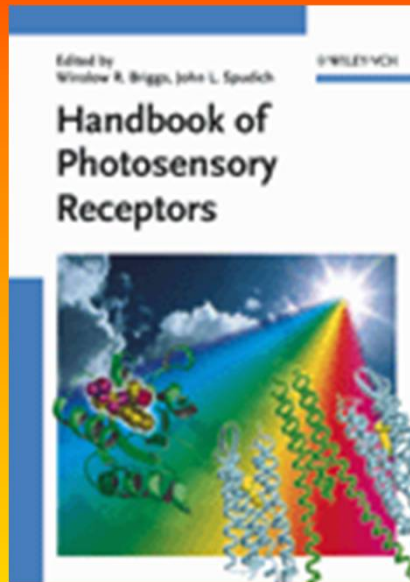


5) Photomorphogenesis

- a) Phytochromes
- b) Plant responses mediated by phytochromes
- c) Ecological functions of phytochromes
- d) Cellular and molecular mechanisms of phytochrome functions



Briggs WR, Spudis JL (eds) (2005)
Handbook of Photosensory
Receptors, Wiley-VCH



Schäfer E, Nagy F (eds) (2006)
Photomorphogenesis in Plants
and Bacteria, 3rd ed., Springer



Whitelam GC, Halliday KJ (eds) (2007)
Light and Plant Development
Blackwell Publishing

**Growth in the dark
(etiolated plants, skotomorphogenesis)**



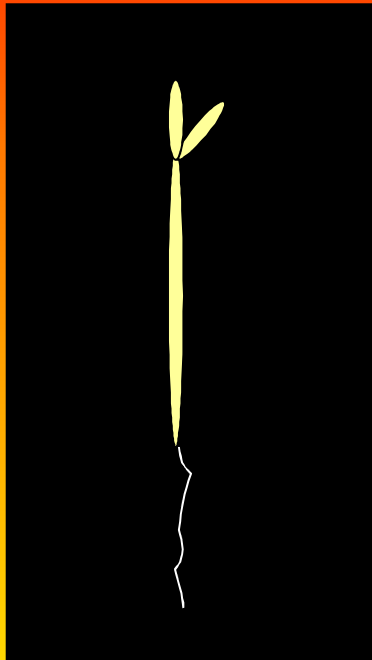
**Growth in light
(photomorphogenesis)**



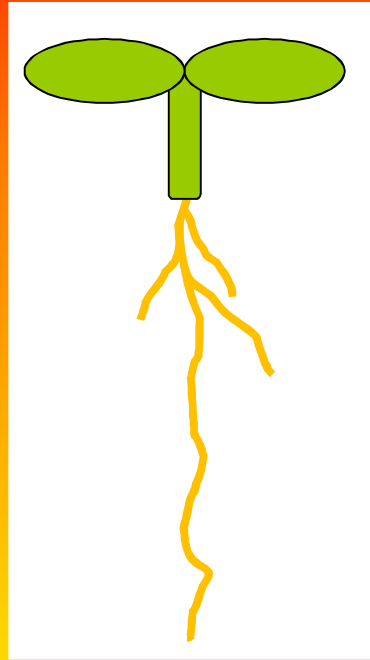
Photomorphogenesis

A process in which light as a signal alters development of the plant to the form, at which the plant can use light as source of energy.

Dark



Light



Basic photomorphogenic responses:

- Inhibition of elongation
- Stimulation of chlorophyll synthesis
- Stimulation of leaf growth

For the process of photomorphogenesis, light is perceived by pigments that are a part of **photoreceptors**:

- red light: phytochromes A to E
- blue light and UV-A: cryptochromes, phototropins

a) Phytochromes

Phytochrome = protein pigment of blue light identified in 1959

Plant responses induced by phytochromes:

- promotion of germination
- stimulation of de-etiolization (e.g. leaf opening)
- stimulation of formation of leaf primordia and leaf growth
- inhibition of elongation

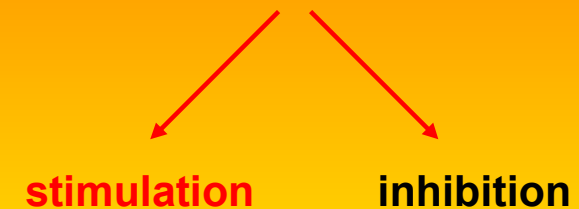
TABLE 17.1

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

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

PLANT PHYSIOLOGY, Third Edition, Table 17.1 © 2002 Sinauer Associates, Inc.

