Phytochrome and Photomorphogenesis

Plants, like all organisms, must be able to monitor and respond to environmental conditions. For example, a dark-grown seedling "knows" that it has a limited time to get to light until it runs out of energy. In response, an "etiolated" plant exhibits a variety of features in response to darkness including expanded internodes for rapid growth, an apical hook (in eudicots), unexpanded leaves, no chlorophyll. A brief exposure to light causes internode elongation to slow, the hook to uncurl, leaves to expand and chlorophyll synthesis to begin. Thus, light has an obvious impact on the form of the plant (*photomorphogenesis*).

II. The signal: red & far-red light

In many photomorphogenetic responses, red and far-red light are important environmental signals. For example, from studies of light sensitive lettuce seed germination (see Table 1), Borthwick and Hendricks concluded that: (1) germination is dependent upon which wavelength of light is received last; and that (2) this response is the product of a photo-reversible pigment.

Table 1: Germination of Grand Rapids Lettuce Seeds after brief exposures to red or far-red light

Treatment	Percent Germination
none	8.5
red	98
red, far red	54
r, fr, r	100
r, fr, r, fr	43
r, fr, r, fr, r	99
r, fr, r, fr, r, fr	54
r, fr, r, fr, r, fr, r	98

III. The receptor: Phytochrome

The best characterized, and most important receptor for light-induced growth responses is phytochrome. The absorption spectrum of phytochrome closely matches the action spectrum implicating it in these processes.

IV. Nature of phytochrome

A. Chemistry

Phytochrome is a pigment-protein complex (called a holoprotein = chromophore + apoprotein)

1. Pigment (chromophore)

    - blue-green
    - open chain tetrapyrolle; called phytochromobilin (overhead)
    - made in the plastids

2. Protein (apoprotein)

    - glycoprotein
    - soluble
    - dimer (MW 240,000 D = 240 kD); each of the two peptides are identical with a MW *ca*. 124,000 D and comprised of *ca*. 1128 amino acids
    - gene(s) have been cloned and the amino acid sequence is known; large proportion of hydrophobic amino acids; suggests phytochrome is associated with membranes
    - tetrapyrolle is covalently-bonded to the protein via a thioether linkage involving a cysteine
    - one chromophore per dimer
    - holochrome apparently "self assembles" (autocatalytic) after the individual components are synthesized. For example, the protein is made by ribosomes associated with the ER and the tetrapyrolle is synthesized in the plastids, which is not too surprising considering the similarity it has to the structure of chlorophyll.
    - coded by phy genes

3. Types of phytochrome - phytochrome in dark-grown seedlings is distinctly different than that in light-grown plants. These two forms are:

Type I - found in dark grown, etiolated seedlings; most studied (MW 124 kD; maximum absorption - Pr 666 nm, Pfr 730 nm)

Type II - found in light grown plants; (118 kD, Pr 654; Pfr 724)

4. Conclusions

    - phytochrome in etiolated plants (type I) is slightly larger and absorbs light maximally at a longer wavelength than phytochrome from light-grown plants (type II).
    - differ mainly in apoprotein
    - at least five different proteins that can be identified immunologically; proteins are about 50% similar to one another.
    - proteins are coded by 5 different genes called phy A - phy E. The phytochrome they make is called PHY A etc
    - PHY A is found only in dark-grown seedlings (Type 1); the other four types occur in both etiolated and green (light-grown) plants. Interestingly, PHY A is unstable. The

PHY A is probably important for detecting the presence of light while the others monitor its quality.

B. Photoreversibility

The unique feature of phytochrome is that it exhibits photoreversibility; it exists in two forms that are interchangeable. Pr - red light absorbing form and Pfr - far red light absorbing form. When Pr absorbs red light (*ca*. 660 nm) it is converted into Pfr. When Pfr absorbs far red light (*ca*. 730 nm) it is converted into Pr. In short, phytochrome acts like a light switch. This can be depicted:

$$Pr \leftrightarrow Pfr$$

The absorption spectrum for phytochrome will be provided. Note that there is some overlap in the spectra and also note that there is some absorption of blue light.

C. Chemical Changes during photoreversibility

The main difference between the two forms is a cis-trans isomerization that occurs between one pair of tetrapyrolles. This change has the effect of extending or opening up the chromophore. The protein also undergoes a conformation change. One piece of evidence that supports this is that the protein is more readily digested in the Pfr form.

D. Efficiency of photoconversion

Phytochrome acts like a weird light switch that only turns off/on a portion of the lights. In other words, red light treatment of Pr results in about 85% Pfr + 15% Pr; far red light treatment of Pfr results in 97% Pr + 3% Pfr. Thus, at photo-equilibrium not all the phytochrome is interconverted. The reason for this is because the absorption spectra for the two pigments overlap and they are essentially competing reactions.

