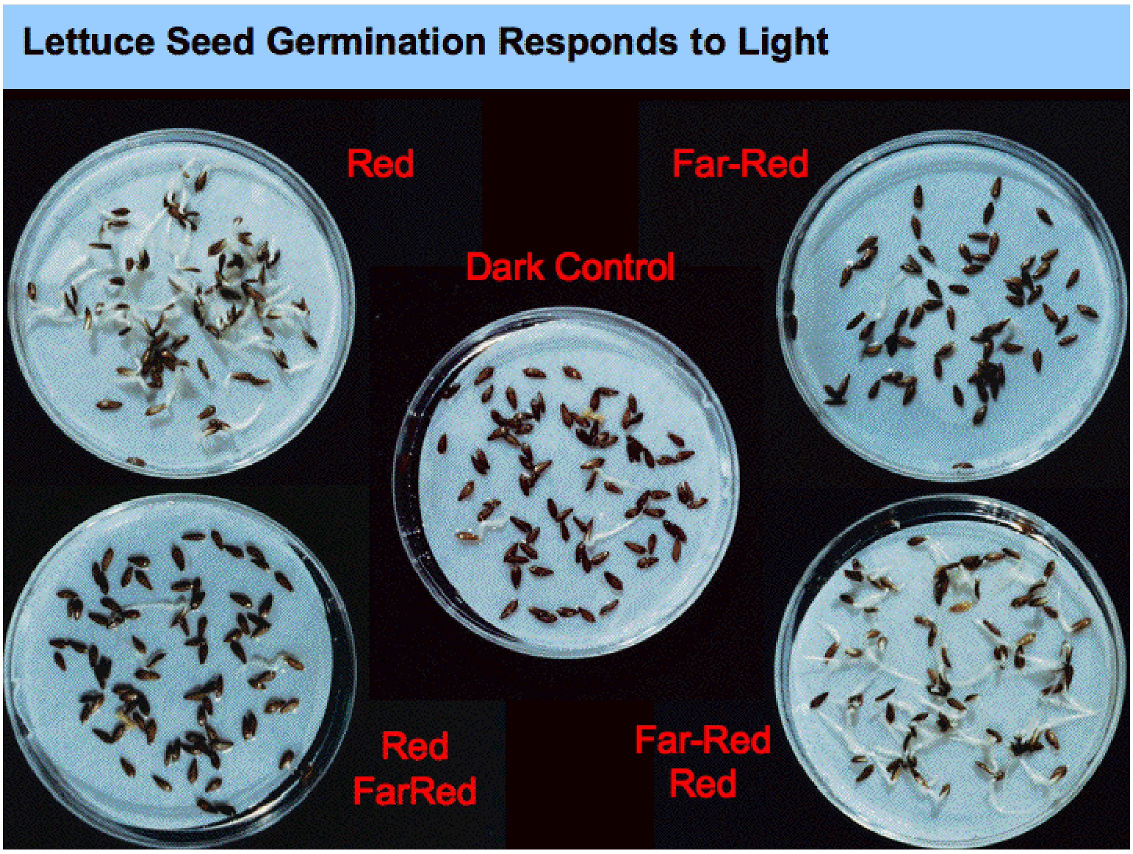
Phytochrome

Introducing Phytochrome

You have already learned much about pigments, especially chlorophyll. You know that a pigment absorbs light and is altered electronically at least for a instant. This alteration results in a change in other chemicals in the immediate environment to drive photosynthesis. Chlorophyll is not the only important pigment, you learned about antenna pigments too. Today we are examining a pigment that fundamentally alters plant behavior. It is phytochrome.

Just as in botanical history, you knew about phytochrome effects before we discussed the identity and functions of this critical pigment. Your seed germination laboratory exercise with lettuce demonstrates a classic phytochrome effect. The seeds germinate better in red light and fail in far-red light compared to control seeds in kept in darkness. Here is a figure to extend what you observed in lab...

