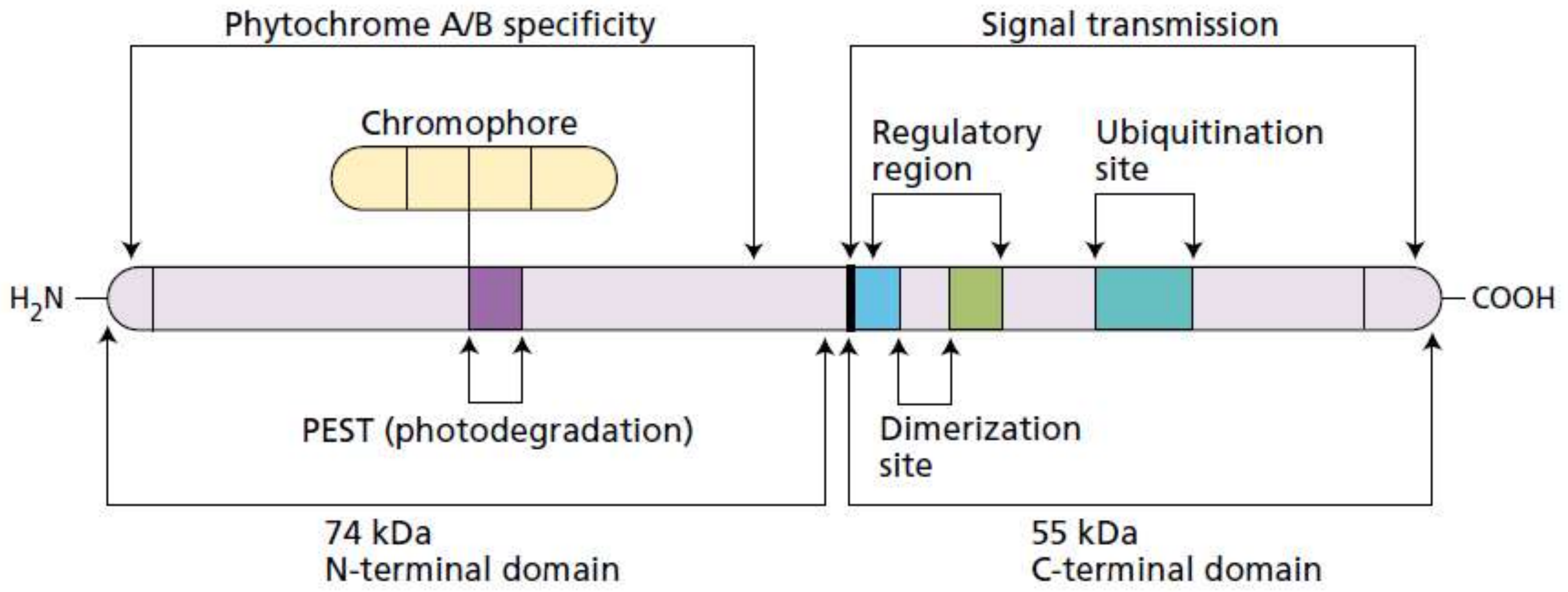


FIGURE 17.15 Mutually antagonistic roles of phyA and phyB. (After Quail et al. 1995.)

- Continuous far-red light stimulates de-etiolationwhen absorbed by the phyA system; continuous red light inhibits the response.

PHYTOCHROME FUNCTIONAL DOMAINS

- Each phytochrome-mediated response is regulated by a specific phytochrome, or by interactions between specific phytochromes
- specific regions of the PHY proteins must be specialized to allow them to perform their distinct functions
- Just as mutations *reducing* the amount of a particular phytochrome have yielded information about its role, plants genetically engineered to *overexpress* a specific phytochrome are also useful.
- phytochrome as a molecule having two domains linked by a hinge: an N-terminal lights ensing domain in which the light specificity and stability reside, and a C-terminal domain that contains the signal transmitting sequences



- The C-terminal domain also contains the site for the formation of phytochrome dimers and the site for the addition of ubiquitin, a tag for degradation.

