Plant Growth *Phytochromes*

Plant Growth

Phytochrome Action

Phytochrome, Photoperiodism and Flowering

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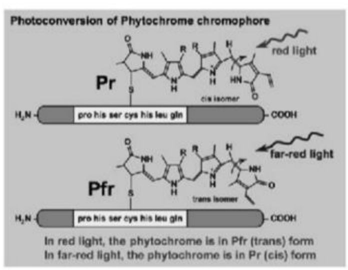
PHYTOCHROME



Photoreversibility is the most distinctive property of phytochrome

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Initial Studies

- The discovery of phytochrome is closely associated with studies on flowering.
- **Phytochrome** is a blue protein pigment with a molecular mass of about 125 kDa that plants, and some bacteria and fungi, use to detect light.
  - The term phytochrome ("plant color") was originally coined to describe the proteinous pigment that controls photoperiod detection and floral induction of certain short-day plants (e.g. cocklebur and soybean) (Garner and Allard, 1920).

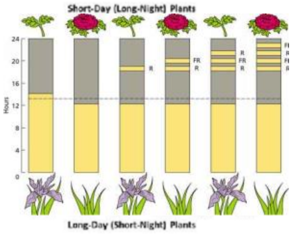

Fig. 24.22 A flash of **red** (R) light during the dark period induces flowering in an LDP but prevents flowering in an SDP (1), and the effect is reversed by a flash of **far-red** (Fr) light (2). This response indicates the involvement of phytochrome.

- Essentially all proteins absorb light in the near-UV region due to the presence of aromatic amino acids, and proteins that sense visible light possess chromophore cofactors that confer the desired wavelength sensitivity.

- In 1932, Beltsville research group of the USDA headed by Borthwick and Hendricks showed that red light (630 to 680 nm) elicits the germination of lettuce seeds, whereas far-red light (710 to 740 nm) inhibits the process.

Irradiations	Germination (%)
R	88
R, Fr	22
R, Fr, R	84
R, Fr, R, Fr	18
R, Fr, R, Fr, R	72
R, Fr, R, Fr, R, Fr	22

R = red, Fr = Far red

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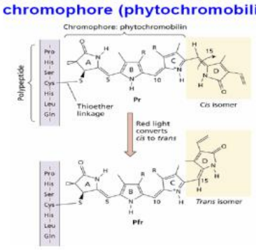
Chemical Structure

- Phytochromes are soluble proteins and exist as homodimers. Each monomer of phytochrome molecule has two components: a protein part (the apoprotein) and a chromophore.
- The chromophore is an open–chain tetrapyrrole which is covalently linked to the protein moiety through a cysteine residue (a sulfur linkage).
  - Apoprotein consists of a 60 kDa amino-terminal domain, and a 55 kDa carboxyl-terminal domain.

Structure of the Pr and Pfr forms of the chromophore (phytochromobilin)



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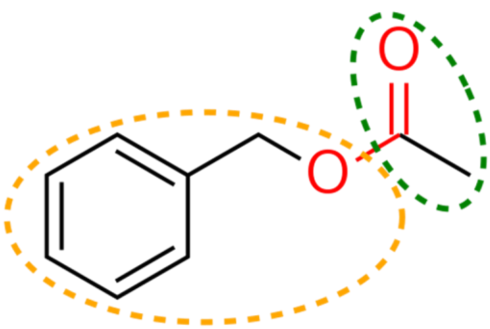
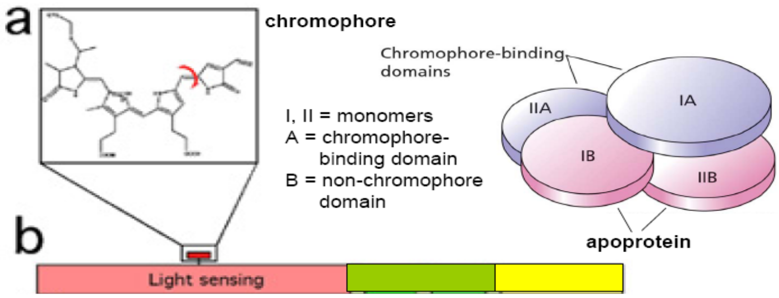
A moiety is a part of a molecule that is given a name because it *is identified* as a part of other molecules as well.



Benzyl acetate contains a benzyloxy moiety (encircled with light orange). It also contains an ester functional group (in red), and an acetyl functional group (encircled with dark green).

Phytochrome structure

- soluble protein of ~ 250 kDa
- Dimer composed of two polypeptides
- each monomer consists of two components: chromophore and apoprotein = holoprotein



- On absorption of light, the Pr chromophore undergoes a *cis-trans* isomerisation of the double bond between carbons 15 and 16 and the rotation of the C14 and C15 single bond.
- The absorption of red light appears to provide the energy required to overcome high activation energy for rotation around double bond, a transition that is not possible at normal temperature.