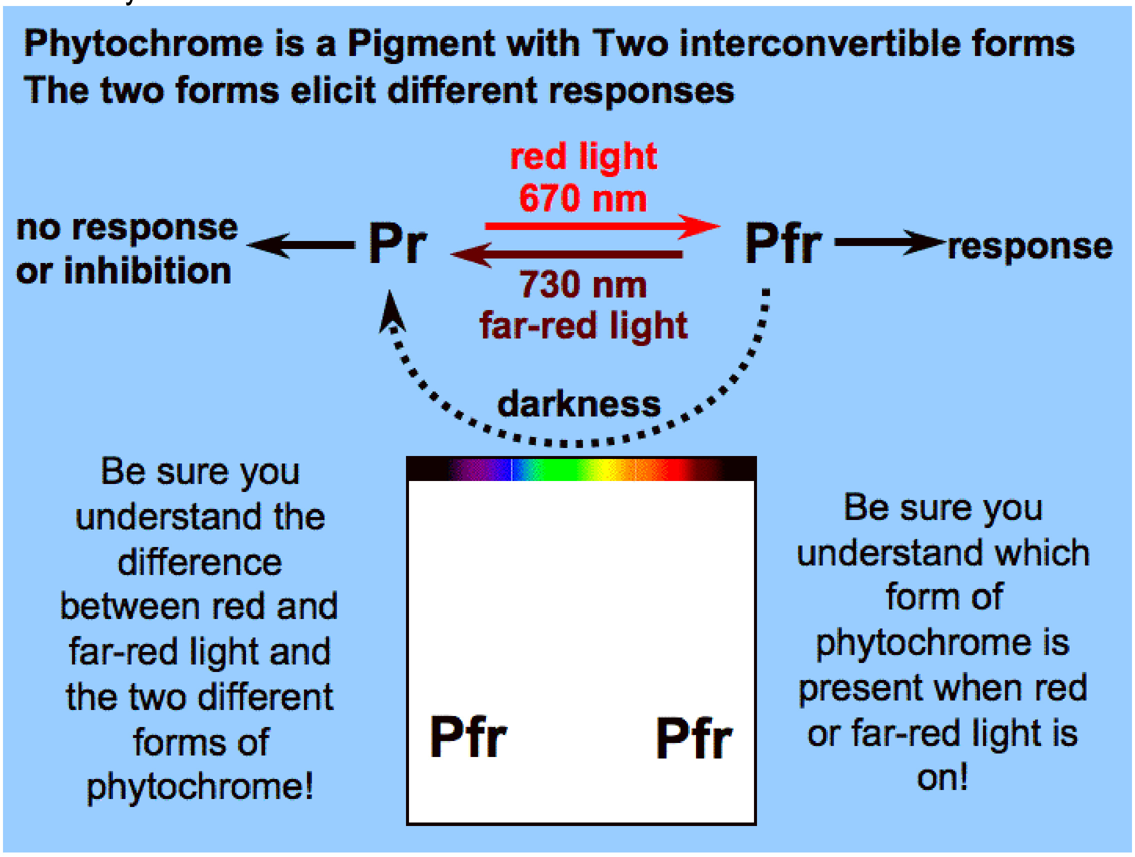


Lettuce seeds kept in the dark germinate at low frequency. Seeds kept in the dark but briefly exposed, after imbibing water, to red light results in considerable germination. Seeds kept in the dark but briefly exposed, after imbibing water, to far-red light results in virtually no germination. Seeds kept in the dark but briefly exposed, after imbibing water, to red light and then briefly exposed to far-red light results in virtually no germination. The FR exposure appears to reverse the R response. Seeds kept in the dark but briefly exposed, after imbibing water, to far-red light and then briefly exposed to red light results in considerable germination. The R exposure appears to reverse the FR response.

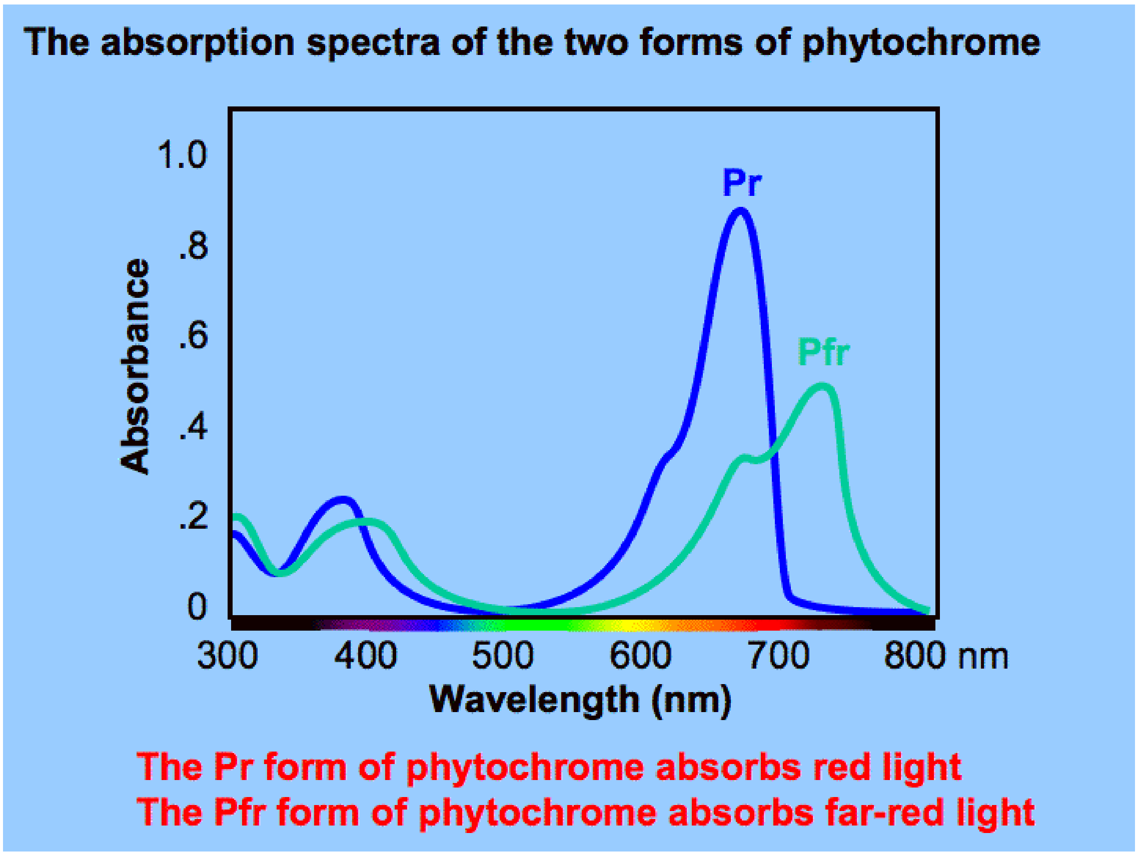
Lettuce seeds kept in the dark but exposed, after imbibing water, to any sequence of red and far-red light ending in FR, results in very low germination. Lettuce seeds kept in the dark but exposed, after imbibing water, to any sequence of red and far-red light ending in R, results in considerable germination.

