

Plant Developmental Biology

Analysis of Photomorphogenesis

- Plants exhibit different growth habits in dark and light
- In the dark they have elongated stems, undifferentiated chloroplasts and unexpanded leaves. This is called skotomorphogenesis.
- Photomorphogenesis (light grown) involves the inhibition of stem elongation, the differentiation of chloroplasts and accumulation of chlorophyll, and the expansion of leaves.

Photomorphogenesis cont...

- Light has profound effects on the development of plants .
- Most striking effects of light are observed when a germinating seedling emerges from the soil and exposed to light for first time .
- Normally the seedling radicle (root) emerge first from the seed,

photo receptor reads the information contained in the light by selectively absorbing different wave length of light.

There are 4 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

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

Phytochromes are widely expressed across many tissues and developmental stages. [\[2\]](#)

Other plant photoreceptors include cryptochromes and phototropins, which respond to blue and ultraviolet-A light and UVR8, which is sensitive to ultraviolet-B light.

DISCOVERY OF PHYTOCHROME

The phytochrome pigment was discovered by **Sterling Hendricks** and **Harry Borthwick** at the **USDA-ARS Beltsville Agricultural Research Center** in **Maryland** during a period from the late 1940s to the early 1960s. Using a **spectrograph** built from borrowed and war-surplus parts, they discovered that red light was very effective for promoting germination or triggering flowering responses. The red light responses were reversible by far-red light, indicating the presence of a photoreversible pigment.

The phytochrome pigment was identified using a **spectrophotometer** in 1959 by biophysicist **Warren Butler** and biochemist **Harold Siegelman**.

Warren Butler coined the term phytochrome.



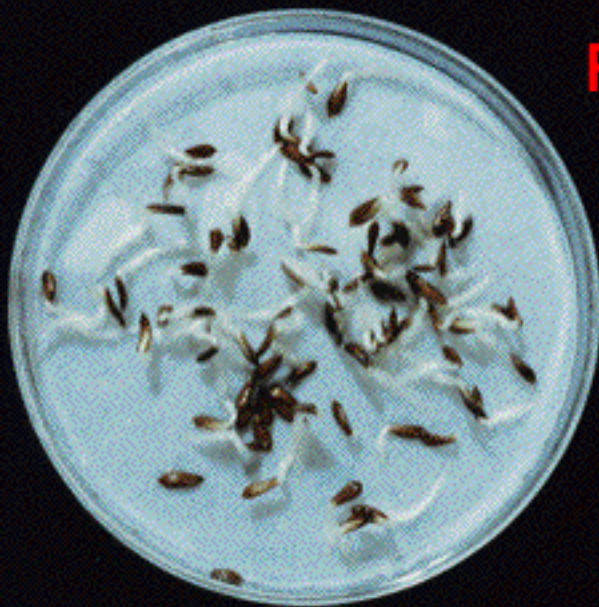
The famous lettuce seed germination experiment

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 reverses 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 reverses 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.

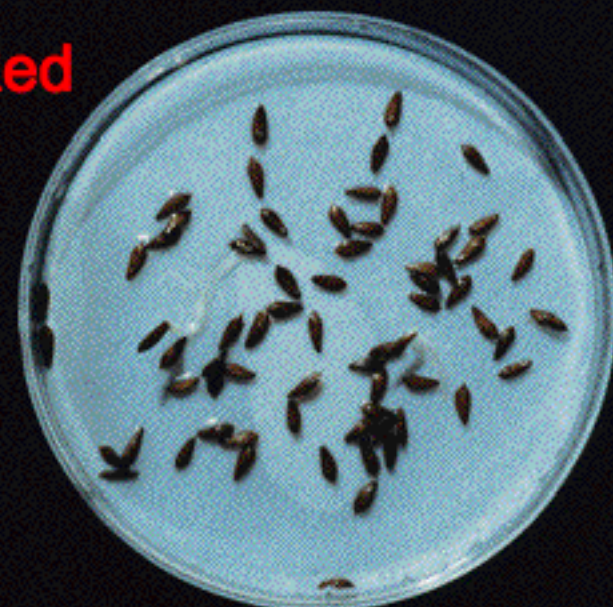
Lettuce seeds kept in the dark but exposed, after imbibing water, to any sequence of red and far-red light ending in R, results in germination.