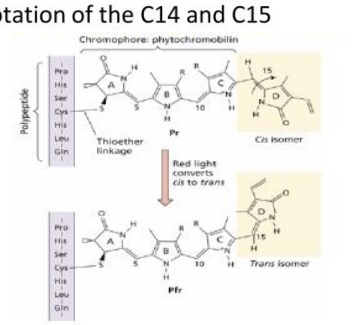
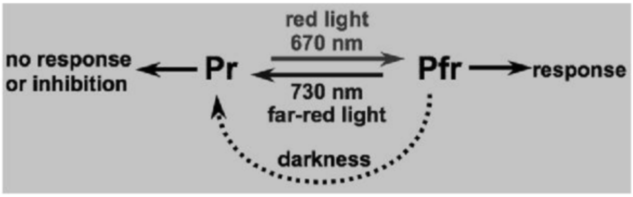


Fig. 17.6 Structure of the Pr and Pfr forms of the **chromophore** (phytochromobilin) and the peptide region bound to the chromophore through a thioether linkage. The chromophore undergoes a *cis-trans* isomerization at carbon 15 in response to red and far-red light. (After Andel et al. 1997.)

PHYTOCHEMISTRY AND BIOCHEMISTRY OF PHYTOCROME

Photoreversibility

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



- It is important to note that the phytochrome pool is never fully converted to the Pfr or Pr forms following-red or far-red irradiation, because the absorption spectra of the Pfr and Pr forms overlap.
- Thus, when Pr molecules are exposed to red light, most of them absorb the photons and are converted to Pfr, but some of the Pfr made also absorbs the red light and is converted back to Pr (Fig. 17.3).
- The proportion of phytochrome in the Pfr form after saturating irradiation by red light is only about 88%.
- Similarly, the very small amount of far-red light absorbed by Pr makes it impossible to convert Pfr entirely to Pr by broad-spectrum far-red light. Instead, an equilibrium of 98% Pr and 2% Pfr is achieved. This equilibrium is termed the **photostationary state**.

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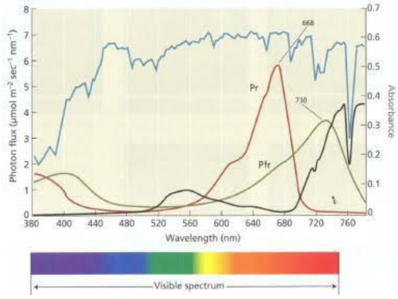
Fig. 17.3 Absorption spectra of purified oat phytochrome in the Pr (red line) and Pfr (green line) forms overlap. At the top of the canopy, there is a relatively uniform distribution of visible spectrum light (blue line), but under a dense canopy much of the red light is absorbed by plant pigments, resulting in transmittance of mostly far-red light.



The black line shows the spectral properties of light that is filtered through a leaf. Thus, the relative proportions of Pr and Pfr are determined by the degree of vegetative shading in the canopy. (After Kelly and Lagarias 1985, courtesy of Patrice Dubois.)

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- In addition to absorbing red light, both forms of phyto-chrome absorb light in the blue region of the spectrum that can convert Pr to Pfr and vice versa.
- Blue-light responses can also result from the action of one or more specific blue-light photoreceptors.

The Physiologically Active Form of Phytochrome

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

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- Evidence such as this has led to the conclusion that Pfr is the physiologically active form of phytochrome.
- The use of narrow waveband red and far-red light was central to the discovery and eventual isolation of phytochrome.
- However, a plant growing outdoors is never exposed to strictly "red" or "far-red" light, as are plants used in laboratory-based photobiological experiments.
- In natural settings plants are exposed to a much broader spectrum of light, and it is under these conditions that phytochrome must work to regulate developmental responses to changes in the light environment.

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

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

CHARACTERISTICS OF PHYTOCROME-INDUCED RESPONSES

Variety of Phytochrome Responses

- The variety of different phytochrome responses in intact plants is extensive, in terms of both the kinds of responses (see Table 17.1) and the quantity of light needed to induce the responses.
- These phytochrome-induced responses, for ease of discussion, may be logically grouped into two types:
  - Rapid biochemical events
  - Slower morphological changes, including movements and growth
- Such responses can be classified into various types depending on the amount and duration of light required and on their action spectra.

Lag and Escape Time of Phytochrome Responses

- Morphological responses to the photoactivation of phytochrome may be observed visually after **a lag time**-the time between stimulation and observed response.
- The lag time may be as brief as a few minutes or as long as several weeks.
- The more rapid of these responses are usually reversible movements of organelles or reversible volume changes (swelling, shrinking) in cells, but even some growth responses are remarkably fast.
- Variety in phytochrome responses can also be seen in the phenomenon called **escape from photoreversibility**.
  - Red light-induced events are reversible by far-red light for only a limited period of time, after which the response is said to have "escaped" from reversal control by light.

- This escape phenomenon can be explained by a model based on the assumption that phytochrome-controlled morphological responses are the end result of a multi-step sequence of linked biochemical reactions in the responding cells.
- Early stages in the sequence may be fully reversible by removing Pfr, but at some point in the sequence a point of no return is reached, beyond which the reactions proceed irreversibly toward the response.
- Therefore *the escape time represents the amount of time it takes before the overall sequence of reactions becomes irreversible*; essentially, the time it takes for Pfr to complete its primary action.
- The escape time for different responses ranges remarkably, from less than a minute to hours.