Phytochrome exists in two interconvertible forms

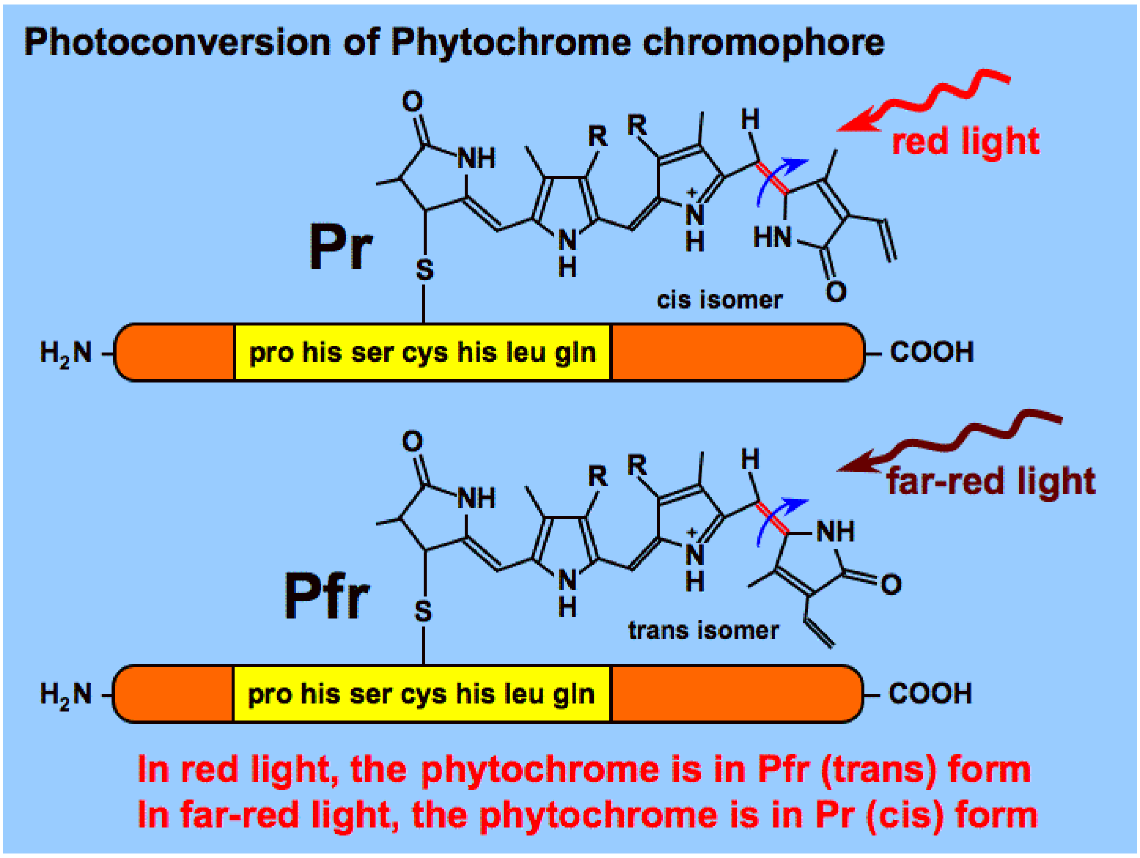
Unlike other pigments you have met so far, phytochrome has two different chemical structures that are inter-convertible. The forms are named by the color of light that they absorb maximally: Pr is a blue form that absorbs red light (660 nm) and Pfr is a blue-green form that absorbs far-red light (730 nm). What is strange about these pigments is that when they DO absorb these photons, they change chemically into the OTHER form. This is shown in the following diagram which you should commit to memory:



The two forms of phytochrome differ in their absorption spectra...



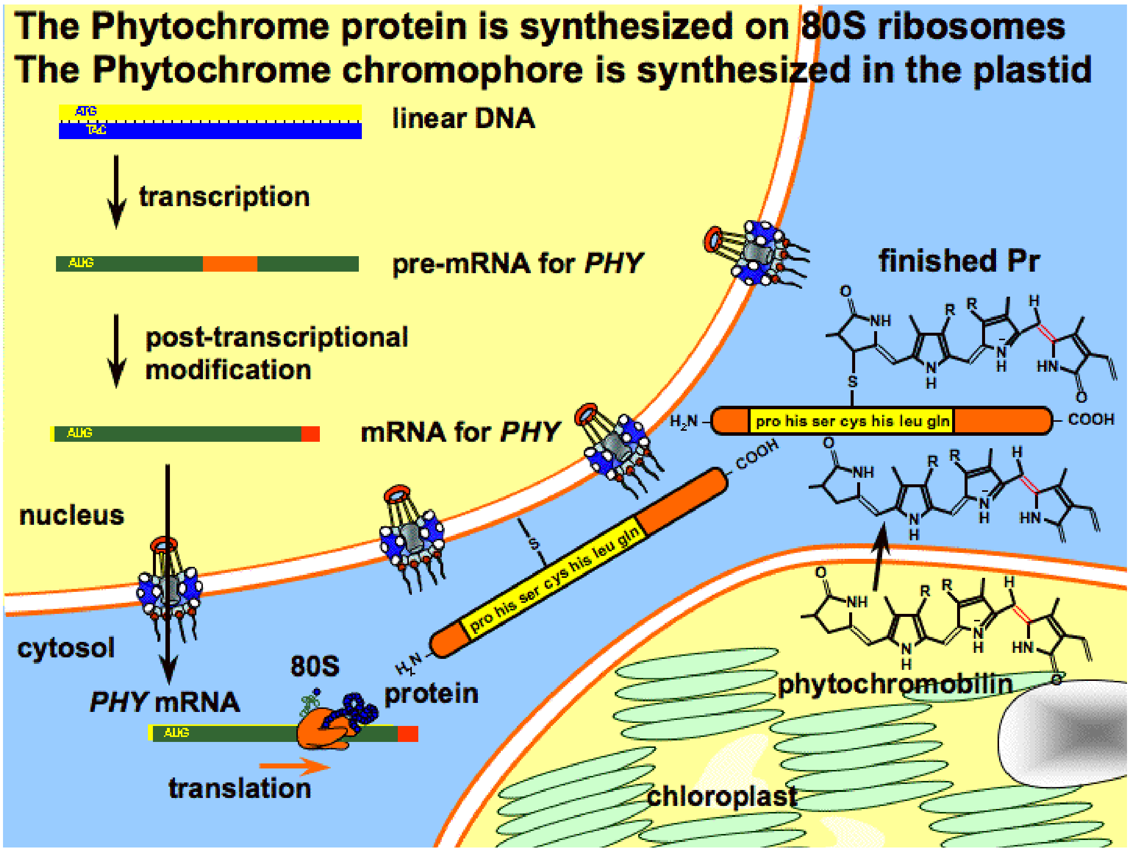
Obviously Pr absorbs red light (660 nm) very strongly, while Pfr absorbs far-red light (730 nm) very strongly. Both have some absorption in the blue-end of the spectrum. These differences in absorption have to do with differences in the chemical structures of these two forms.



