PHYTOCHROME (PC)

Skotomorphogenesis/photomorphogenesis

A

I Photochemical and Biochemical properties

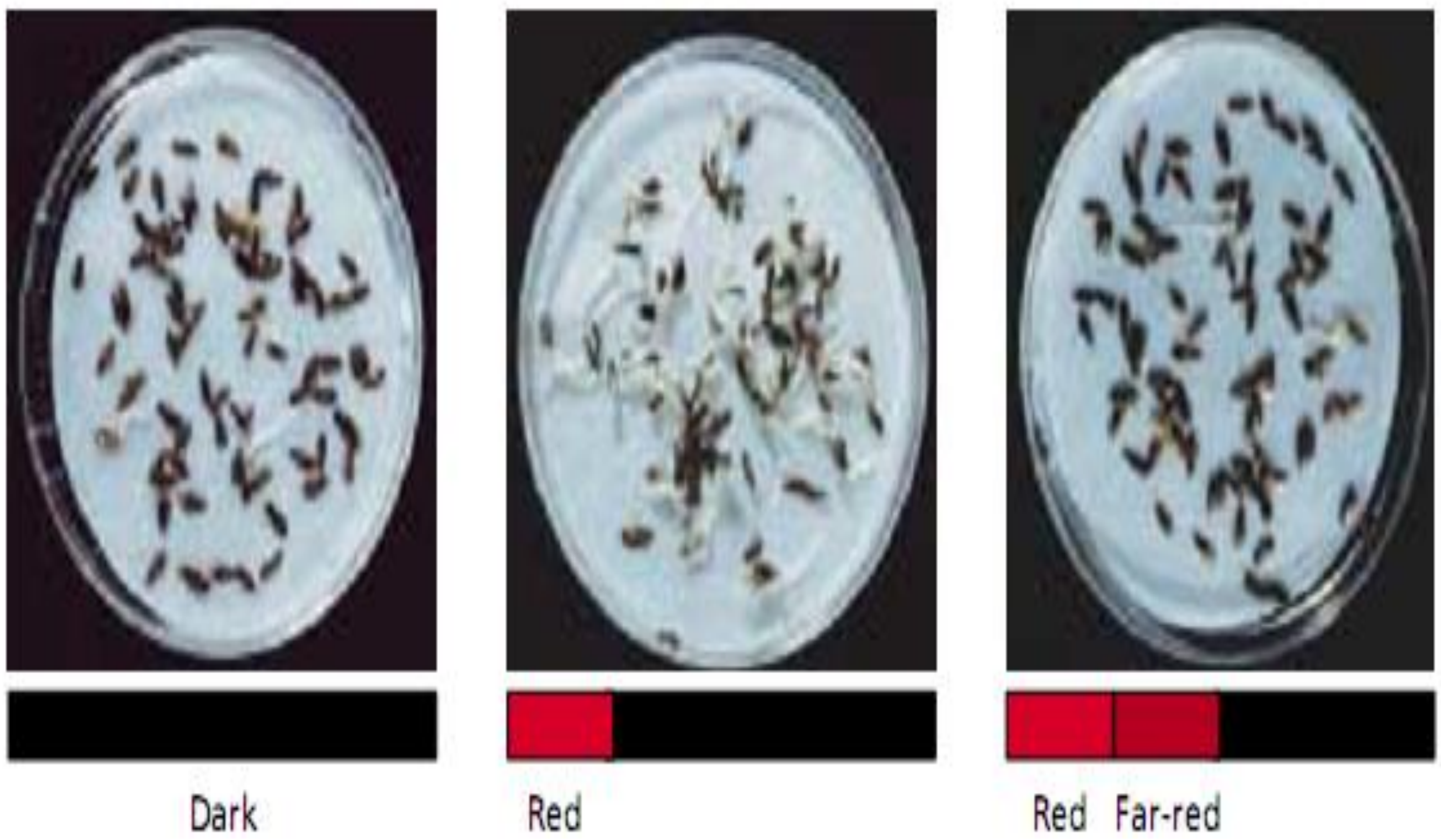
PC – blue protein pigment – 125 kDA - 1959

1930 – red light induced – seed germination

Lewis Flint – USDA – lettuce seed germination stimulated by R and inhibited by FR

Borthwick - 1952 - single pigment existing in 2 interconvertible forms (R & FR absorbing forms)

Lettuce seed germination is a typical photoreversible response controlled by phytochrome. Red light promotes lettuce seed germination, but this effect is reversed by far-red light.