Phytochrome Responses and Light Quantity

- Phytochrome responses can be distinguished by the amount of light required to induce them.
- The amount of light is referred to as the **fluence**, *the number of photons impinging on a unit surface area*.
  - The standard units for fluence are micromoles of quanta per square meter (μmol m^{-2}).
  - Some phytochrome responses are sensitive not only to the fluence, but also to the **irradiance*** or **fluence rate** of light. The units of irradiance are micromoles of quanta per square meter per second (μmol m^{-2} s^{-1}).
- Phytochrome responses fall into three major categories based on the amount of light required: very low-fluence responses (VLFRs), low-fluence responses (LFRs), and high-irradiance responses (HIRs) (Fig. 17.4).

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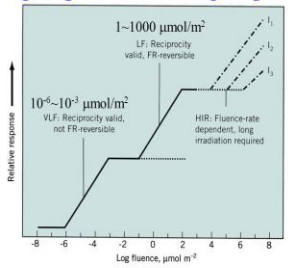
- ***VLFRs are nonphotoreversible***
  - Some phytochrome responses can be initiated by fluences as low as 0.0001 μmol m^{-2} (one-tenth of the amount of light emitted by a firefly in a single flash), and saturated (i.e., reach a maximum) at about 0.05 μmol m^{-2}. For example, Arabidopsis seeds can be induced to germinate with red fight (0.001 to 0.1 μmol m^{-2}).

Fig. 17.4 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 proportional to irradiance as well as to fluence, the effects of three different irradiances !aiven continuously are illustrated (11 > 12 > 1 3). (After Briggs et al. 1984.)

Phytochrome responses can be grouped into three groups



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- ***LFRs are photoreversible***.
  - These phytochrome responses cannot be initiated until the fluence reaches 1.0 μmol m^{-2}, and are saturated at about 1000 μmol m^{-2}.
  - These include most of the red/far-red photoreversible responses, such as the promotion of lettuce and Arabidopsis seed germination (Fig. 17.5).
  - 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).

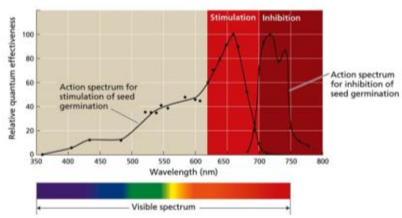

Fig. 17.5 LFR action spectra for the photoreversible stimulation and inhibition of seed germination in Arabidopsis. (After Shropshire et al. 1961.)

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

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- HIRs require prolonged or continuous exposure to light of relatively high irradiance. The response is proportional to the irradiance until the response saturates and additional light has no further effect.
- These responses are proportional to fluence rate–the number of photons striking the plant tissue per second–rather than fluence–the total number of photons striking it in a given period of illumination that leads to the term of HIRs (high-irradiance responses).
- HIRs saturate at much higher fluences than LFRs–at least 100 times higher.
- HIRs obey the law of reciprocity as suggested by inhibition of hypocotyl elongation in response to short pulses of FR light, suggesting that photoperception by phytochrome is rate-limiting for this response.

So, Phytochromes

– **Phytochromes** are a class of photoreceptor in plants, bacteria and fungi used to detect light. They are sensitive to light in the red and far-red region of the visible spectrum and can be classed as either Type I, which are activated by far-red light, or Type II that are activated by red light.

Action

- Phytochromes control many aspects of plant development. They regulate the germination of seeds (photoblasty), the synthesis of chlorophyll, the elongation of seedlings, the size, shape and number and movement of leaves and the timing of flowering in adult plants.

Photoblasty

• Seeds that are light stimulated to germinate by are described as positively photoblastic seeds whose germination is inhibited by light are said to be negatively photoblastic . The response to light is apparently mediated by phytochrome regulated production of the plant hormone gibberellin .

The pigment phytochrome

- Detects R and FR light
- Provides information about environment
- Answers 3 questions for plant
  - – Am I in the light?
  - – Do I have plants as neighbors or above me?
  - – Is it time to flower?

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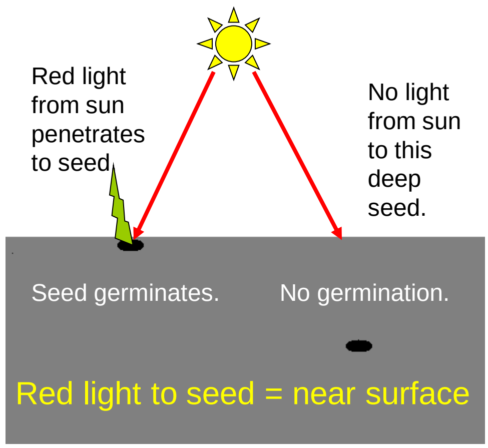
Seed location?