Light perception by receptors and signal transduction differ in various organs



Effect of red light (R; 650-680 nm) is reversed by far-red light (FR; 710-740 nm)



Dark



R



R

FR



R

FR

R



R

FR

R

FR



2 hypotheses

2 hypotheses explaining the R – FR reversibility

- 1) Existence of two pigments – for R and FR – antagonistically regulate germination
- 2) Existence of one pigment – changes the form from R-absorbing to FR-absorbing

Hypothesis supported. Reversible properties confirmed *in vitro*



3 following topics

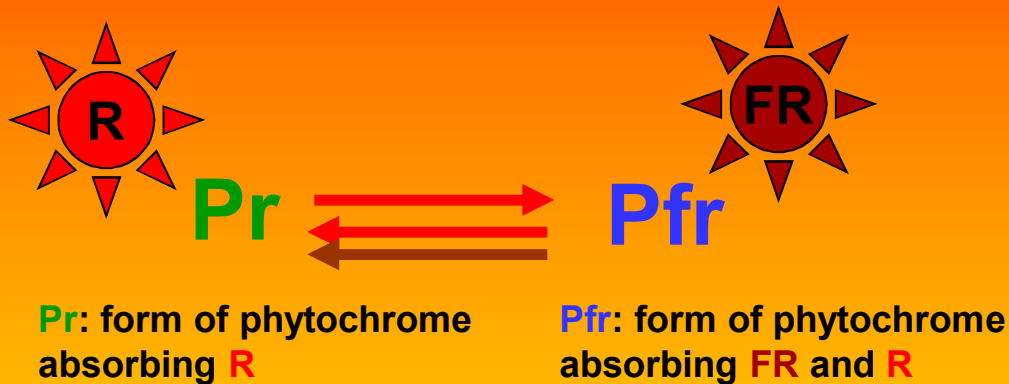
- 1) Photoreversibility and relation to phytochrome responses
- 2) Structure of phytochrome, localization and conformation changes
- 3) Genes coding for phytochromes and their function in photomorphogenesis

1) Photoreversibility and relation to phytochrome responses

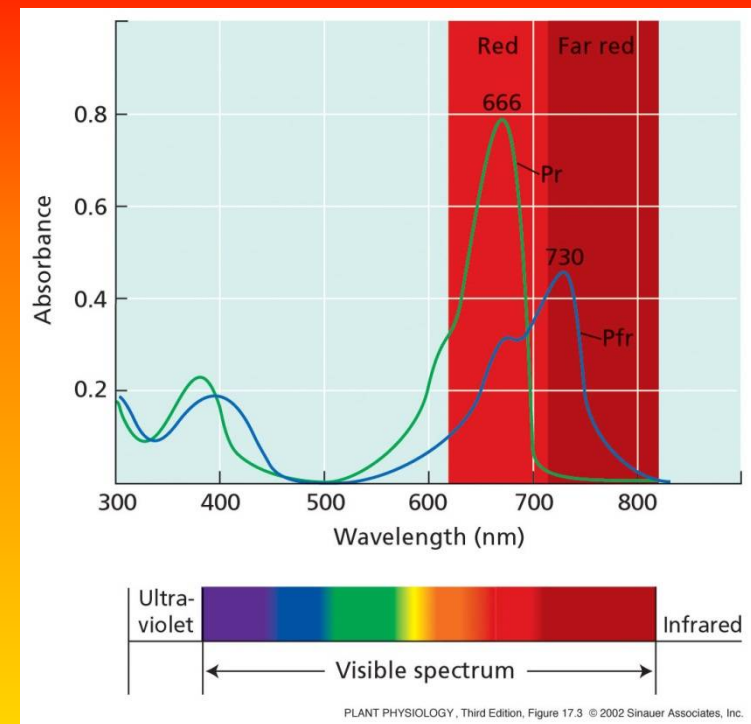


R-absorbing form: **Pr**

Pr is synthesized in the dark *de novo*



Photostationary status: **Pr** : **Pfr** = **98%** : **2%**

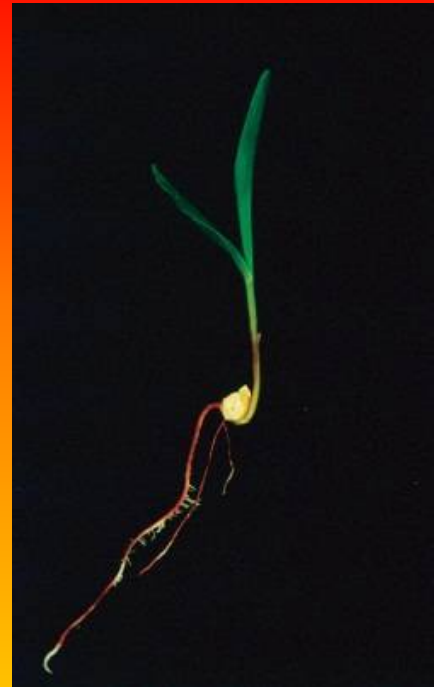


Pfr is physiologically active form of phytochrome => absence of Pfr causes inability of plant to respond to light.