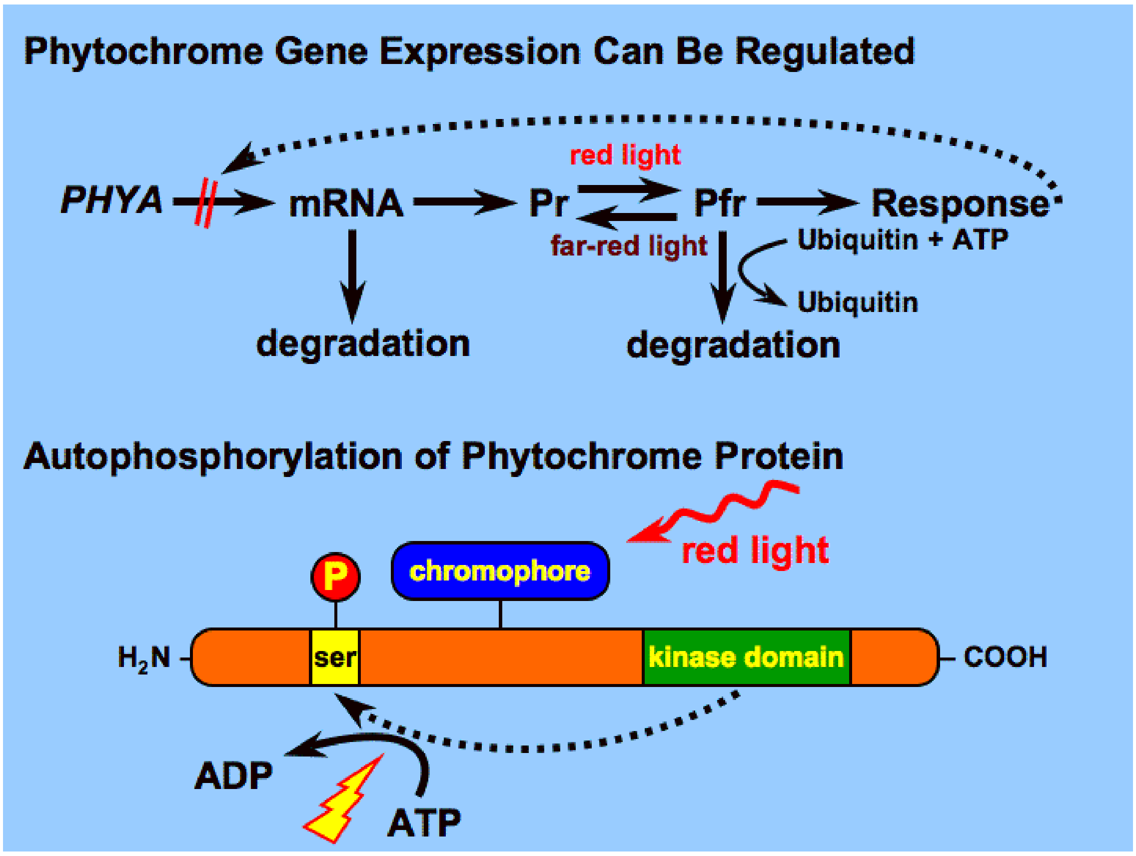
You can see the relationship between chlorophyll and phytochrome, both have evolved from a tetrapyrrole ring system also seen in the phycobilin pigments of bacteria. The chromophore is bound to a protein, just as in the case of chlorophyll. The protein has a mass of 165 kilodaltons. What is interesting is how the chemical structure of phytochrome is altered to its complementary form when struck by photons of the correct energy level (wavelength!).

Biosynthesis of phytochrome

Phytochrome is produced in different parts of the cell and assembled from those parts:



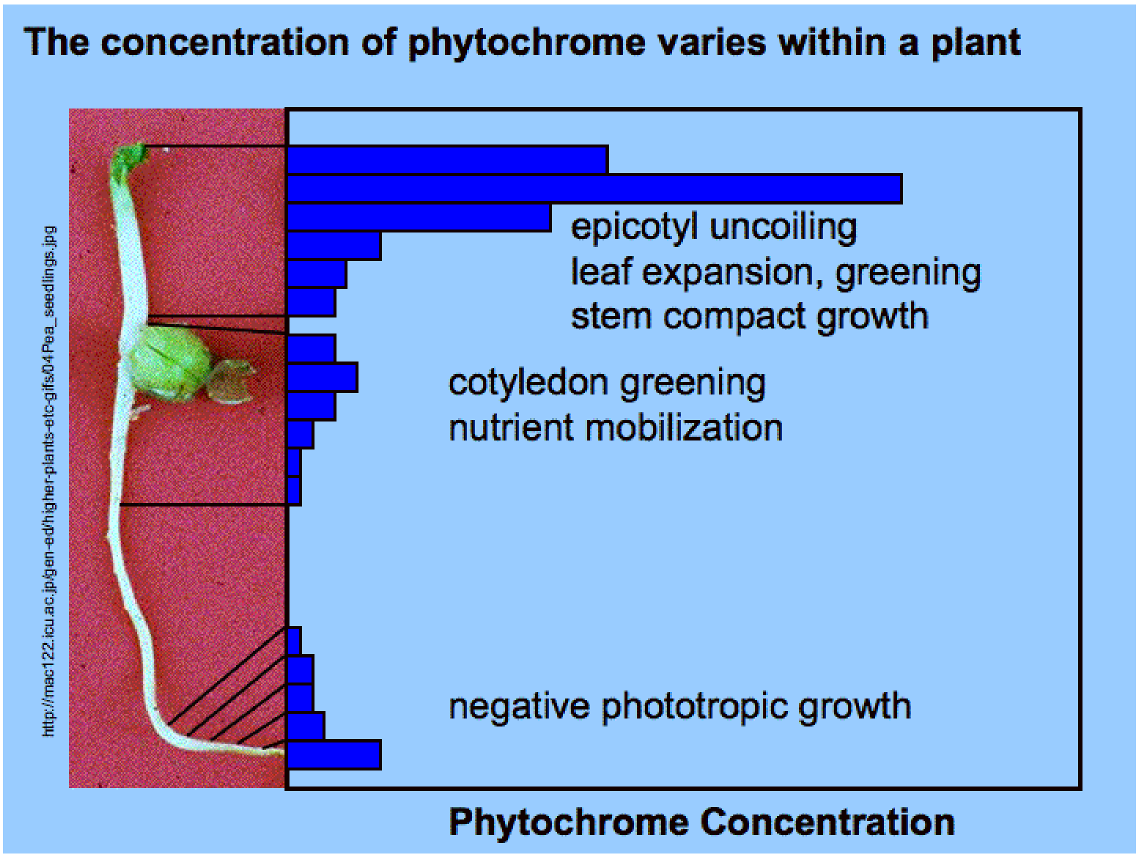
The phytochrome binding protein is coded in nuclear genes, transcribed in the nucleus and translated on cytosolic ribosomes. The phytochrome chromophore is produced in the plastid. These are assembled in the cytosol. However, phytochrome has been found to be associated with plastids in terms of final destination. The regulation of the "central dogma" for phytochrome is shown below. Obviously there are feedback mechanisms so that phytochrome levels are kept to essential and not excessive levels.



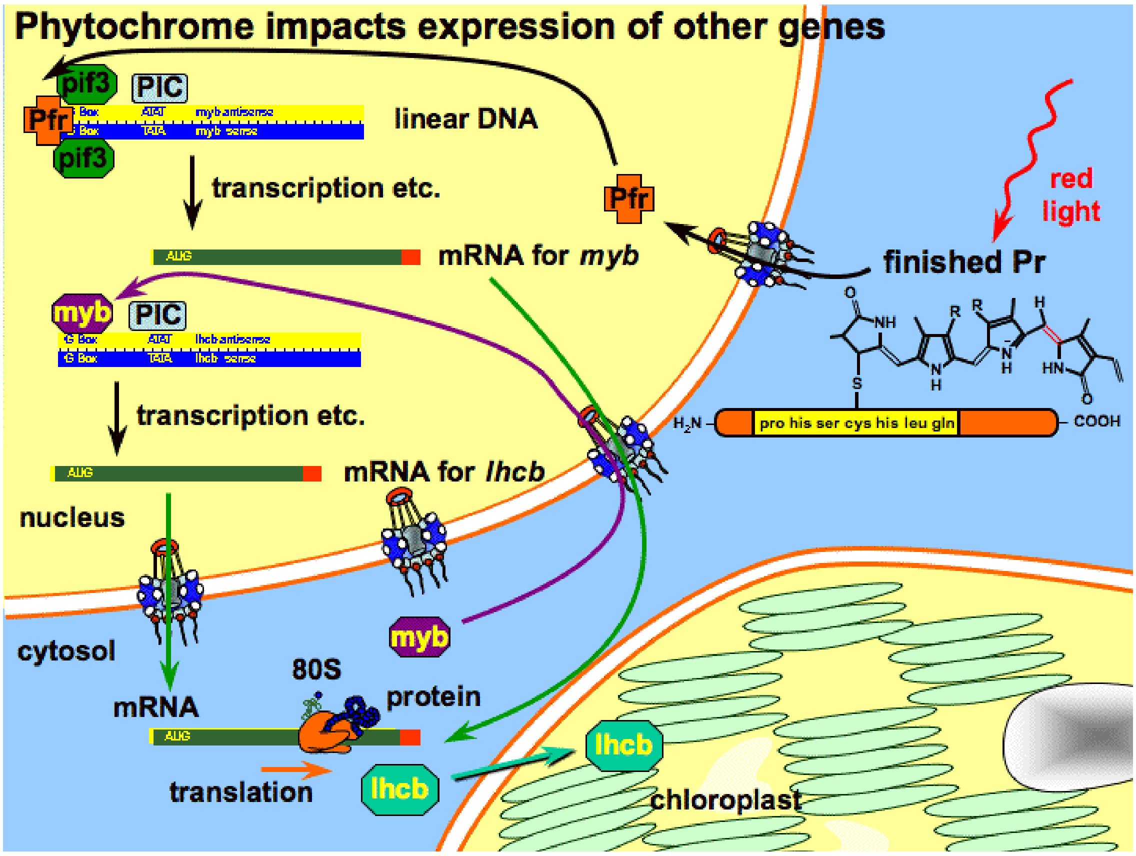
The phytochrome protein includes a kinase domain that, after exposure to red light (i.e. when the chromophore is in Pfr form), allows the protein to phosphorylate itself. This way the autophosphorylation of phytochrome protein activates it. It may not proceed to somewhere else in the cell to activate other proteins that need to be phosphorylated to become activated. This is the beginning of the response part of our phytochrome mediated physiology.