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- Seeds store materials to start growth
- Must reach light before running out of stored materials
- Small seeds
  - Need to be very near surface
  - Often need light for germination
- Germinating plants straighten & open leaves at surface, too

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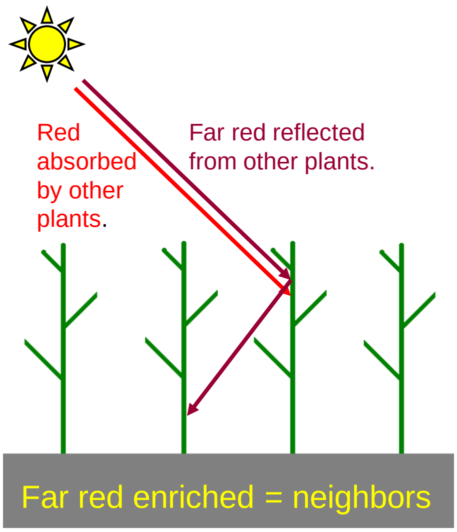
Plant neighbors?



Why does this matter?

- Neighboring plants are threats
  – Might grow taller, shade you
- Solution
  – Grow at least as tall as neighbors
  – Need to know that you have neighbors
- Isolated plants typically shorter than crowded plants
  – Other reasons, too

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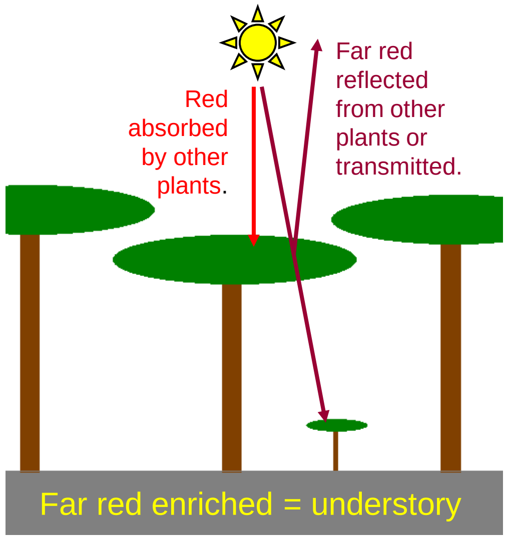
Under other plants?



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Why important?

- Best growth strategy for understory plants is different than for plants in open
- Need to know whether
  – Shaded by other plants
  OR
  – Just cloudy
  OR
  – Late in day (low light)

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Right time to flower?

* Unreliable indicators of time of year
  - Temperature
  - Moisture
  - Light levels
* Reliable: length of day/night
  - Varies with season
  - Varies with latitude

Detected by phytochrome

Phytochrome has 2 forms

- Red-absorbing phytochrome

  P_r

- Far red absorbing phytochrome

  P_{fr}

- Interconverted P_r P_{fr}
- Two forms of the same compound
- Total amount same

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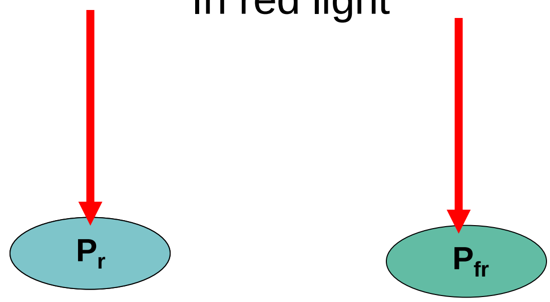
In red light



P_r absorbs red light, changes to P_{fr} form.

P_{fr} doesn't absorb red light, stays the same.

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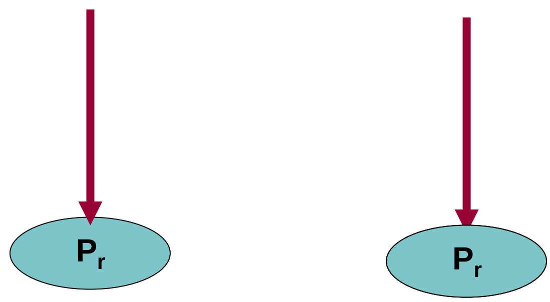
In far red light



P_r doesn't absorb far red light, stays the same.

P_{fr} absorbs far red light, changes to P_r form.

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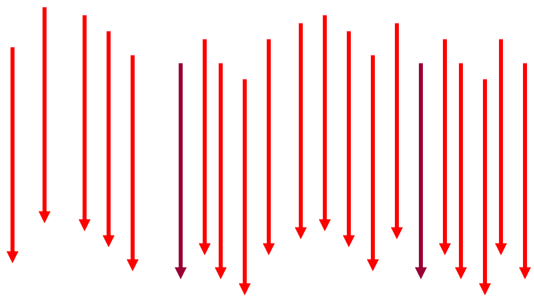
Sunlight