A measure of the efficiency conversion is the ratio of the Pfr to the total which is expressed as follows:

$$\text{efficiency} = Pfr/(Pr+Pfr = \text{phytochrome total})$$

In red light = 0.85; far red 720 nm = 0.03; this varies with the environment (see text and overhead)

E. Intermediates

There are a series of intermediates in the conversion from Pr to Pfr. The intermediates are unstable but there is probably always a small pool of them available. There are apparently three intermediate stages in conversion of Pr to Pfr; and 2 stages in conversion of Pfr to Pr. Thus we can modify the original equation:

$$Pr \rightarrow \text{red light} \rightarrow Pr^* \rightarrow [1] \rightarrow [2] \rightarrow [3] \rightarrow Pfr \rightarrow \text{far red} \rightarrow Pfr^*\ [1] \rightarrow [2] \rightarrow Pr$$

(The "" refers to initial excited stage of phytochrome when it absorbs a photon.)*

V. Regulation of Phytochrome levels

A. Synthesis

Phytochrome makes up about 0.2% of the total protein in a dark grown plant. And, there is about 50x more phytochrome in an etiolated plant than a green one. Pr is the form synthesized by the plant; only form in the dark; light inhibits synthesis of Pr. Thus:

phy gene → mRNA → Pr ↔ Pfr

B. Destruction

Both are degraded *in vivo*. Pfr is more labile (unstable). Protease digestion is an important route of hydrolysis; Pfr is more accessible to hydrolysis, perhaps because it gets tagged by proteins like ubiquitin for disposal. Thus:

phy gene → mRNA → Pr ↔ Pfr → destruction

VI. Pfr is the active form

The first hint of this came from lettuce seed germination experiments. Since the seeds only germinated in the light and since Pr is only found in dark grown plants, Pfr must be the active form. Thus:

phy gene → mRNA → Pr ↔ Pfr → destruction or physiological action

VII. Localization

- wide distribution (including angiosperms, gymnosperms, algae, bryophytes)
- meristems and leaves
- found throughout cell (in cytoplasm and associated with organelles)
- Pr seems to be dispersed through cytosol and in nuclei and plastids
- Pfr seems to be sequestered in clumps - granules about 300 nm in size with no membranes

VIII. Actions - there are many phytochrome-mediated responses (see listing). These can be roughly grouped into three major categories:

A. Induction-Reversion Responses or, Low Fluence (LF) Responses

These are the "classic" responses that are induced by red light and reversed by far red. These responses are sensitive to 1 μmol m^{-2} , are photoreversible, and saturate at 1000 μmol m^{-2}. Examples include:

1. Light-sensitive seed germination
   Note that many seeds require light for germination (like lettuce), whereas others are inhibited by light (wild oats, *Phacelia*, Royal Paulownia). Phytochrome presumably stimulates GA synthesis/release which in turn, stimulates the mobilization of stored reserves (recall the GA lecture). This provides energy for the germinating seed and also decreases the solute potential for water uptake necessary for providing the force for the radicle to push its way through the seed.

2. Reversal of etiolation
   Recall that etiolated plants are those that have been grown in the dark and exhibit a series of characteristics including elongated internodes, no chlorophyll, apical hook or unopened coleoptile, unexpanded or coiled leaves. These are all adaptations to save energy and get to the light asap. Light stimulates increased [cytokinin] which stimulates cell division and greening; increases [GA] which presumably stimulates IAA oxidase production to get rid of excess IAA that causes the long spindly growth. Also, IAA stimulates ethylene synthesis which maintains the apical hook. Treating the apical hook with red light + ethylene maintains the hook.
3. Change surface charge
   In a classic experiment, Tanada (1968) observed that red light-treated barley root tips adhered to the sides of a beaker (with a negatively charged surface) but released after far red treatment. It was subsequently show that red light caused the surface to become positively charged and far red light negatively charged. Red light causes a depolarization of membranes and far-red reverses or even causes a slight hyperpolarization.
4. Chloroplast rotation in *Mougeotia* (Haupt)

B. Very Low Fluence (VLF) Responses

These are extremely sensitive to low levels of light (0.1 nmol m^{-2}. Less than 0.02% is Pfr). These are not photo-reversible and saturate at 50 nmol m^{-2}. Some examples include

1. mesocotyl elongation in cereals; and
2. *Arabidopsis* seed germination

C. High fluence (HF) or High Irradiance Response (HIR)

Prolonged or continuous light (10 mmol m^{-2}); fluence rate is important; and not fully photoreversible. Apparently what is important is maintaining a low level of Pfr over time. Another pigment is likely involved - a blue-UVA receptor (cryptochrome?). Examples of phenomena involved in this reaction include:

- anthocyanin synthesis in seedlings and apple skins
- inhibition of hypocotyl elongation in mustard, lettuce and petunia
- plume hook opening in lettuce
- cotyledon enlargement in lettuce
- ethylene production in sorghum

VI. Mechanism of Action

A. Stimulates transcription

Jaffee (1969) found RNA levels in pea increased after red light treatment. Rubisco is light-stimulated, other genes inhibited. Phytochrome involved at the transcriptional level.

The mechanism of action is not clear, but the Pfr may activate an inactive regulatory protein that moves into the nucleus to bind to a site on the DNA near the genes of interest to promote their transcription.

B. Calmodulin

This calcium binding protein may be involved in phytochrome action. Pfr has been shown to stimulate calcium uptake in *Mougeotia*. Calcium complexes with calmodulin and can stimulate enzymes.

Predictions:

1. Calcium should increase after red light treatment - it does (*Mougeotia*)
2. Calcium ionophores like A23187 can substitute for red light - it does
3. Valinomycin - a potassium ionophore has no effect
4. Calmodulin treatment should stimulate activity in absence of red light
5. Calmodulin inhibitors should prevent the response - they do