Phytochrome concentrations vary within the plant

The homeostatic regulation of amount of phytochrome can be observed by measuring its level throughout the plant. The famous example of pea seed germination is shown below.



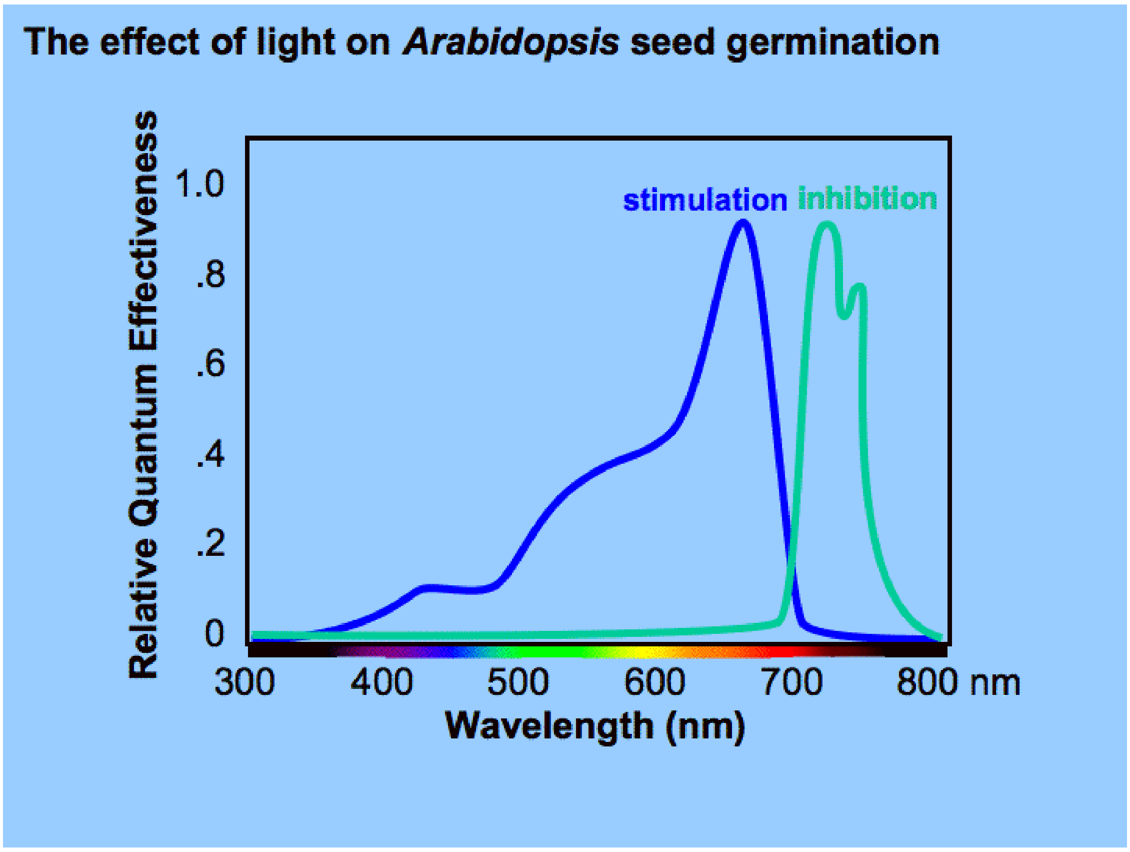
The plant maintains higher levels of phytochrome at its growing points where phytochrome plays important roles in growth responses to light. You will also notice the correlation with the zones of greening. Many of the genes for photosynthesis related proteins are regulated by phytochrome. The mechanism for this is shown below for the light harvesting complex b protein.