Mostly red

A little far red

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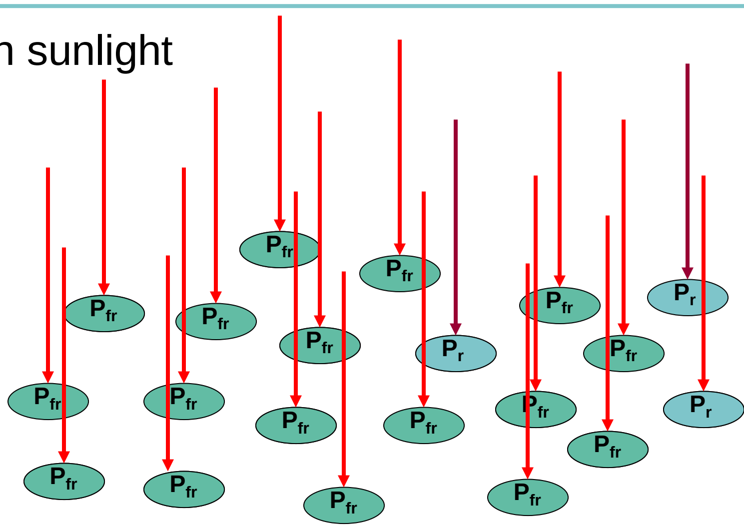
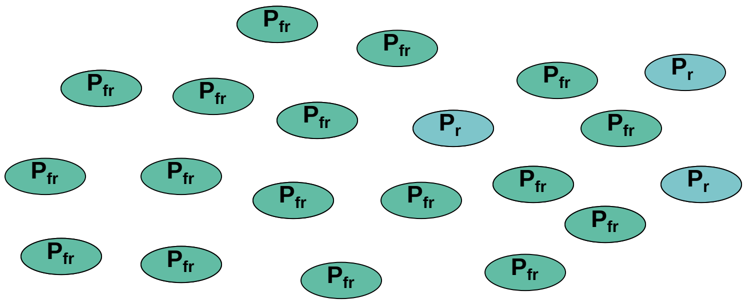
In sunlight



In sunlight most P gets converted to P_{fr} form.

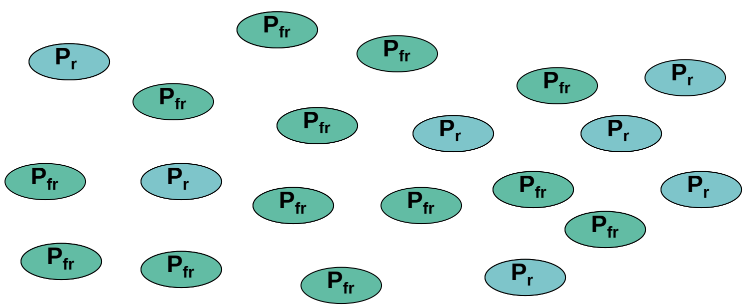
Start of night

Most P in P_{fr} form.



In the dark

P_{fr} form changes gradually to P_r form.



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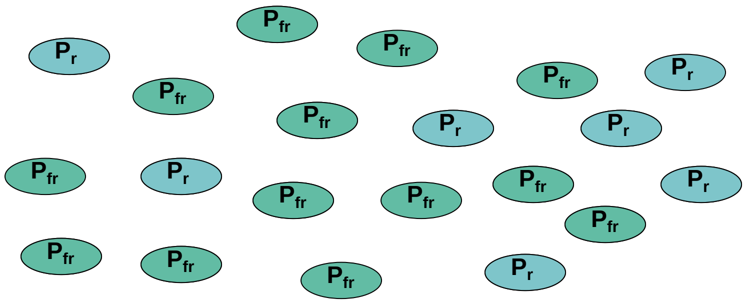
After a short night

P_{fr} still left.



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LDP = SNP

- Needs short night
- P_{fr} still present at end of night
- P_{fr} promotes flowering for LDPs

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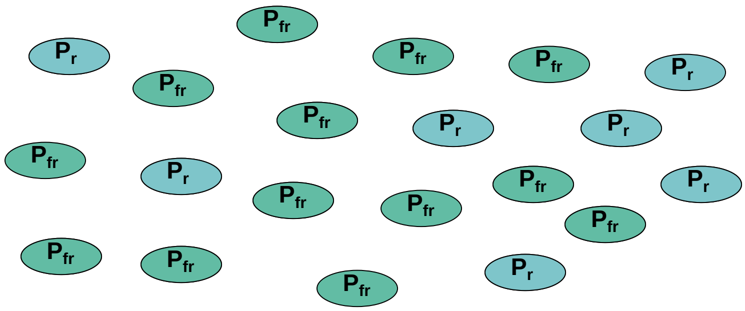
Later in the night

More P_{fr} changes to P_r.



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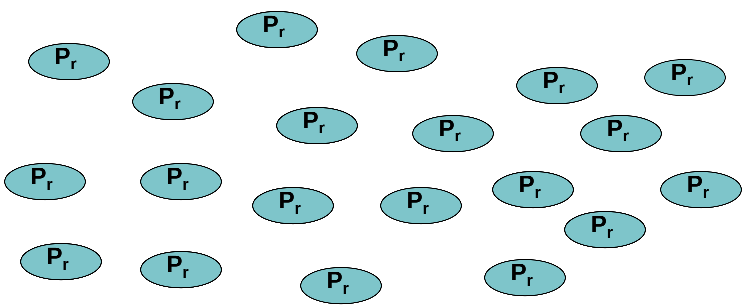
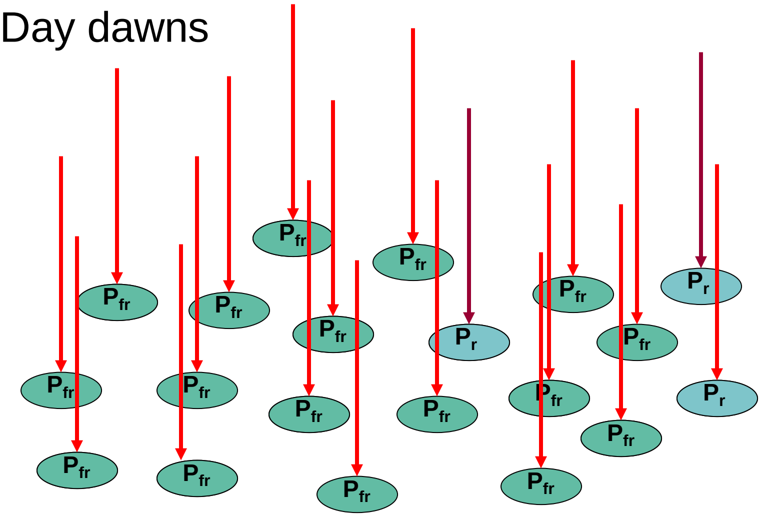
After a long night