Dark = elongation
(stimulation)

Pr ↑ → Pfr ↓



Light = shortening
(inhibition)

Pr ↓ → Pfr ↑

2) Structure of phytochrome, localization and conformation changes

Phytochrome = soluble protein, ~ 250 kDa, 2 subunits = dimer

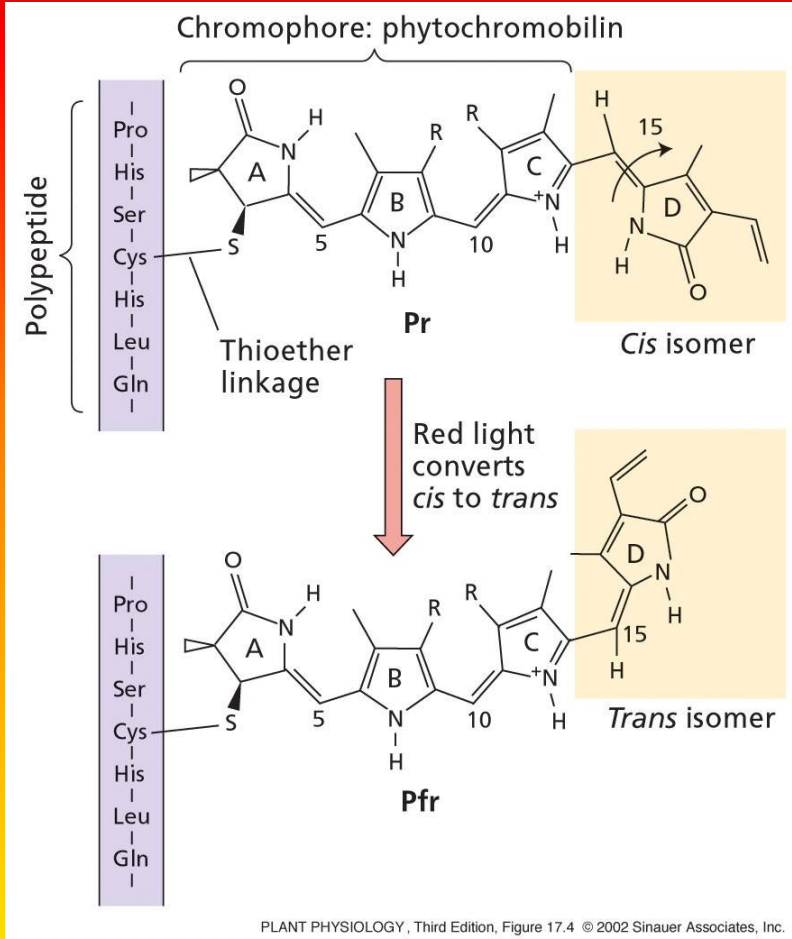
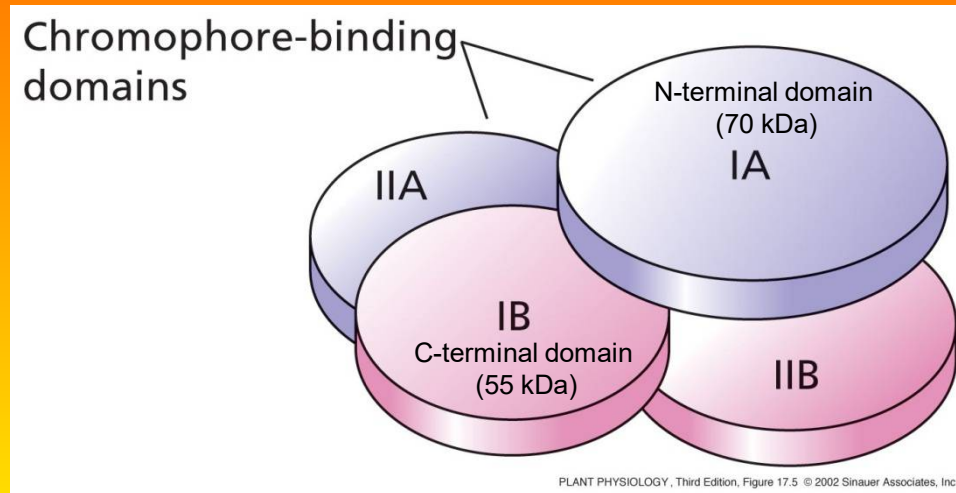
Phytochrome = chromophore + apoprotein
 (pigment) (polypeptide, 125 kDa)

Higher plants:

Chromophore = linear tetrapyrrole = phytochromobilin

Phytochromobilin + apoprotein = holoprotein

Phytochrome dimer

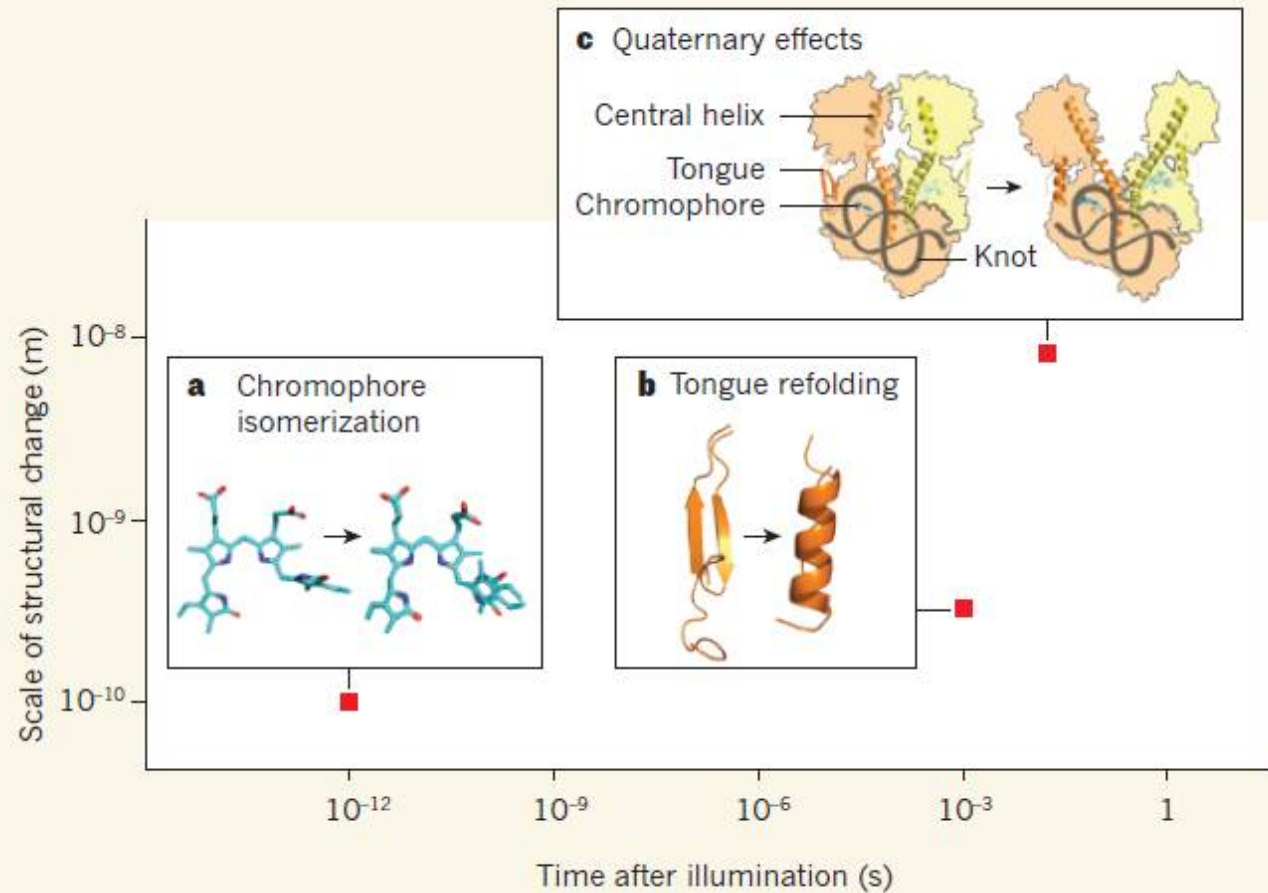


Update 2014

Baker AW, Forest KT (2014)
Nature 509: 174–175

Takala et al. (2014)
Nature 509: 245-258

a) Light-induced conformation changes of chromophore from the form *cis* to *trans*



b) Reorganization of key secondary structure „tongue“: structure of β -hairpin changes to the α -helix structure

c) Closed quaternary structure of phytochrome (occurring in the dark) open and Y conformation is formed, typical for phytochrome in cells in the light.