Lettuce Seed Germination Responds to Light



Red

Far-Red

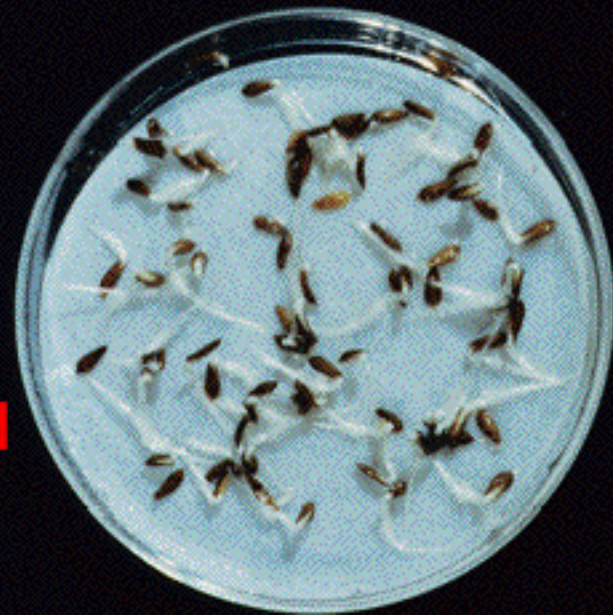


Dark Control



Red
FarRed

Far-Red
Red



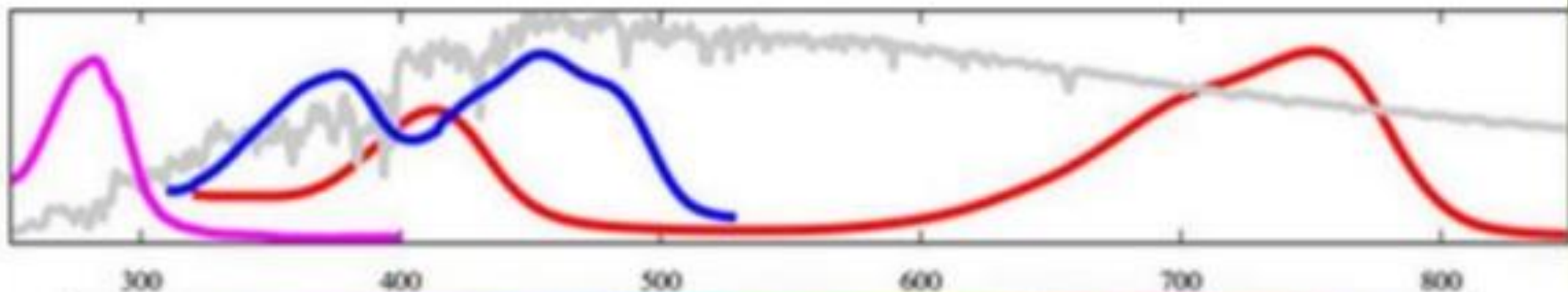
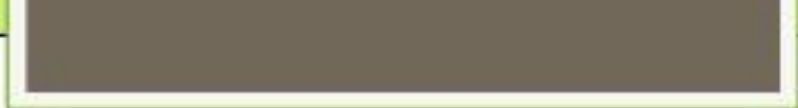
In 1983 the laboratories of Peter Quail and Clark Lagarias reported the chemical purification of the intact phytochrome molecule, and in 1985 the first phytochrome **gene sequence** was published by Howard Hershey and Peter Quail.