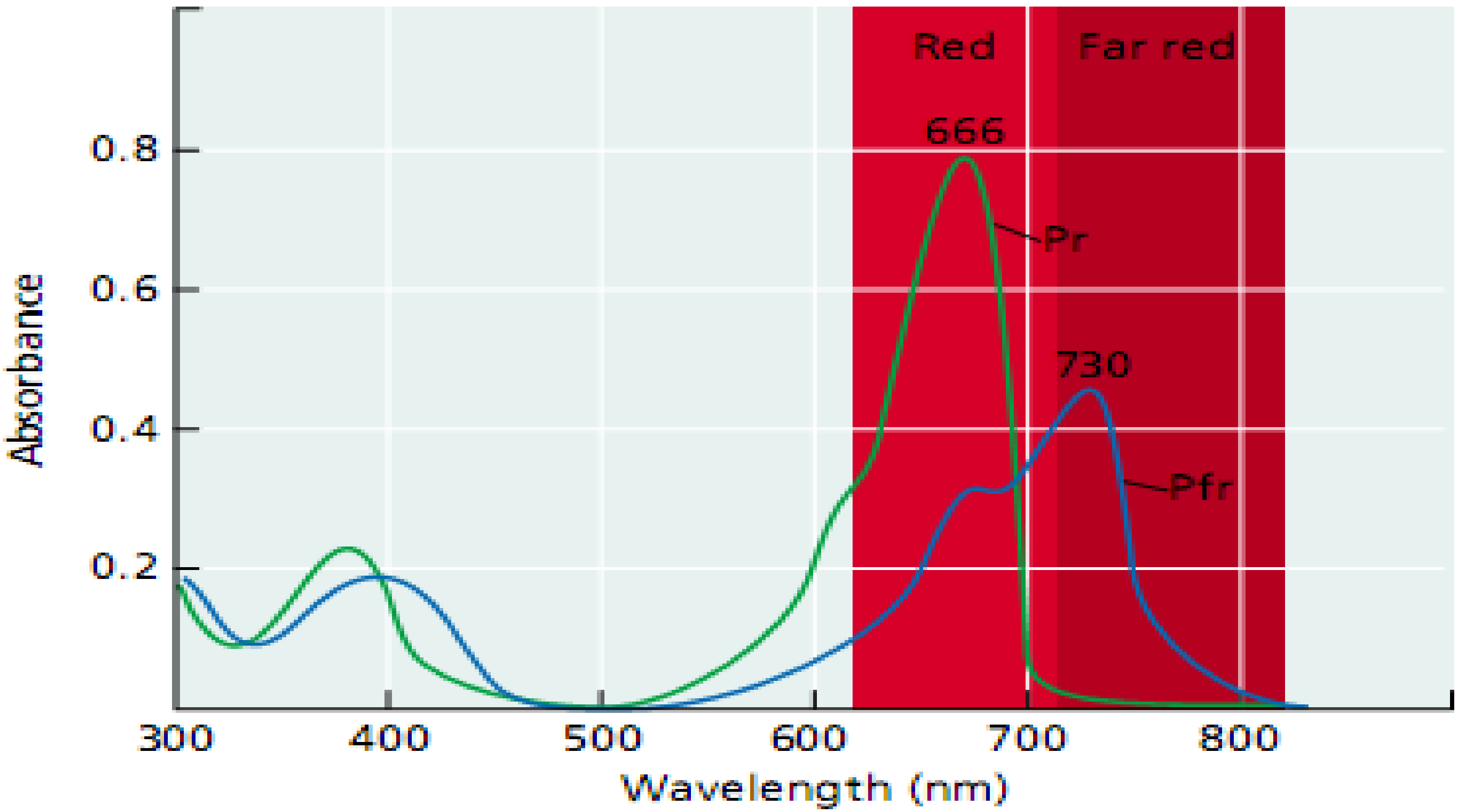


II Phytochrome interconverts between Pr & Pfr forms

$$\underset{\text{(Blue)}}{Pr} \underset{\text{Far red}}{\overset{\text{red}}{\rightleftharpoons}} \underset{\text{(Green)}}{Pfr}$$

A dynamic equilibrium exist between the 2 states, called photostationery state

Short lived PC intermediates: - whether intermediates play role in initiating or amplifying PC responses under sunlight which contains mixture of all visible wave lengths?



Red
Far red
666
Pr
730
Pfr
0.8
0.6
0.4
0.2
Absorbance
300
400
500
600
700
800
Wavelength (nm)

III Pfr is the physiologically active form

Ratio between Pfr and Pr, determines the magnitude of response

Arabidopsis mutants

IV PC is adimer composed of 2 polypeptides

- William Siegelman - USDA Beltsiville, Maryland - young etiolated seedlings of cereals rich source of PC
- 0.2% of total extractable protein
- precautions to be taken while purifying
- Native PC – soluble protein - 250 kDA

- A dimmer - 2 subunits → chromophore (light absorbing part) / → Apoprotein (polypeptide chain) } haloproetin

V Chromophore /phytochromobilin is synthesized in plastids

- light absorbed when polypeptide is linked with phytochromobilin to form haloprotein