3) Genes coding for phytochromes and their function in photomorphogenesis

Type I *PHYA*

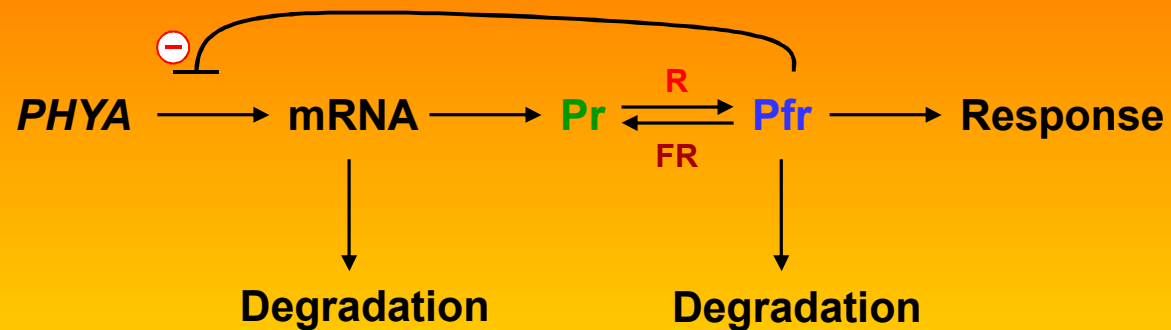
Type II *PHYB*

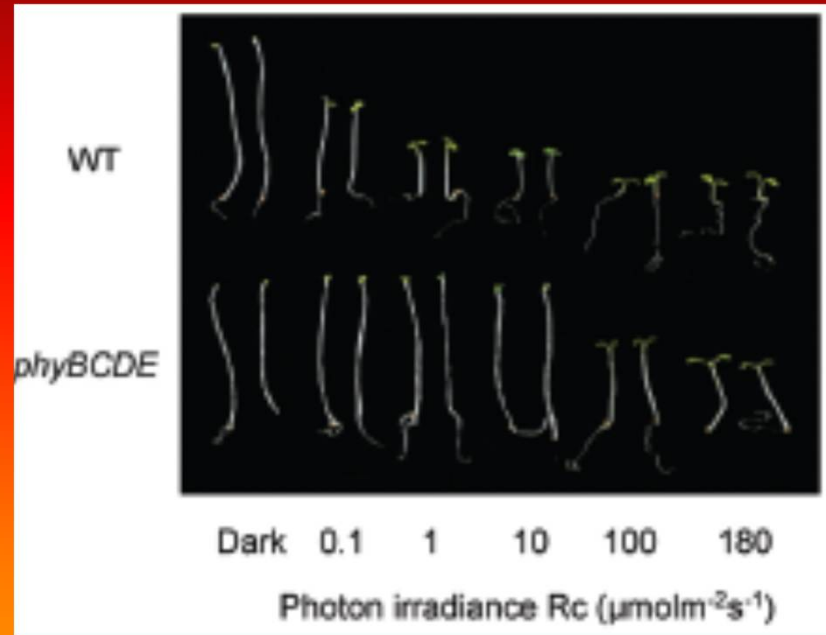
PHYC

PHYD

PHYE

PHYA – expression is inhibited by light => transcriptionally active in etiolated plants (monocotyledons)





Analysis of quadruple mutant at $160 \mu\text{mol.m}^{-2}.\text{s}^{-1}$
phyBphyCphyDphyE – de-etiolation and plant development till flowering



At high irradiance (over $100 \mu\text{mol.m}^{-2}.\text{s}^{-1}$):

- *phyA* is not degraded
- *phyA* functions as light sensor

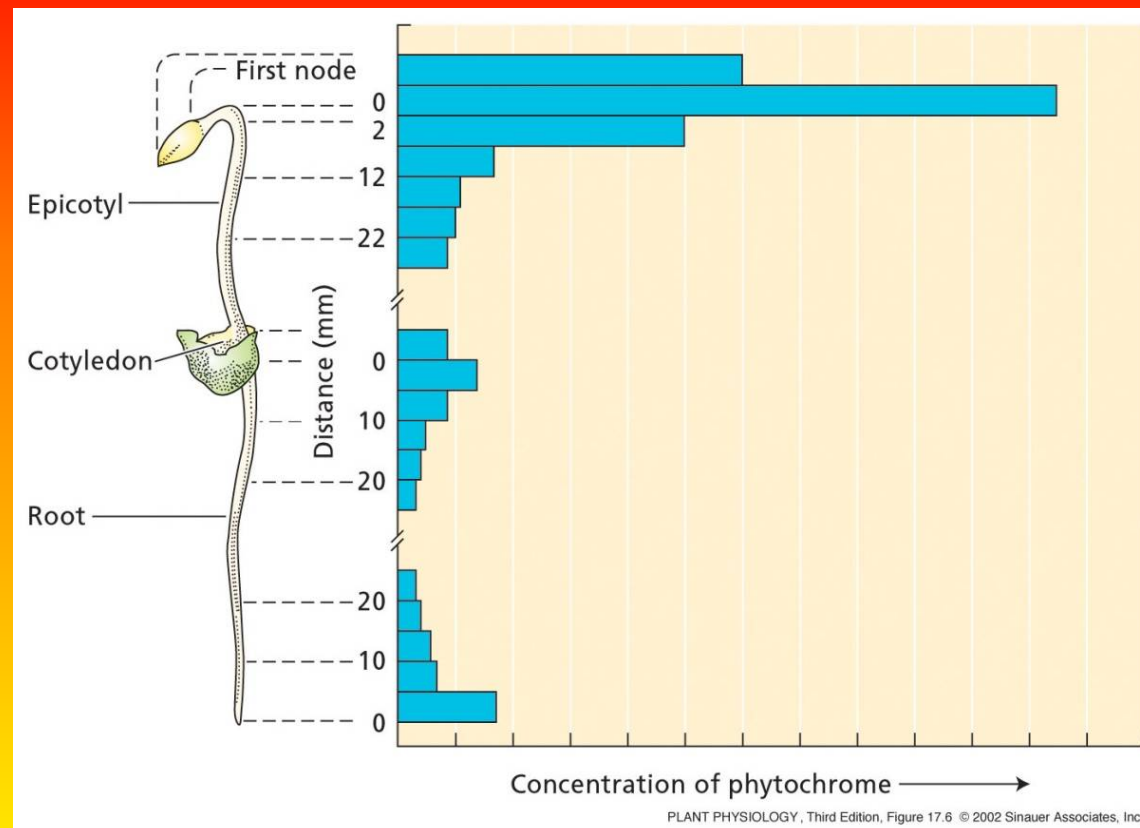
PHYB - E – expression is not affected by light => transcriptionally active in etiolated and green plants; proteins *phyB - E* are more stable



Phytochrome localization in cells and tissues

Knowledge of phytochrome localization suggests phytochrome functions

- Spectrophotometrically – etiolated plants
- Visualization of gene expression using reporter gene GUS



b) Plant responses mediated by phytochromes

- 1) Rapid biochemical responses
- 2) Slower morphological changes (+ movement and growth)

Lag phase = time between light stimulation and the observed response

Short – minutes (cell expansion and shrinking)

Long – several weeks (flowering)

a) Very-low-fluence responses (VLFRs)