All the P_{fr} is gone



Day dawns



Most P gets converted to P_{fr} form again.

SDP = LNP

- Needs long night
- P_{fr} gone at end of night
- P_{fr} inhibits flowering for SDPs

LDP SDP



Long day: P_{fr} left at end of short night.

P_{fr} promotes flowering for LDPs.

P_{fr} inhibits flowering for SDPs.



Short day: P_{fr} gone at end of long night.

No P_{fr} to promote flowering for LDPs.

No P_{fr} to inhibit flowering for SDPs.

Waiting for the right time

- Plants grow leaves until it is time to flower
- LDPs wait until the day is long enough
  – Really night short enough

- SPDs wait until the day is short enough
  – Really night long enough

  Flower opening happens later

Day neutral plants

- Flower when mature enough
- Maybe other environmental signals (temp?)
- Day length (dark length) doesn't matter

Phytochrome tells plants

- If they are near the surface
- About their plant neighbors
- Whether it is time to flower
- And lots more

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So, photoperiodism is any response of plant to the duration and timing of light and dark.

Note:- in many plants.

- long-day plants.
- Short-day plants.
- Intermediate- day plants.

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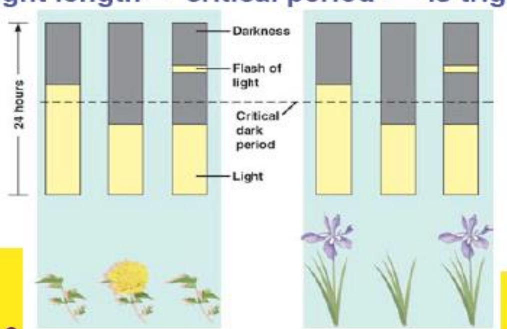
Flowering Response

- **Triggered by photoperiod**
  - relative lengths of day & night
  - night length—"critical period"— is trigger



Short-day plants Long-day plants

Plant is sensitive to red light exposure

What is the evolutionary advantage of photoperiodism?

Synchronizes plant responses to season

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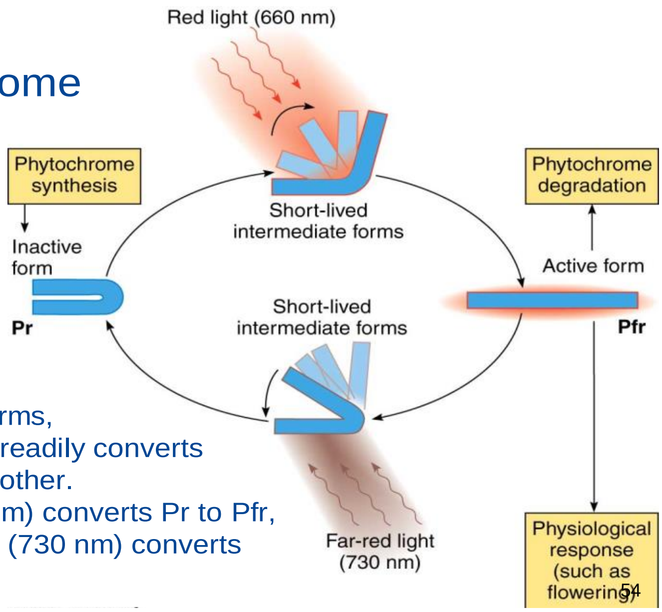
Phytochrome



This pigment
occurs in two forms,
Pr and Pfr, and readily converts
from one to the other.
Red light (660 nm) converts Pr to Pfr,
and far-red light (730 nm) converts
Pfr to Pr

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Practical Application

- **Why do you plant lettuce seed by scattering them on the ground instead of burying seed?**
- **What is the evolutionary advantage to lettuce seeds?**