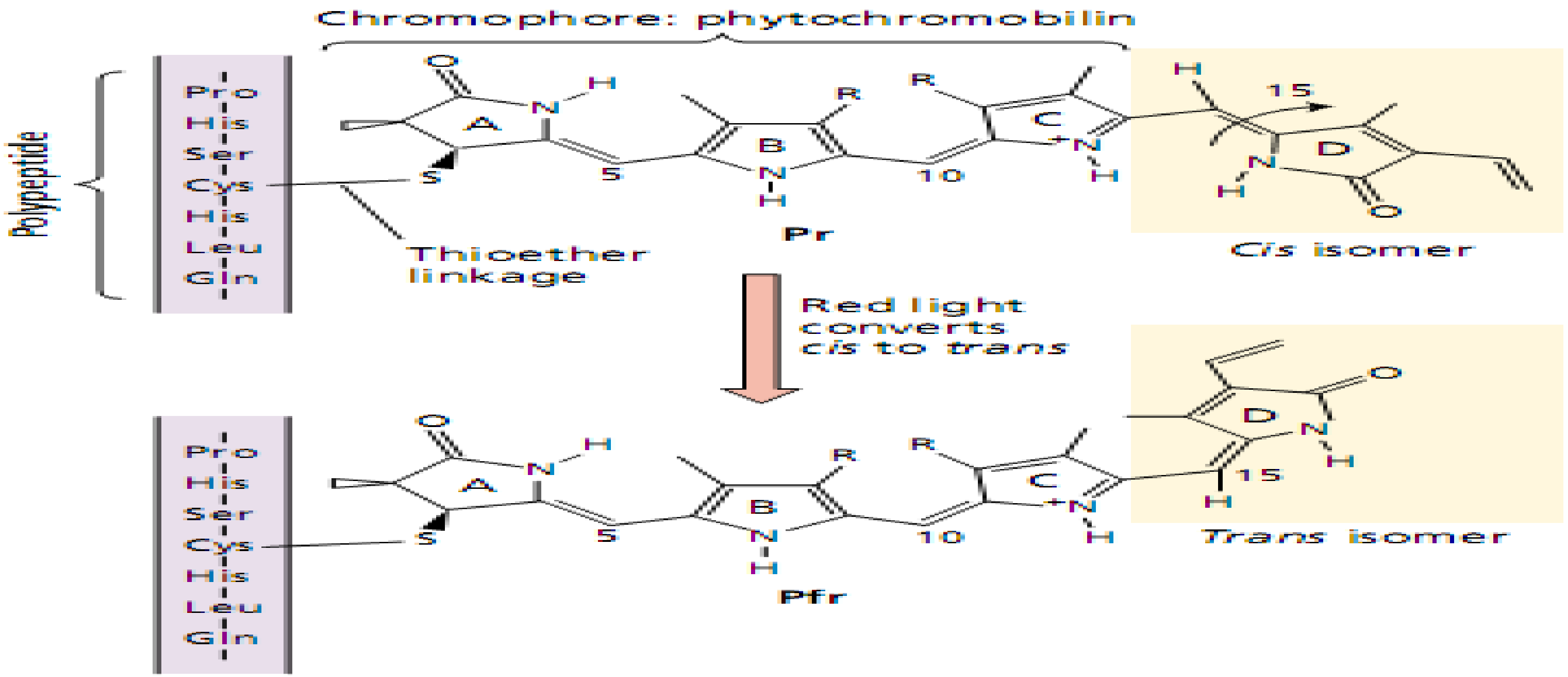
5 aminolevulinic acid → Chl

5 aminolevulinic acid → PC

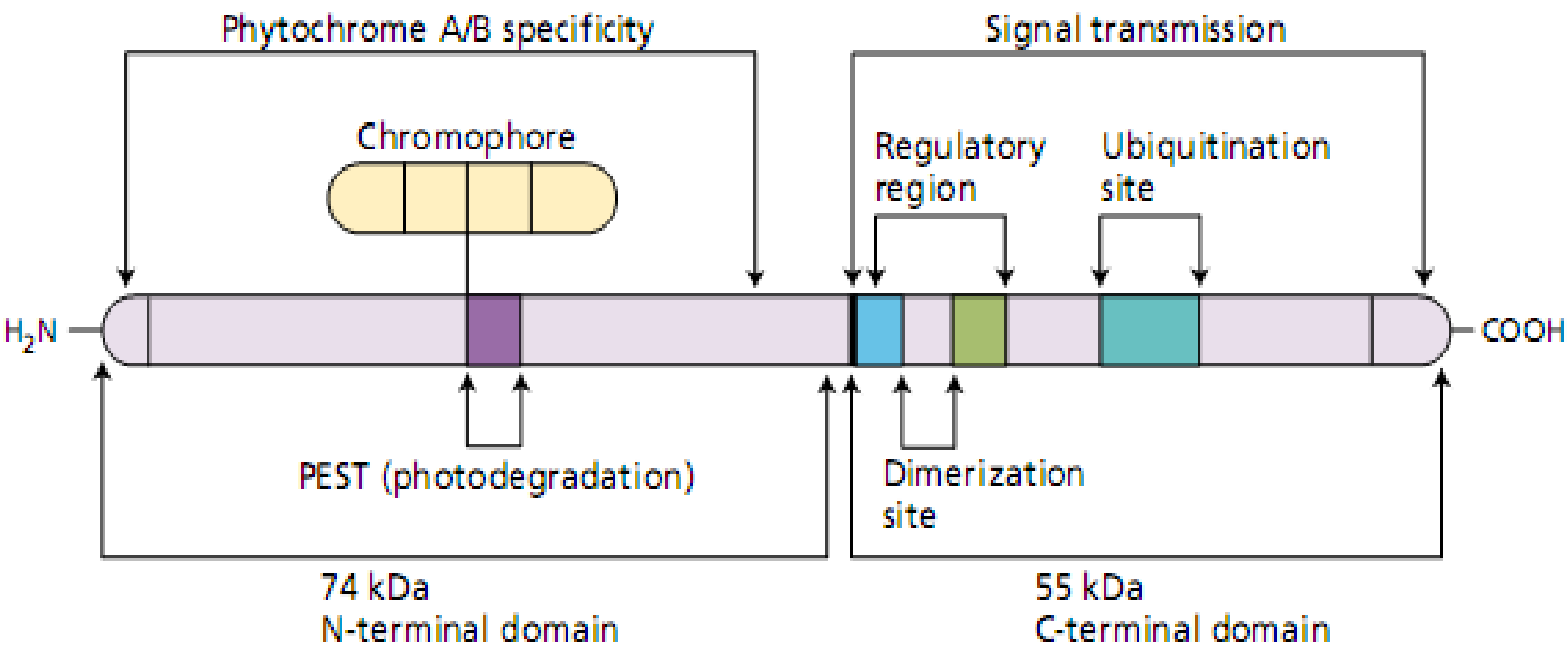
- Assembly of apoprotein with its chromophore is autocatalytic
- Pc apoprotein is synthesized by expressing in saccharomyces (yeast) cells

VI Chromophore and protein undergo conformational changes

- Upon absorption of light, the Pr chromphore undergoes cis-trans isomerization by rotation around double bonds between C-15 & C- 16.
- Apoprotein also undergoes change - in far red from the difference in their susceptilities to proteases and in phosphorylation of exogenous protein kinases
- Pr & Pfr can be distinguished immunologically