0.0001 mmol.m^{-2} to 0.05 mmol.m^{-2}

Stimulation of coleoptile growth, inhibition of mesocotyl growth, promotion of germination

b) Low-fluence responses (LFRs)

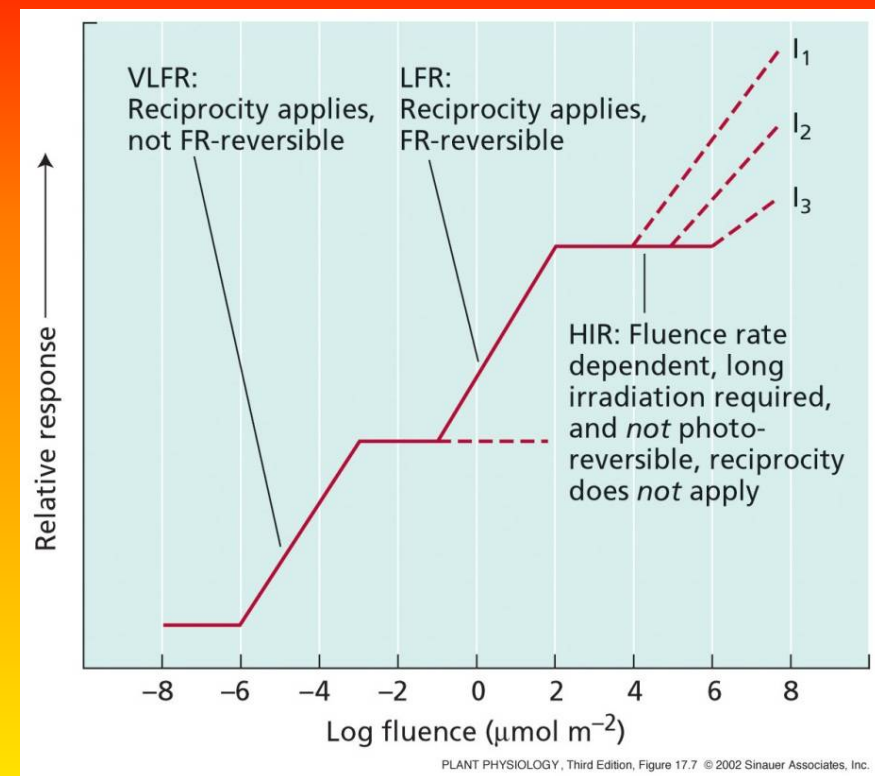
1.0 mmol.m^{-2} to 1000 mmol.m^{-2}

Stimulation of lettuce seed germination, regulation of leaf movement

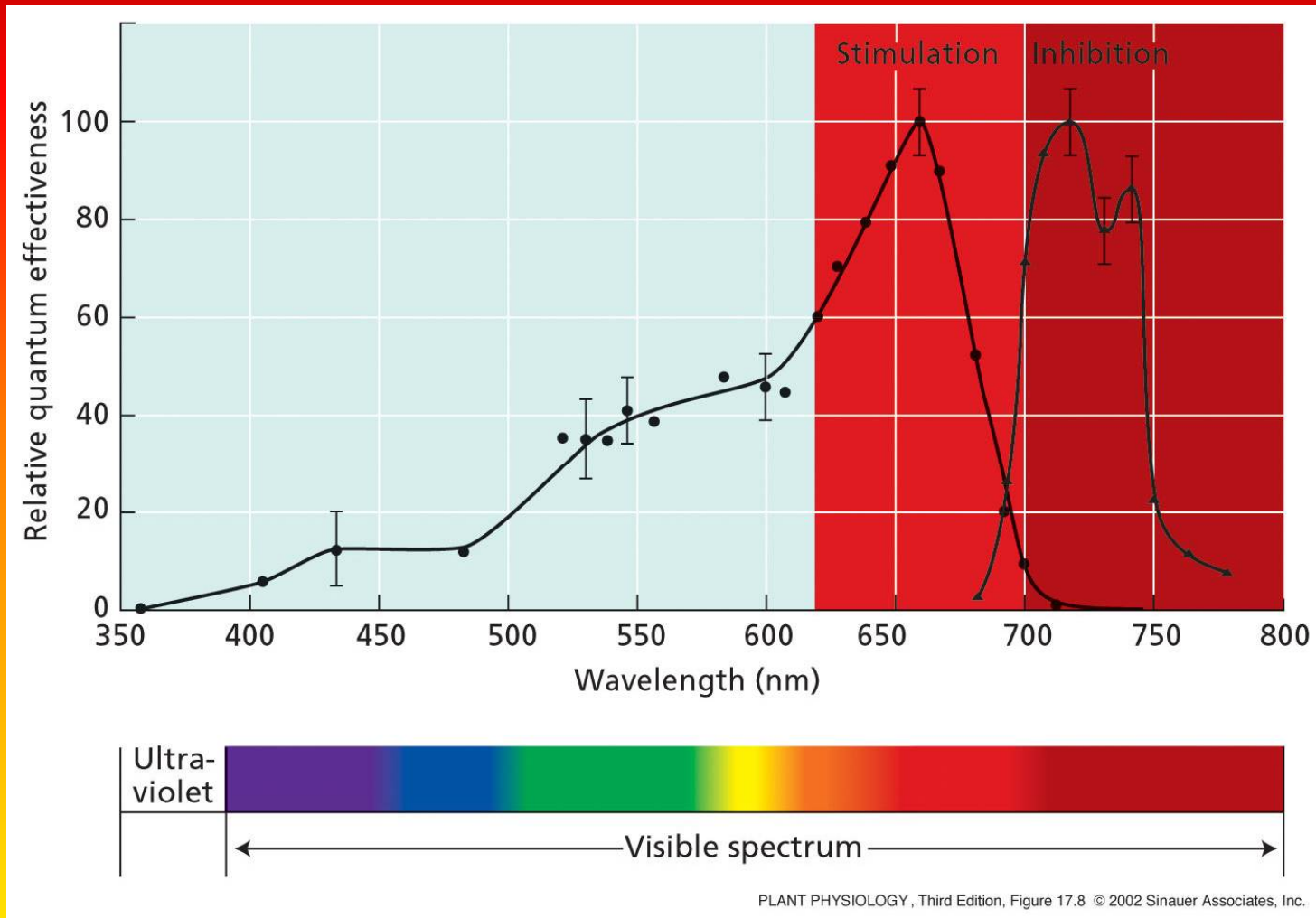
c) High-irradiance responses (HIRs)