By 1989, molecular genetics and work with **monoclonal antibodies** that more than one type of phytochrome existed; for example, the **pea** plant was shown to have at least two phytochrome types (then called type I (found predominantly in dark-grown seedlings) and type II (predominant in green plants)). It is now known by **genome sequencing** that *Arabidopsis* has five phytochrome genes (PHYA, PHYB, PHYC, PHYD & PHYE).

Rice has only three (PHYA, PHYB, PHYC). While this probably represents the condition in several di- and monocotyledonous plants, many plants are **polyploid**. Hence **maize**, for example, has six phytochromes - phyA1, phyA2, phyB1, phyB2, phyC1 and phyC2.

While all these phytochromes have significantly different protein components, they all use phytochromobilin as their light-absorbing chromophore. Phytochrome A or phyA is rapidly degraded in the Pfr form - much more so than the other members of the family.

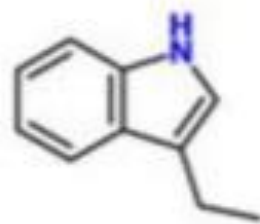
In the late 1980s, the Vierstra lab showed that phyA is degraded by the ubiquitin system, the first natural target of the system to be identified in eukaryotes.



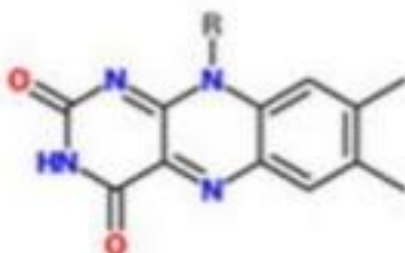
(λ) 280 315
UV-B
UVR8

400 500
Blue Green
CRYPTOCHROME, HAL3
Phototropin, ZEITLUPE

650 700(nm)
Red Far-red
PHYTOCHROME



Tryptophan

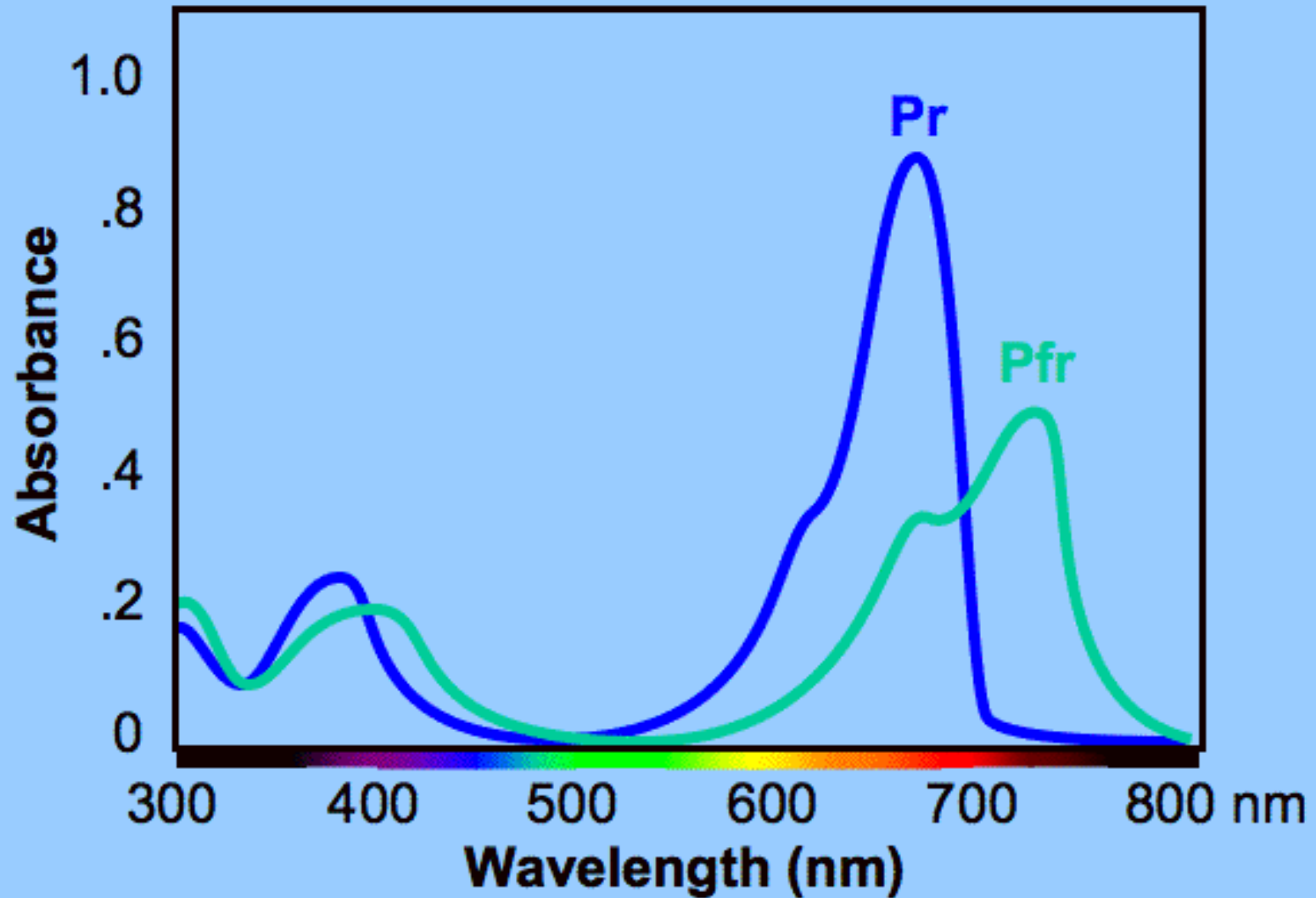


Flavin



Bilin

The absorption spectra of the two forms of phytochrome



The Pr form of phytochrome absorbs red light

The Pfr form of phytochrome absorbs far-red light

❖ The Pfr form:

- Absorbs at a peak of 730 nm.