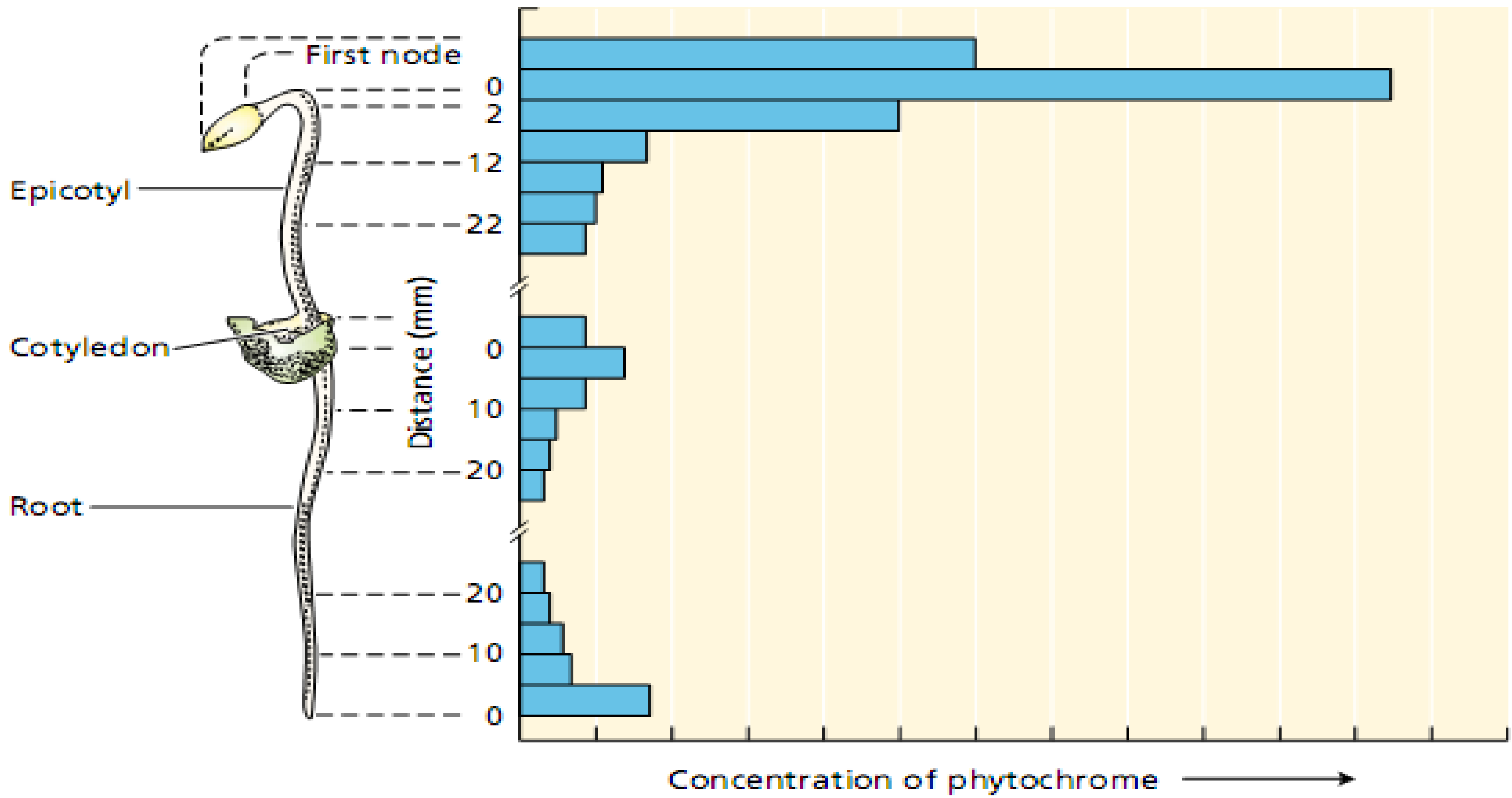
Phytochrome holoprotein, showing the various functional domains: The chromophore-binding site and PEST sequence are located in the N-terminal domain, which confers photosensory specificity to the molecule that is, whether it responds to continuous red or far-red light.
The C-terminal domain contains a dimerization site, a ubiquitination site, and a regulatory region. The C-terminal domain transmits signals to proteins that act downstream of phytochrome.



VIII Two types of PC identified
Type I & II
Type I is 9 times more abundant than Type II in dark grown seedlings, in light grown pea seedlings the amounts of 2 types are equal. The two types are distint proteins

Localization of PC in tissues and cells

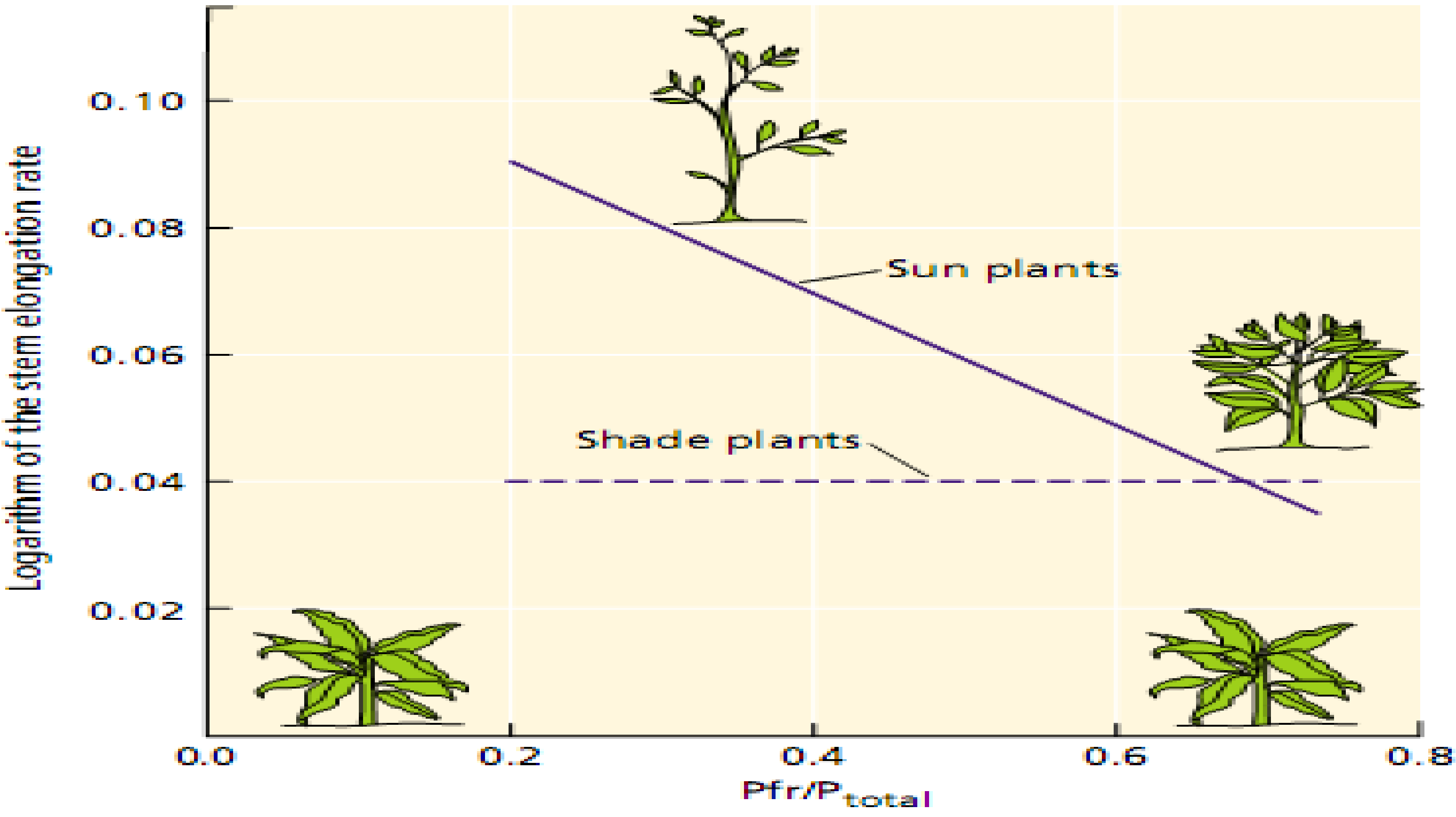
1) PC can be detected in tissues spectrophotometrically – high PC in meristematic regions or regions which were recently meristematic
2) Through antibodies – showed subcellular distribution – in cytosol and in association with organelles (plastids & nuclei)
   - Pr is diffused throughout cytosol, Pfr is mainly sequestered in discrete regions
     - sequestered Pfr is associated with discrete structures (globular to oval shape)
3) Through promoter genes - of PHY genes attached to reported genes of GUS