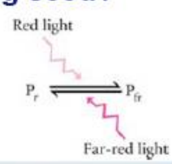

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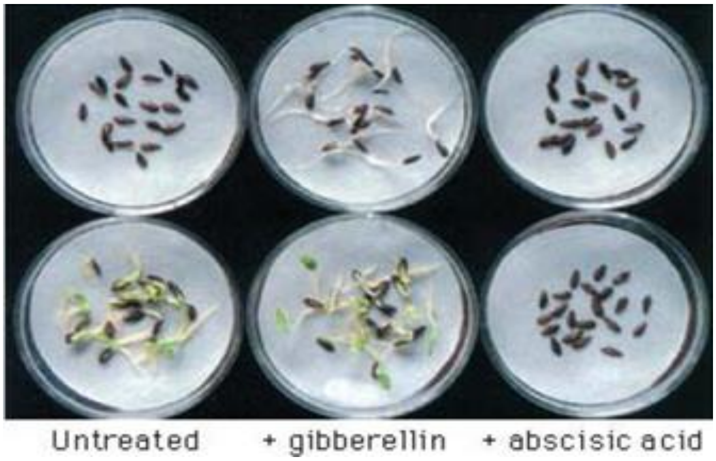
Figure shows the effect of light on germination of seeds



Light –induced germination of lettuce seeds. The seeds in top row were placed in darkness, and the seeds in the bottom row were placed in light.

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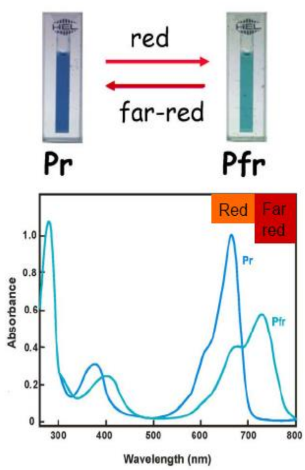
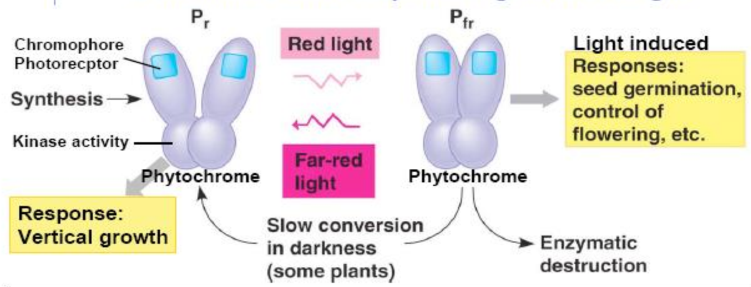
Absorption spectra of purified phytochrome



Phytochrome photoreceptors

- **Molecular switch reaction to red light**
  - conversion of $P_r \rightarrow P_{fr}$ in sunlight stimulates germination, flowering, branching…
  - conversion of $P_{fr} \rightarrow P_r$ in dark inhibits response, & stimulates other responses: growth in height



Phytochrome responses can be distinguished by the amount of light required

- **Very low fluence response (VLFR):** PHYA, PHYE
  Example: **seed germination**
- **Low fluence response (LFR):** PHYB, PHYC, PHYD, PHYE
  Example: **hypocotyl and petiole elongation**
- **High irradiance response (HIR):** PHYA, PHYB
  Example: **flowering**

Fluence = amount of light, the number of photons impinging on a unit surface area

Plant Growth *Phytochromes*

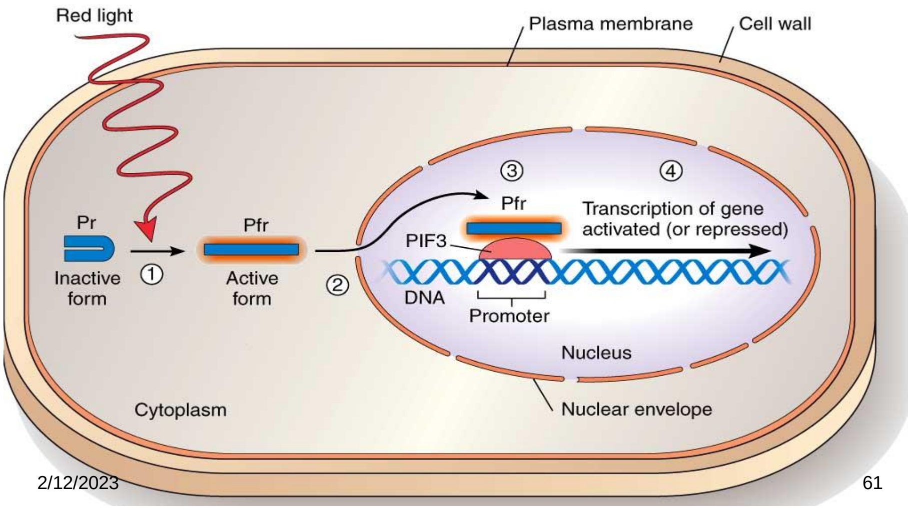
SIGNALING PATHWAYS

1. Early Steps in Phytochrome Action

- All phytochrome-regulated changes in plants begin with absorption of light by the pigment.
- After light absorption, the molecular properties of phytochrome are altered, probably affecting the interaction of the phytochrome protein with other cellular components that ultimately bring about changes in the growth, development, or position of an organ.
- The early steps in phytochrome action and the signal transduction pathways that lead to physiological or developmental responses fall into two general categories:
  - Ion fluxes, which cause relatively rapid turgor responses
  - Altered gene expression, which result in slower, long-term processes.