- The Pfr form is the active form that initiates biological responses.
- When Pfr absorbs far red light, it is converted to the Pr form in the dark over time = dark reversion; Pfr is also susceptible to proteinases.

- Pfr absorbs some red light, so in red light, there is a balance of 85% Pfr and 15% Pr.
- Pr absorbs very little far red light, so in far red light, there is a balance of 97% Pr to 3% Pfr.

In 1996 laboratory of Arthur Grossman at the Carnegie Institution at Stanford University identified the proteins, in the filamentous cyanobacterium *Fremyella diplosiphon*. called RcaE with similarity to plant phytochrome that controlled a red-green photoreversible response called chromatic acclimation and identified a gene in the sequenced, published genome of the cyanobacterium *Synechocystis* with closer similarity to those of plant phytochrome. This was the first evidence of phytochromes outside the plant kingdom.

Jon Hughes in Berlin and Clark Lagarias at UC Davis subsequently showed that this *Synechocystis* gene indeed encoded a *bona fide* phytochrome (named Cph1) in the sense that it is a red/far-red reversible chromoprotein.

Most likely, plant phytochromes are derived from an ancestral cyanobacterial phytochrome, perhaps by gene migration from the chloroplast to the nucleus

Subsequently, phytochromes have been found in other **prokaryotes** including *Deinococcus radiodurans* and *Agrobacterium tumefaciens*. In *Deinococcus* phytochrome regulates the production of light-protective pigments, however in *Synechocystis* and *Agrobacterium* the biological function of these pigments is still unknown.