First node
Epicotyl
Cotyledon
Root
0
2
12
22
0
10
20
20
10
0
Distance (mm)
Concentration of phytochrome

ECOLOGICAL FUNCTIONS: SHADE AVOIDANCE

- The R:FR ratio and shading. 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.
- 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.
- When simulated natural radiation was used to vary the far-red content, it was found that for so-called sun plants, the higher the far-red content (i.e., the lower the Pfr:Ptotal ratio),the higher the rate of stem extension. Simulated canopy shading (high levels of far-red light) induced these plants to allocate more of their resources to growing taller.
- This correlation did not hold for shade plants, which normally grow in a shaded environment. Shade plants showed little or no reduction in their stem extension rate as they were exposed to higher R/FR values.

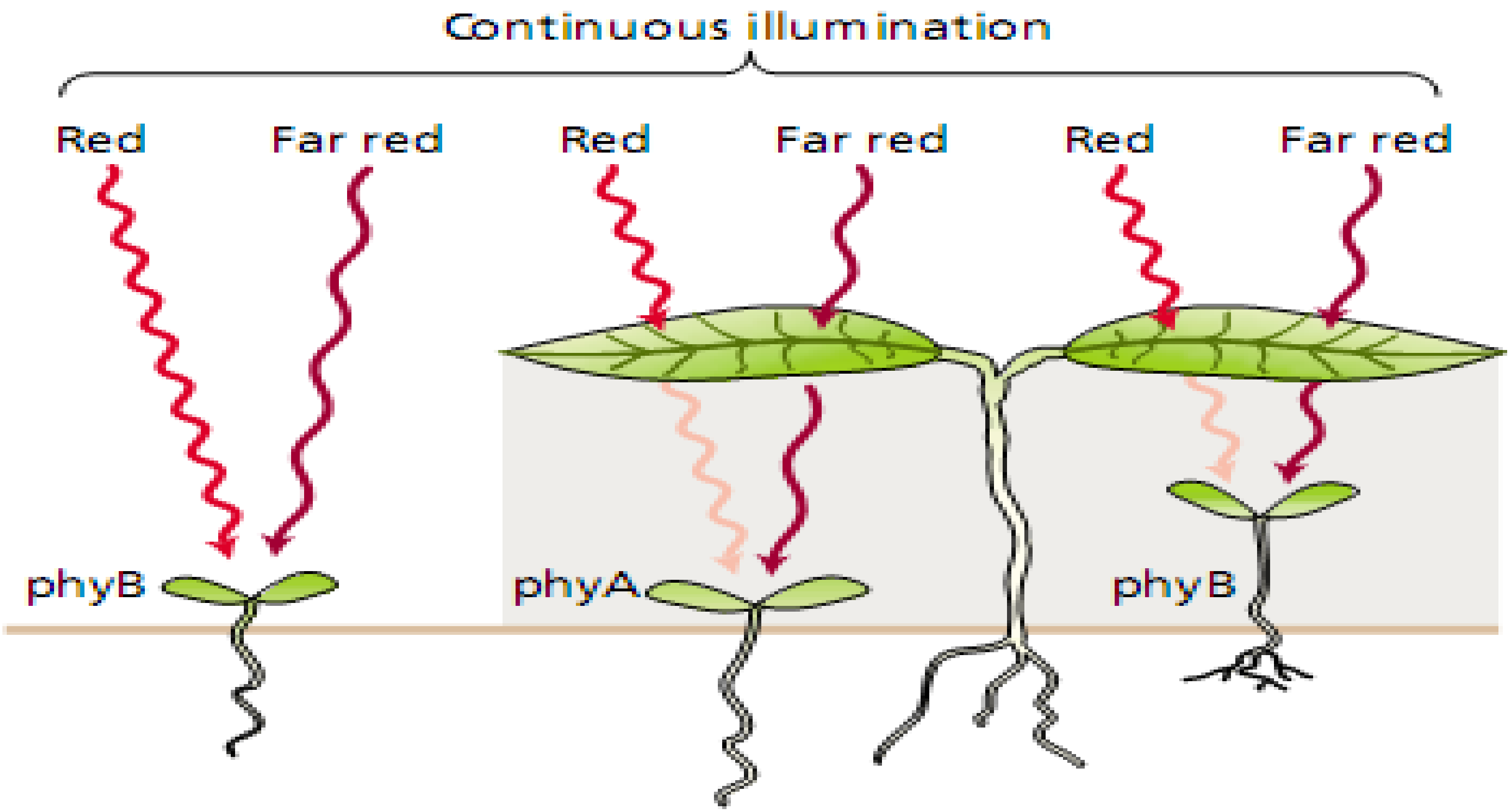


Logarithm of the stem elongation rate
0.10
0.08
0.06
0.04
0.02
0.0
0.2
0.4
0.6
0.8
Sun plants
Shade plants
Pfr/Ptotal

ECOLOGICAL FUNCTIONS: 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.
- phyA:Type I
- phyB,C,D,E: Type 2
- phyA & phyB have mutually antagonistic roles

(B)


Continuous illumination
Red
Far red
Red
Far red
Red
Far red
phyB
phyA
phyB

Developmental Roles for Phytochromes C, D, and E Are Also Emerging

- like phyB, phyD plays a role in regulating leaf petiole elongation, as well as in flowering time .
- Similar analyses support the idea that phyE acts redundantly with phyB and phyD in these processes, but also acts redundantly with phyA and phyB in inhibition of internode elongation.
- phyC, phyD, and phyE appear to play roles that are for the most part redundant with those of phyA and phyB.