0.1 mmol.m^{-2}

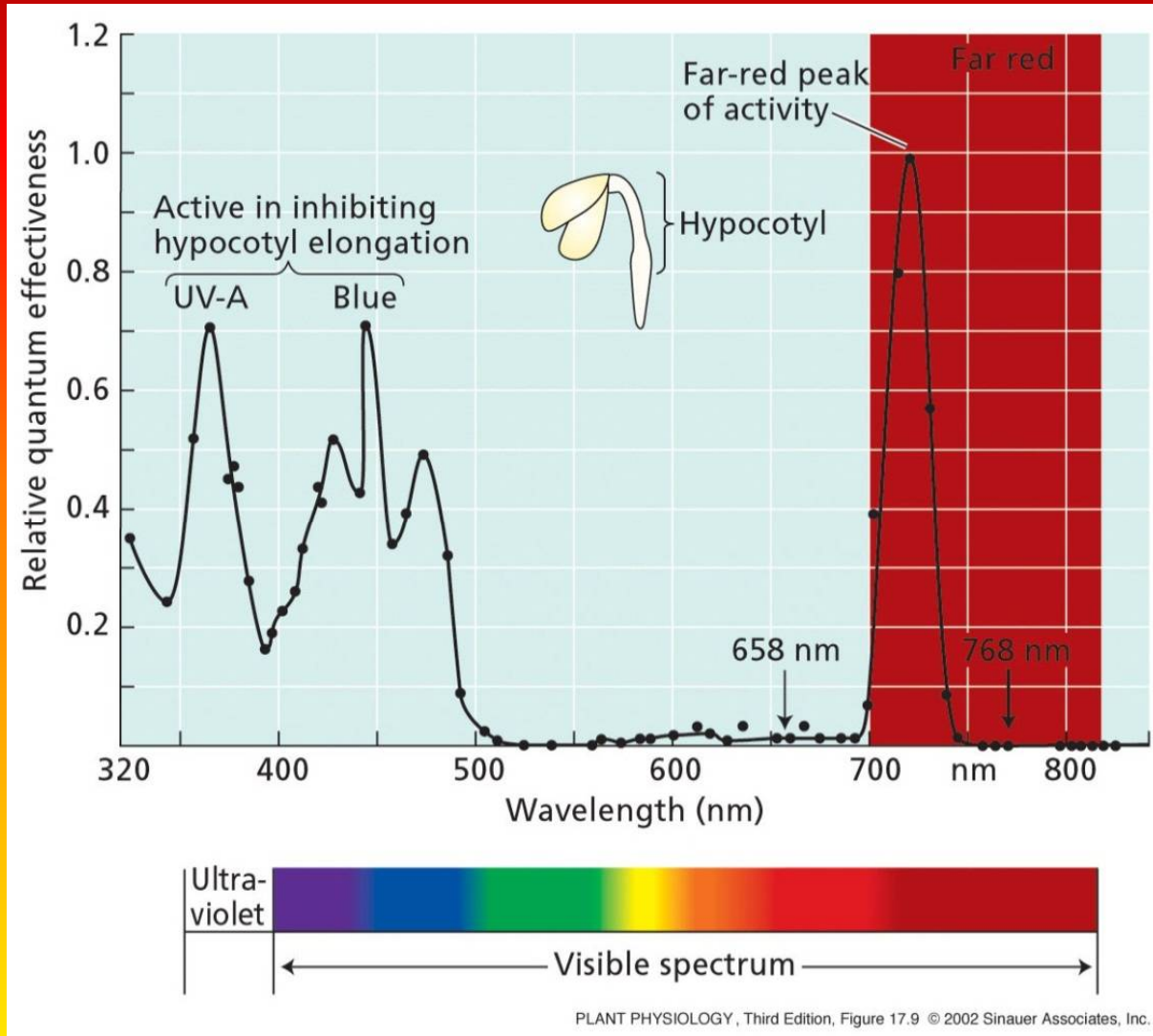
Induction of anthocyanin biosynthesis, inhibition of hypocotyl growth, flowering induction