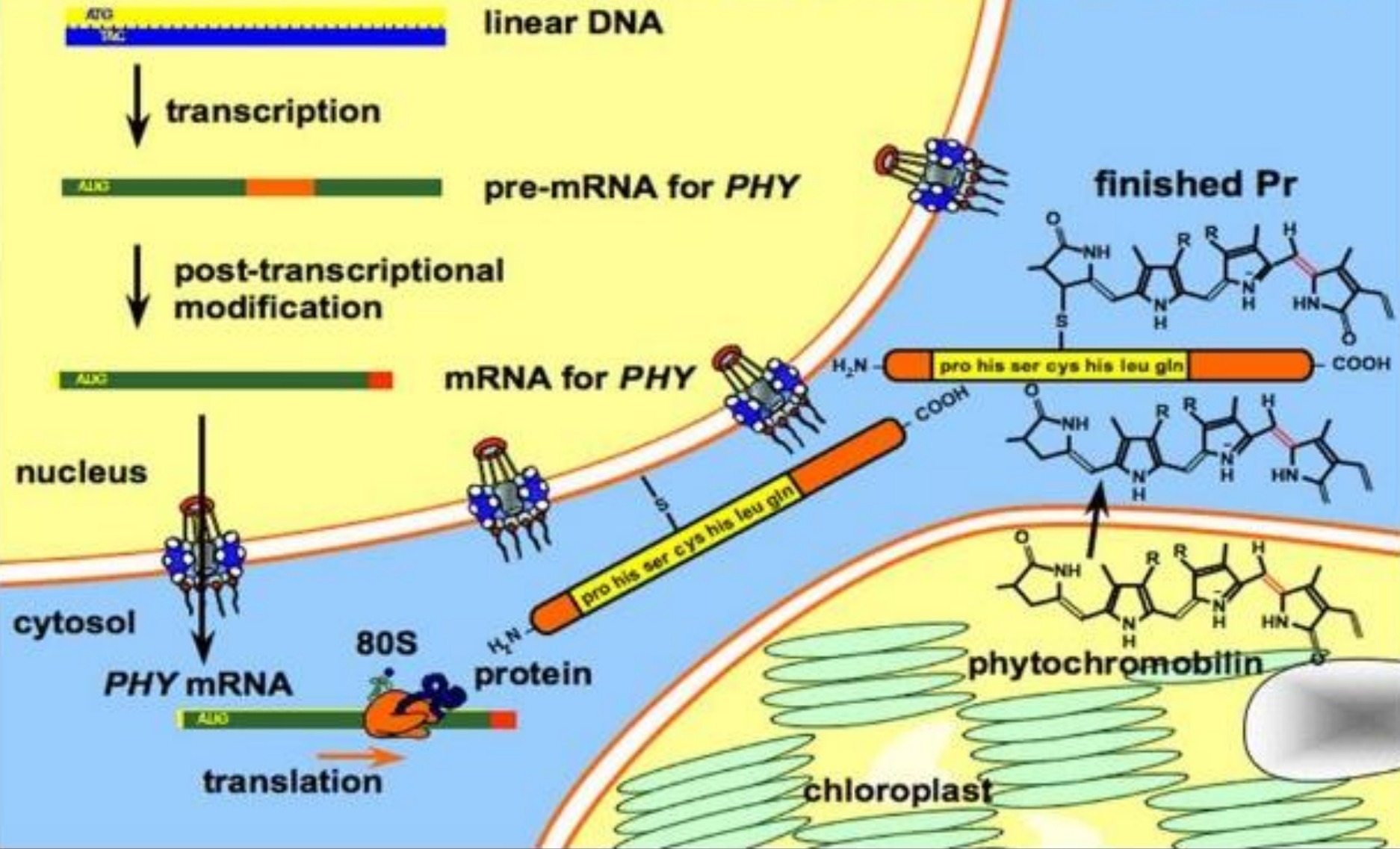
In 2005, the Vierstra and Forest labs at the **University of Wisconsin** published a three-dimensional structure of a truncated *Deinococcus* phytochrome (PAS/GAF domains). This paper revealed that the protein chain forms a knot - a highly unusual structure for a protein.

In 2008, two groups Essen and Hughes in Germany and Yang and Moffat in the US published the three-dimensional structures of the entire photosensory domain. One structure was for the *Synechocystis* sp. (strain PCC 6803) phytochrome in Pr and the other one for the *Pseudomonas aeruginosa* phytochrome in the P_{fr} state.