ECOLOGICAL FUNCTIONS: CIRCADIAN RHYTHMS

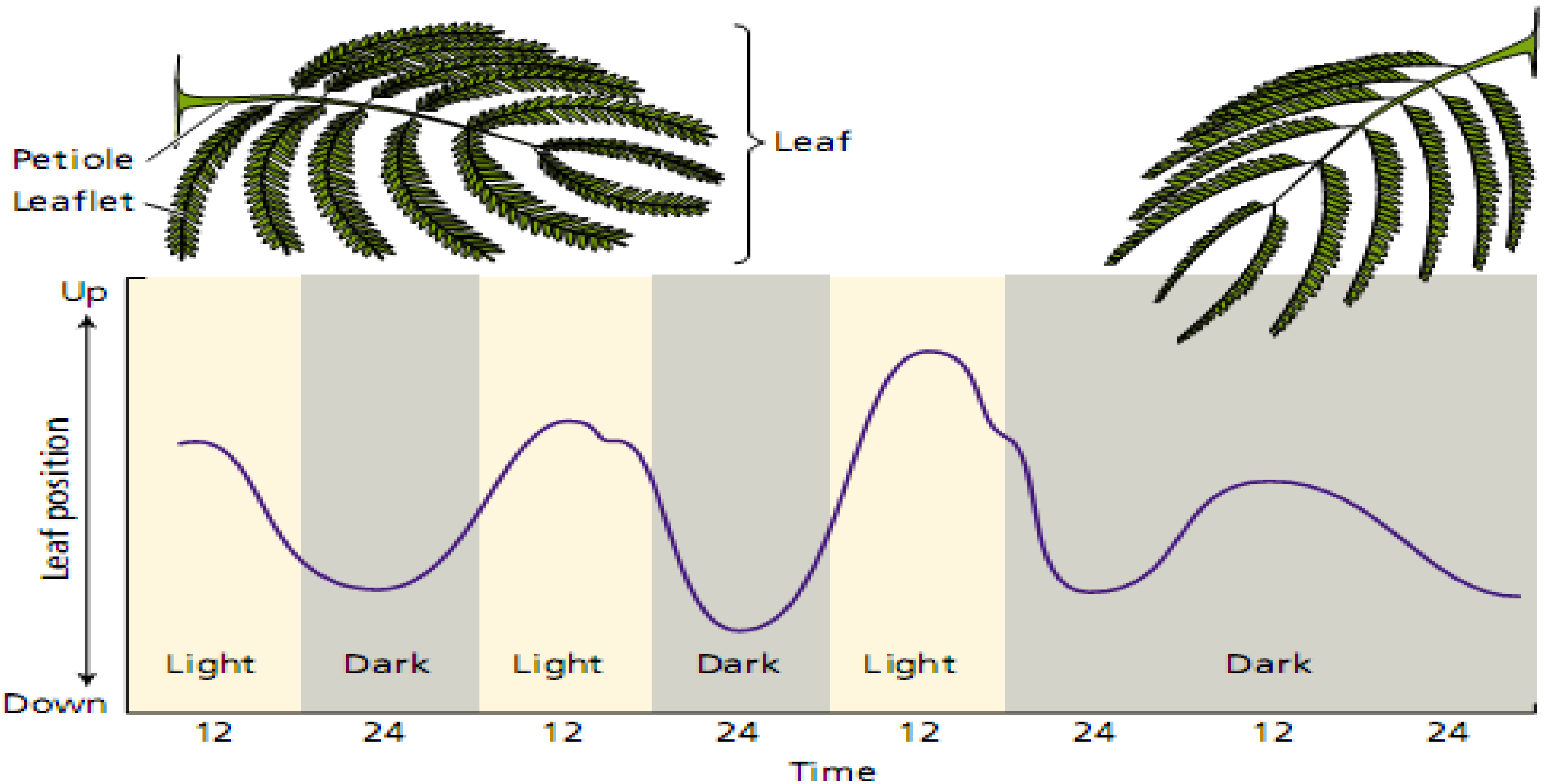
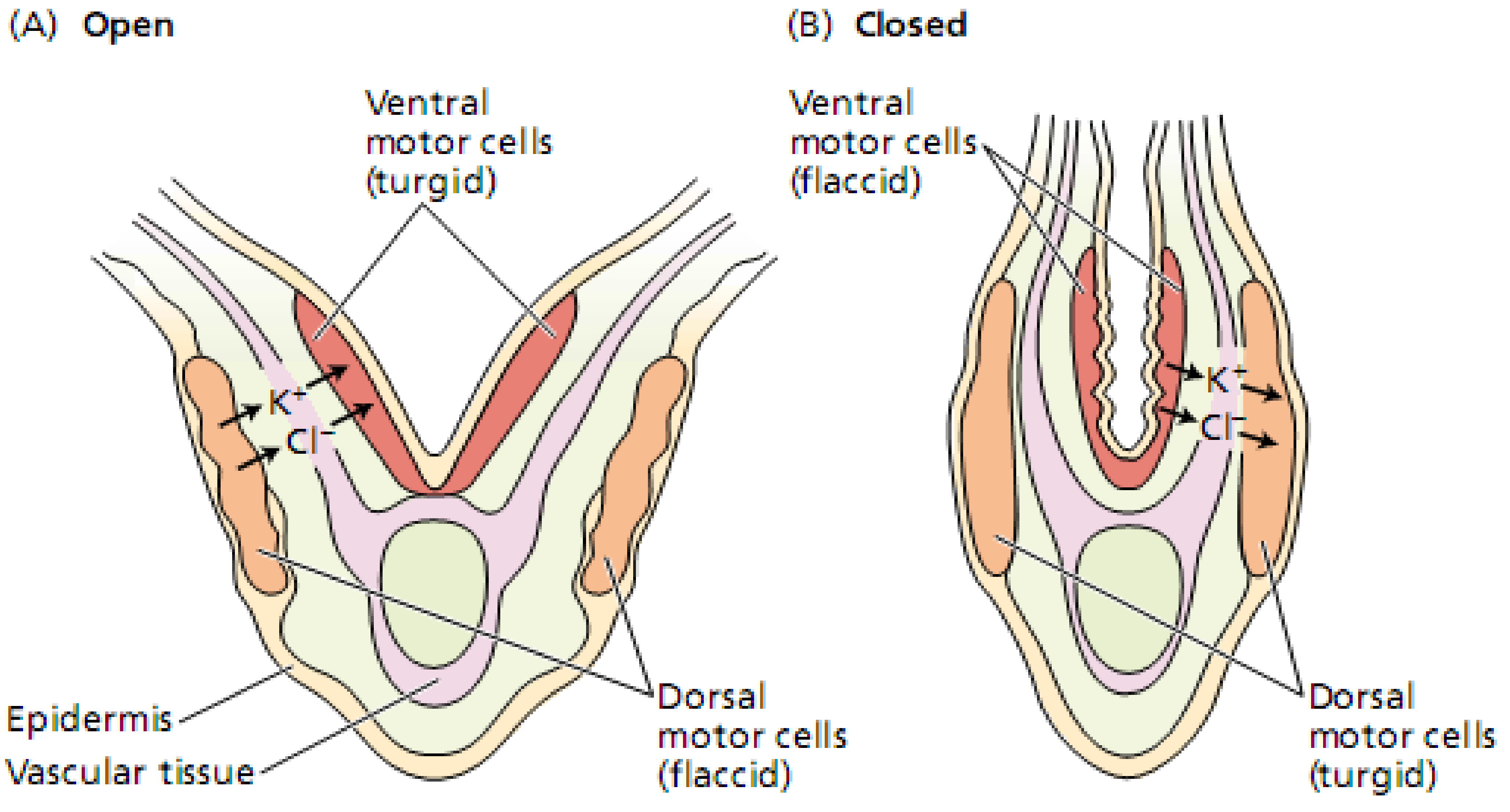