CELLULAR AND MOLECULAR MECHANISMS

- All phytochrome-regulated changes in plants begin with absorption of light by the pigment.
- After light absorption, the molecular properties of phytochrome are altered
- signal-transmitting sequences in the C terminus to interact with one or more components of a signal transduction pathway that ultimately bring about changes in the growth, development, or position of an organ
- signal-transmitting motifs appear to interact with multiple signal transduction pathways; others appear to be unique to a specific pathway

- different phytochrome proteins utilize different sets of signal transduction pathways
- Molecular and biochemical techniques are helping to unravel the early steps in phytochrome action and the signal transduction pathways that lead to physiological or developmental responses.
- These responses fall into two general categories:
  - 1. Relatively rapid turgor responses involving ion fluxes
  - 2. Slower, long-term processes associated with photomorphogenesis,

    involving alterations in gene expression

Phytochrome Regulates Membrane Potentials and Ion Fluxes

- Slide 40

Phytochrome Regulates Gene Expression

- Gene expression and circadian rhythms.

CRYPTOCHROMES AND BLUE LIGHT EFFECT

- Charles Darwin was one of the first to note that plants respond to blue light
- Portions of the spectrum that constitute blue and UV-A light regulate many aspects of growth and development in plants, fungi, and animals
- Plant responses to blue and UV-A light include
  - de-etiolation such as the inhibition of hypocotyl elongation and stimulation of cotyledon expansion
  - the opening and closure of stomata, gene expression, flowering time, the "setting" of endogenous clocks

- Most action spectra for blue-light responses had peaks in the blue and UV-A regions of the spectrum and closely resembled the absorption spectra of flavin molecules, such as riboflavin
- A. W. Galston to postulate flavoprotein was involved in blue light responses
- Others, however, mounted strong arguments in favour of a carotenoid-based photoreceptor, and for many years the flavin–carotenoid controversy was hotly debated
- Because of this elusive, or "cryptic," nature of the pigment and the pervasive blue-light responses in cryptogams, or nonflowering plants, the pigment was referred to as cryptochrome
- The first protein with the characteristics of a blue-light photoreceptor was isolated from *Arabidopsis* in 1993.

PHYTOCHROME ACTION CAN BE MODULATED BY THE ACTION OF OTHER PHOTORECEPTORS

- Additional experiments have confirmed that the other cryptochrome, cry1, also interacts with phytochromes.
- cry1 and cry2 interact with phyA in vitro and can be phosphorylated in a phyA-dependent manner

- Cryptochromes are photolyase, like blue light receptors
- It was originally discovered in Arabidopsis but later found in other plants, microbescand animals
- Arabidopsis has 2 cryptochromes CRY1 and CRY2
- Which primarily mediates blue light inhibition of hypocotyl elongation, photoperiodic control of floral elongation
- In addition to these cryptochromes also regulate dozen of light processes, including circadian rhythm, stomatal opening , guard cell development, cell cycle, programmed cell death, apical dominance ....

PHYTOCHOME ACTS THROUGH MULTIPLE SIGNAL TRANSDUCTION PATHWAYS

- Using biochemical approaches, researchers have shown that signaling involves several different mechanisms, including G-proteins, Ca^{2+}, and phosphorylation.
- ***G-proteins and calcium.***
- Well-characterized signalling pathways in other systems (e.g., mating in yeasts) often include **G-proteins**
- These protein complexes are normally membrane associated, have three different subunits, and bind GTP or GDP on one subunit
- The hydrolysis of GTP to GDP is required for regulation of G-protein function

- *Phosphorylation*
- **Kinases** are enzymes that have the capacity to transfer phosphate groups from ATP to amino acids such as serine or tyrosine, either on themselves or on other proteins
- Kinases are often found in signal transduction pathways in which the addition or removal of phosphate groups regulates enzyme activity
- Phytochrome is now known to be a protein kinase
- they are serine/threonine kinases.

- one potential target is a cytosolic protein termed ***p**hytochrome **k**inase **s**ubstrate 1*, or **PKS1**, that can accept a phosphate from phyA
- Phosphorylation occurs on serines, and to a lesser extent on threonines
- Another protein kinase associated with phytochrome is ***n**ucleoside **d**i**p**hosphate **k**inase* **2 (NDPK2)**
- Phytochrome A has been found to interact with this protein, and its kinase activity is increased about twofold when phyA is bound in the Pfr form.

Thank you