Plant Growth *Phytochromes*

Phytochrome signal transduction



2. Phytochrome and Membrane Potentials

- Phytochrome can rapidly alter the properties of membranes, within seconds of a light pulse.
- Such rapid modulation has been measured in individual cells and has been inferred from the effects of red and far-red light on the surface potential of roots and oat (*Avena*) coleoptiles, in which the lag between the production of Pfr and the onset of measurable hyperpolarization (membrane potential changes) is 4.5 seconds.
- Changes in the bioelectric potential of cells imply changes in the flux of ions across the plasma membrane and suggest that some of the cytosolic responses of phytochrome are initiated at or near the plasma membrane.

3. Phytochrome and Gene Expression

- Phytochrome regulates gene expression, and the stimulation and repression of transcription by light can be very rapid, with lag times as short as 5 minutes.
- Some of the early gene products that are rapidly upregulated following a shift from darkness to light are transcription factors that activate the expression of other genes.
- The genes encoding these rapidly up-regulated proteins are called **primary response genes**.
- Expression of the primary response genes depends on *signal transduction pathways* and is independent of protein synthesis.
- In contrast, the expression of the late genes, or **secondary response genes**, requires the synthesis of new proteins.

4. Phytochrome Interacting Factors (PIFs)

- Several **phytochrome-interacting factors** (**PIFs**) have been identified in Arabidopsis by two methods (yeast two-hybrid library screens and co-immunoprecipitation).
- One of the most extensively characterized of these factors is **PIF3**, a basic helix-loop-helix (bHLH) transcription factor that interacts with both phyA and phyB.
- Recent studies of PIF-family members have indicated that they act primarily as negative regulators of phytochrome response.
- Phytochromes appear to initiate the degradation of PIF proteins through phosphorylation, followed by degradation through the proteasome complex.

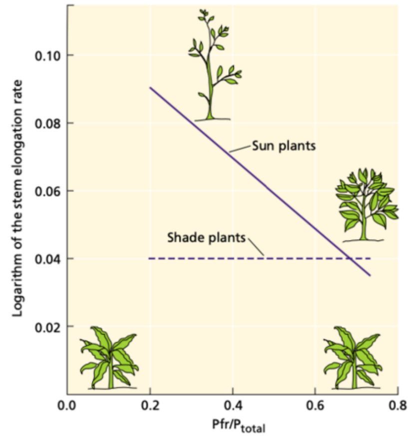
ECOLOGICAL FUNCTIONS

1. Plant Adaptation to Light

- The ratio of red light (R) to far-red light (FR) varies remarkably in different environments
  - As shading increases, the R:FR ratio decreases, and a higher proportion of FR light converts more Pfr to Pr, and the ratio of Pfr/P_{total} decreases.
- An important function of phytochrome is that it enables plants to sense shading by other plants.
- Plants that increase stem extension in response to shading are said to exhibit a **shade avoidance response**.
- When sun plants were grown in natural light with natural F:FR ratio, stem extension rates increased in response to a higher FR content (i.e., a lower $Pfr:P_{total}$ ratio) (Fig. 17.16).

Plant Growth — Phytochromes

Fig.17.16 Phytochromes appear to play a predominant role in controlling stem elongation rate in sun plants (solid line), but not in shade plants (dashed line). (After Morgan and Smith 1979.)



Plant Growth — Phytochromes

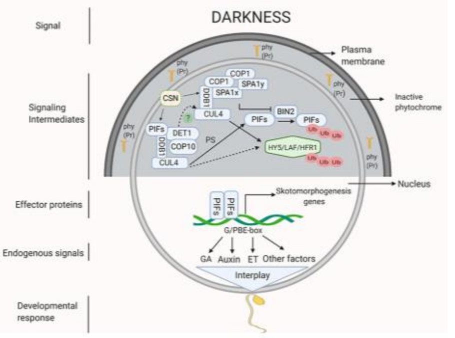
2. Role of Hormones in Shade Avoidance

- Evidence is also emerging for the integration of a number of hormonal pathways in the control of shade avoidance responses including those of auxin, gibberellins, and ethylene.
- Several recent reports have suggested that the PIF proteins play important roles in mediating responses to shade and at least some of these responses are mediated through GA signaling pathways (Fig. 17.17).
- When plants are grown under high R:FR, as in an open canopy, phy proteins become nuclear localized and inactivate PIF proteins.
- In darkness or under low R:FR, a pool of phytochrome is excluded from the nucleus, enabling the accumulation of PIF proteins that promote elongation responses.

Plant Growth Phytochromes

Convergence of light and hormone signaling.

Fig. 17.17 In the dark, the growth-promoting hormone gibberellic acid (GA) binds to its receptor and mediates the ubiquitination of DELLA proteins. The DELLA proteins are then targeted to the 26S proteasome for degradation. In the absence of the DELLA proteins, PIFs can act as both positive and negative regulators of gene expression, likely through interaction with different partners, perhaps mediated through different *cis*-regulatory elements upstream of target genes.



In the light, DELLA proteins bind PIF proteins, preventing them from interacting with genes. PHY proteins also target PIF proteins, through phosphorylation, eventually leading to their ubiquitination and degradation. In the absence of PIF proteins, genes required for cell expansion are not expressed, and plant growth is retarded.