The phytochrome apo protein is coded in nuclear genes, transcribed in the nucleus and translated on cytosolic ribosomes.

The phytochrome chromophore “phytochromobilin” is produced in the plastid that migrates to cytoplasm where it is covalently bound to the cysteine residue of the phytochrome apoprotein by thioether linkage to form the phytochrome holoprotein.

The Phytochrome protein is synthesized on 80S ribosomes
The Phytochrome chromophore is synthesized in the plastid



PHYTOCHROME BIOSYNTHESIS

- Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein.

Phytochromobilin is synthesized inside plastids and is derived from 5-aminolevulinic acid via a pathway that branches from the chlorophyll biosynthetic pathway

- Because the chromophore absorbs the light, conformational changes in the protein are initiated by changes in the chromophore.

Upon absorption of light, the Pr chromophore undergoes a cis–trans isomerization of the double bond between carbons 15 and 16 and rotation of the C14–C15 single bond.

Polar transport require energy and is gravity independent

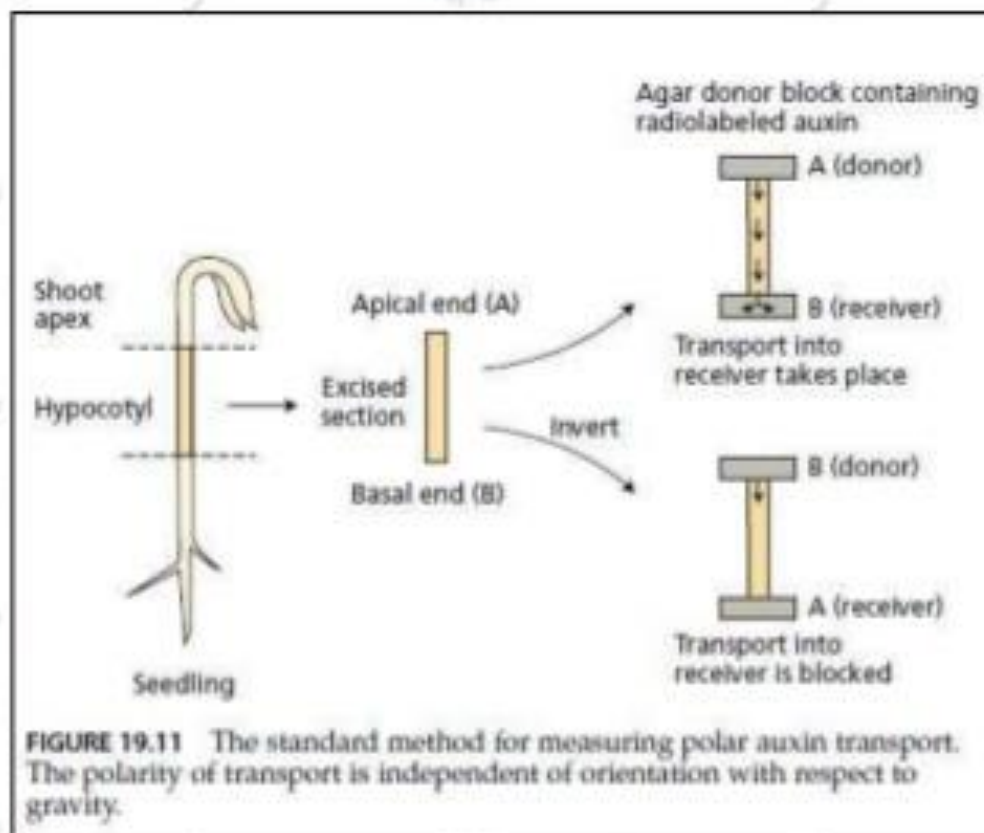




FIGURE 19.12 Roots grow from the basal ends of these bamboo sections, even when they are inverted. The roots form at the basal end because polar auxin transport in the shoot is independent of gravity. (Photo ©M. B. Wilkins.)