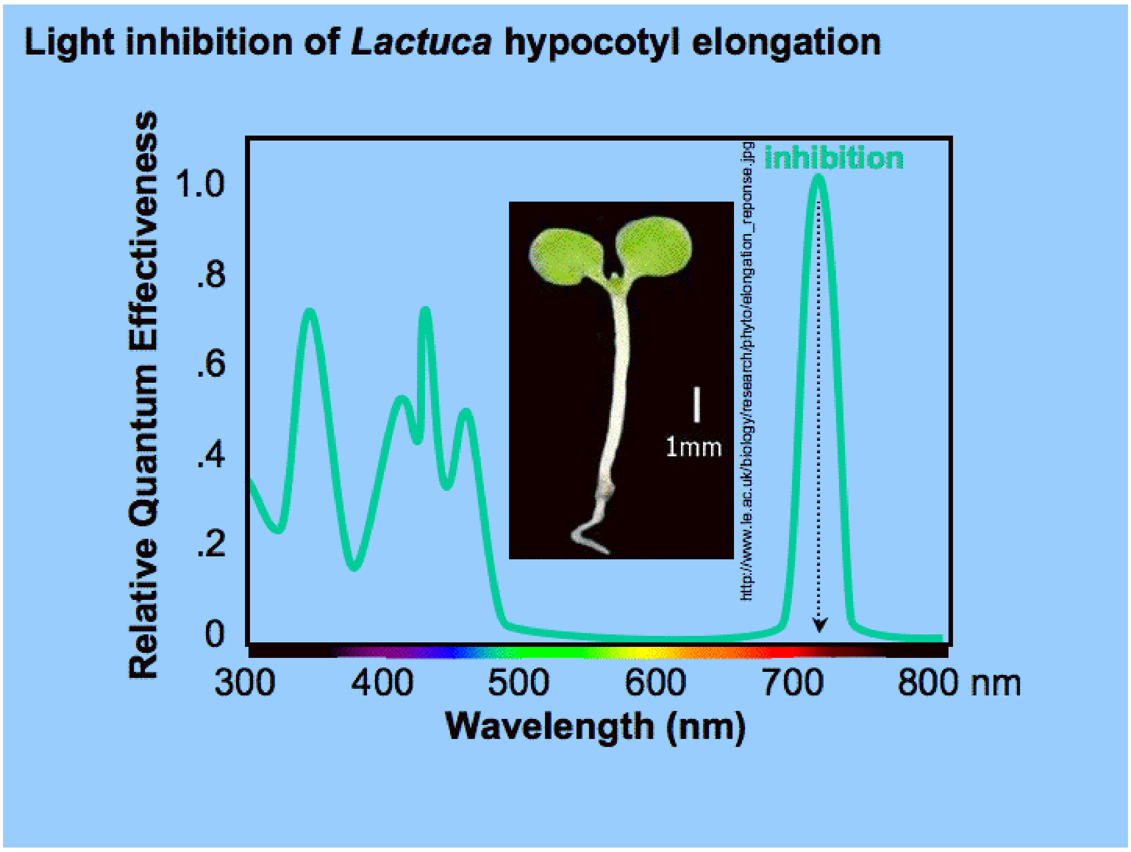
The active phytochrome moves into the nucleus, joins to the dimer of the PIF 3 transcription factors bound to the G-box promoter of the myb genes. The pre-initiation complex (PIC) binds to the TATA box and the myb genes are transcribed and translated. The myb proteins (CCA1 and LHY) are activated and, as transcription factors, bind to the promoter regions of light-stimulated genes...such as LHCB.

Some examples of phytochrome responses

There are several famous examples of phytochrome responses including seed germination in *Arabidopsis*. Is this plant responding in the very same way as lettuce?



After a seed germinates, the hypocotyl lifts the cotyledons above the soil in some species (epigeous). This growth is rapid until the plant penetrates the soil and is exposed to light. This rapid water-uptake growth of a seedling is called **etiolated** growth. The seedling has evolved to include a mechanism to ensure that it rapidly penetrates soil before it runs out of stored nutrients in the seeds. Once in the light, the growth of the hypocotyl is inhibited for strong stocky normal growth of the shoot system. From the diagram below, can you tell which color of light (R or FR) is inhibiting hypocotyl growth? So which form of phytochrome (Pr or Pfr) appears to be active in this case?



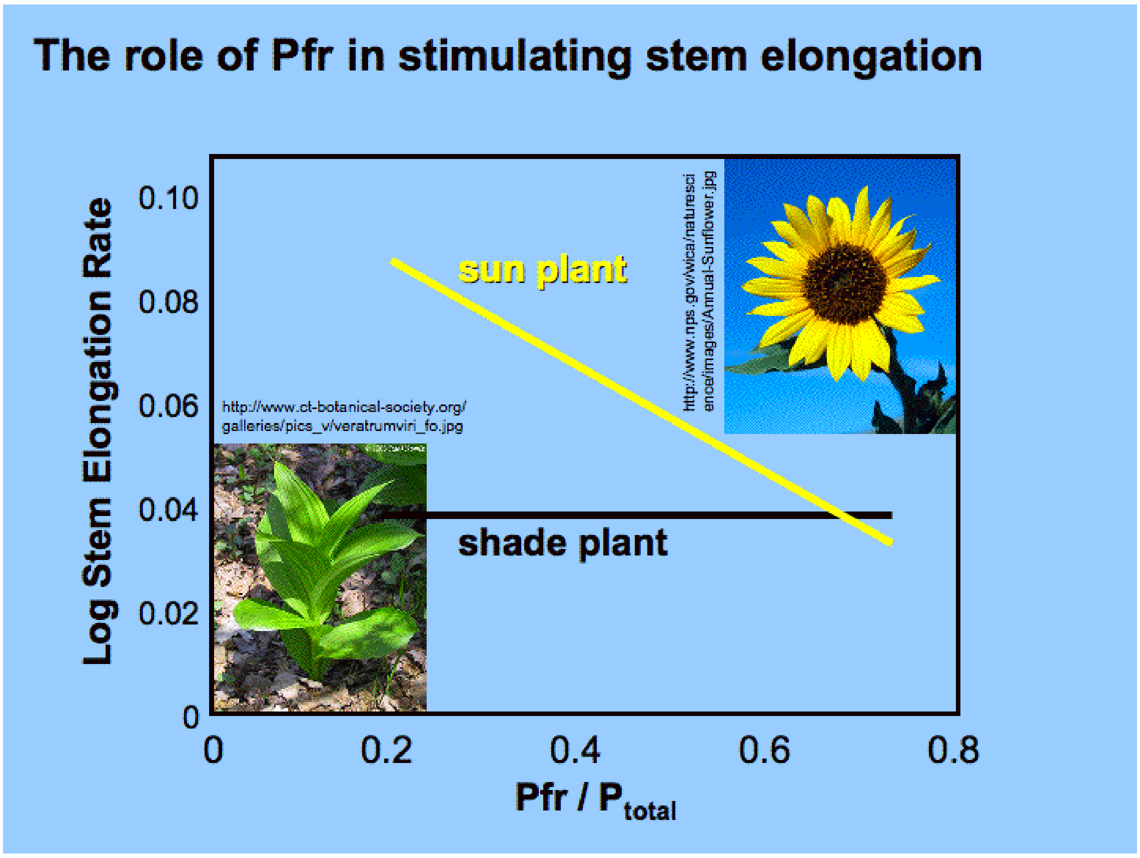
The growth rate of plants is dependent on their genotype and the environment, as you know from any introductory course:

Phenotype = Genotype + Environment

Phytochrome works this way too. Here are some environmental conditions:

	PFD µmol m^{-2} s^{-1}	R/FR ratio
Day	1900	1.19
Sunset	26.5	0.96
Under Canopy	17.7	0.13
5mm deep in Soil	8.6	0.88

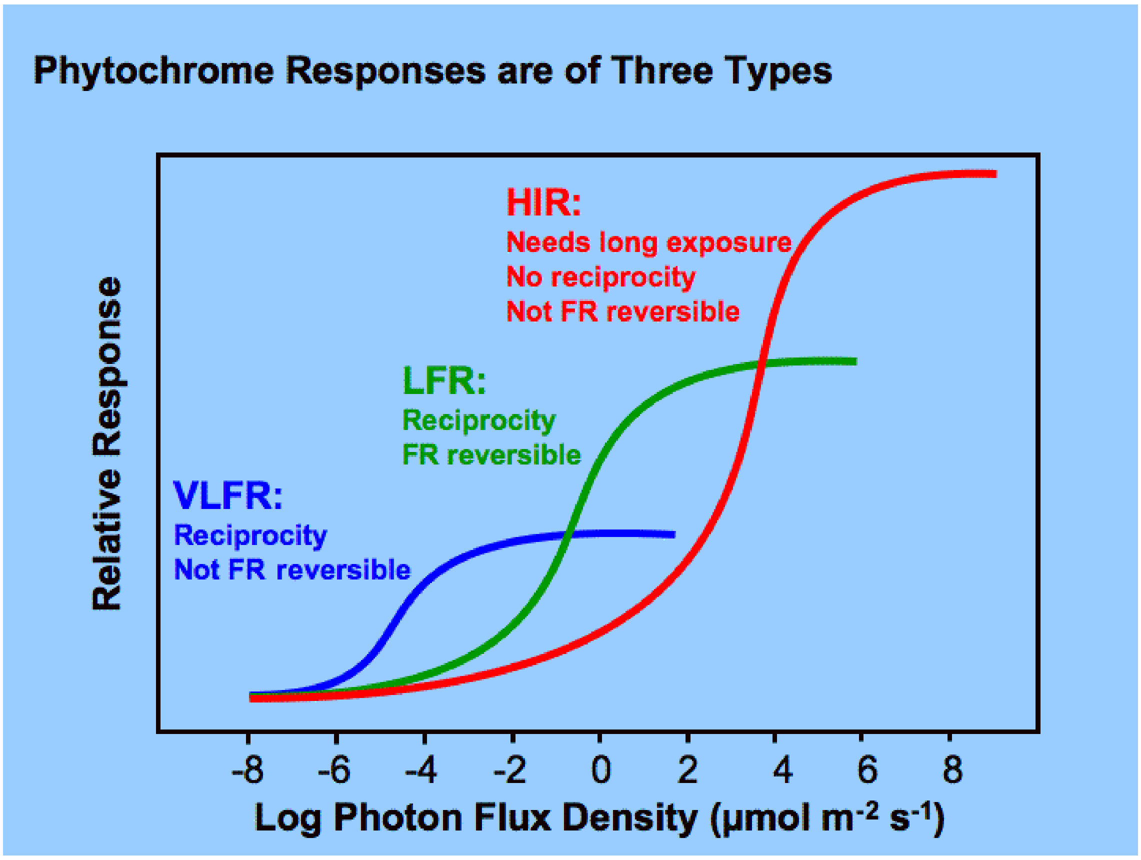
So now we look at the corresponding growth differences between responses of two genotypes of plants. Sun plants are defined here as those with higher light compensation points and requiring greater photon flux density to grow and reproduce. Shade plants are defined here as those with lower light compensation points and requiring lower photon flux density to grow and reproduce. We could think of these as canopy and forest floor species, respectively. Their different responses are shown in the graph below.



As you can see, the canopy species responds to the light available in shaded environments by growing rapidly, and remains compact while in full sun. The shade-tolerant plant does not respond to the different red/far-red light ratio.

Plants respond to light at different flux densities by phytochrome

Phytochrome initiates different responses by the plant at different photon flux densities in terms of the environmental signal perceived. These are classified as: **VLFR** (Very Low Fluence Responses), **LFR** (Low Fluence Responses), and **HIR** (High Irradiance Responses). Thus phytochrome can elicit correct behavior for the lighting conditions found in the plant's environment.



The three different kinds of phytochrome responses, based on photon flux density and wavelength, are summarized in the table below:

	reversible?	Active wavelengths	Protein Form
VLFR	NO	R	phyA
LFR	YES	R/FR	phyB
HIR	NO	Etiolated:FR, B Greened: R	phyA phyB

Hypocotyl elongation in lettuce observed above, is inhibited by the Pr form of phytochrome A under far-red light. In *Sinapis alba* this same inhibition of hypocotyl elongation is a response to the Pfr from of phytochrome B under red light. The difference between phytochroma A nd B has to do with which genes are used to make the phytochrome binding protein. The inhibition of hypocotyl elongation in*Sinapis alba* is an HIR response.

Light inhibition of *Sinapis alba* hypocotyl elongation (HIR)