FIGURE 17.13 Circadian rhythm in the diurnal movements of *Albizia* leaves. The leaves are elevated in the morning and lowered in the evening. In parallel with the raising and lowering of the leaves, the leaflets open and close. The rhythm persists at a lower amplitude for a limited time in total darkness.

Ion fluxes between the dorsal and ventral motor cells of Albizia pulvini regulate leaflet opening and closing.

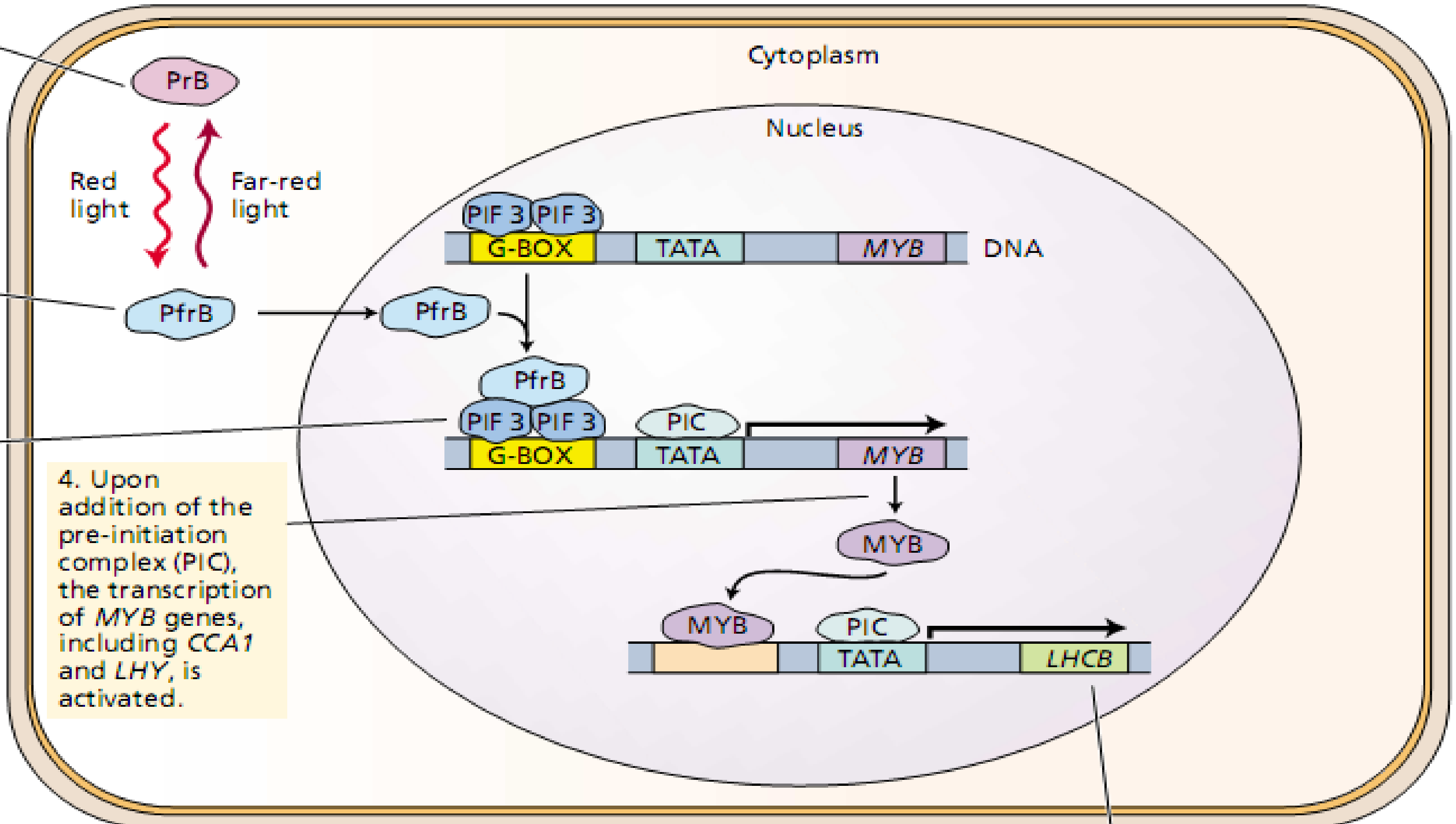


Phytochrome function
1) membrane potential
2) gene expression

1. PhyB is synthesized in the cytoplasm in the inactive PrB form.

2. When converted to the active PfrB form by red light, it moves into the nucleus.

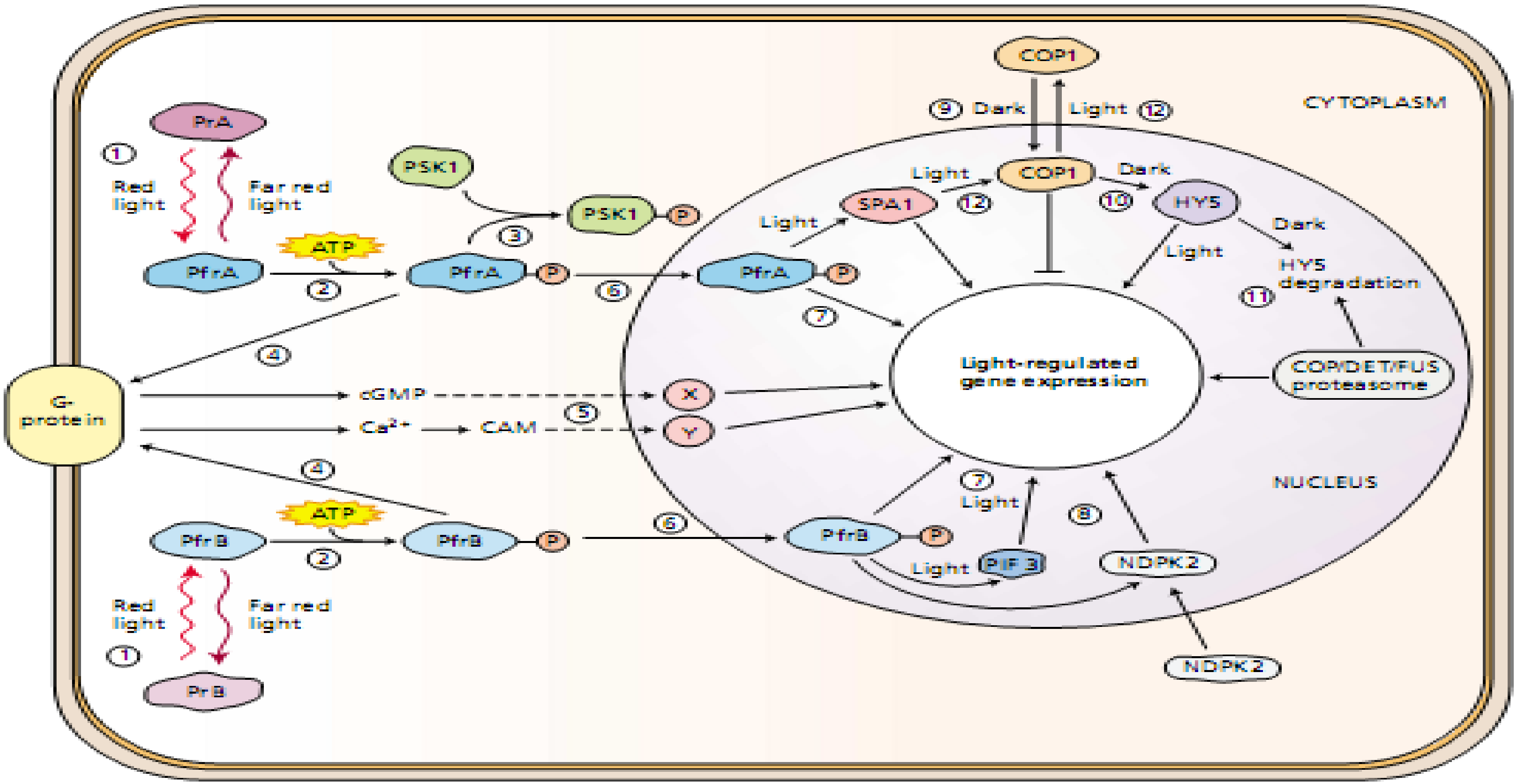
3. PfrB binds to a dimer of the transcription factor, PIF3, which is bound to the G-BOX elements of *MYB* gene promoter.



4. Upon addition of the pre-initiation complex (PIC), the transcription of *MYB* genes, including *CCA1* and *LHY*, is activated.

5. MYB transcription factors in turn activate the transcription of other genes, such as *LHCB*.

FIGURE 17.20 Direct regulation of gene expression by phyB transport to the nucleus. (After Quail 2000.)



1. Red light converts PrA and PrB to their Pfr forms.
2. The Pfr forms of phyA and phyB phytochrome can autophosphorylate.
3. Activated PfrA phosphorylates phytochrome kinase substrate 1 (PKS1).
4. Activated PfrA and PfrB may interact with G-proteins.
5. cGMP, calmodulin (CAM), and calcium (Ca^{2+}) may activate transcription factors (X and Y).
6. Activated PfrA and PfrB enter the nucleus.
7. PfrA and PfrB may regulate transcription directly or through interaction with phytochrome interacting factor 3 (PIF3).
8. Nucleoside diphosphate kinase 2 (NDPK2) is activated by PfrB.
9. In the dark, COP1 enters the nucleus and suppresses light-regulated genes.
10. In the dark, COP1, an E3 ligase, ubiquitinates HY5.
11. In the dark, HY5 is degraded with the assistance of the COP/DET/FUS proteasome complex.
12. In the light, COP1 interacts directly with SPA1 and is exported to the cytoplasm.