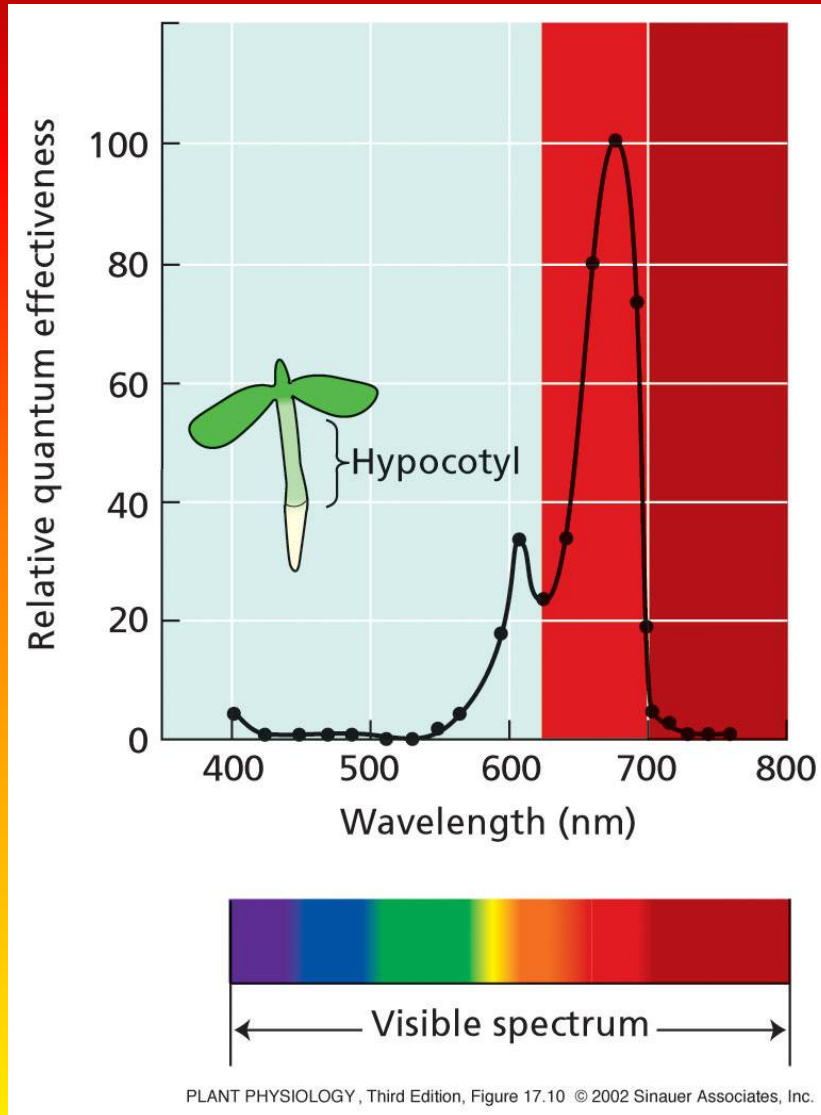
Action spectrum of LFR for photoreversible stimulation and inhibition of *Arabidopsis* seed germination



Action spectrum of HIR for inhibition of elongation of etiolated hypocotyl



Action spectrum of HIR for inhibition of elongation of green hypocotyl



The more **green** plant, the less sensitive to FR



Action spectrum of HIR in **green** plants shifts to R wavelengths
(**Green** plant is more sensitive to R)



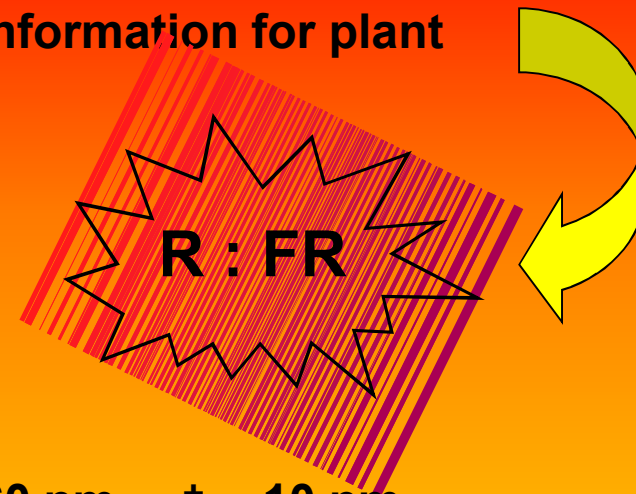
HIR of **green** plant is mediated by phytochrome phyB

c) Ecological functions of phytochromes

R/FR reversible pigment



Wavelengths R and FR = information for plant



$$R : FR = \frac{\text{Photon flow at } 660 \text{ nm } \pm 10 \text{ nm}}{\text{Photon flow at } 730 \text{ nm } \pm 10 \text{ nm}}$$

R : FR in various environments

TABLE 17.3
Ecologically important light parameters

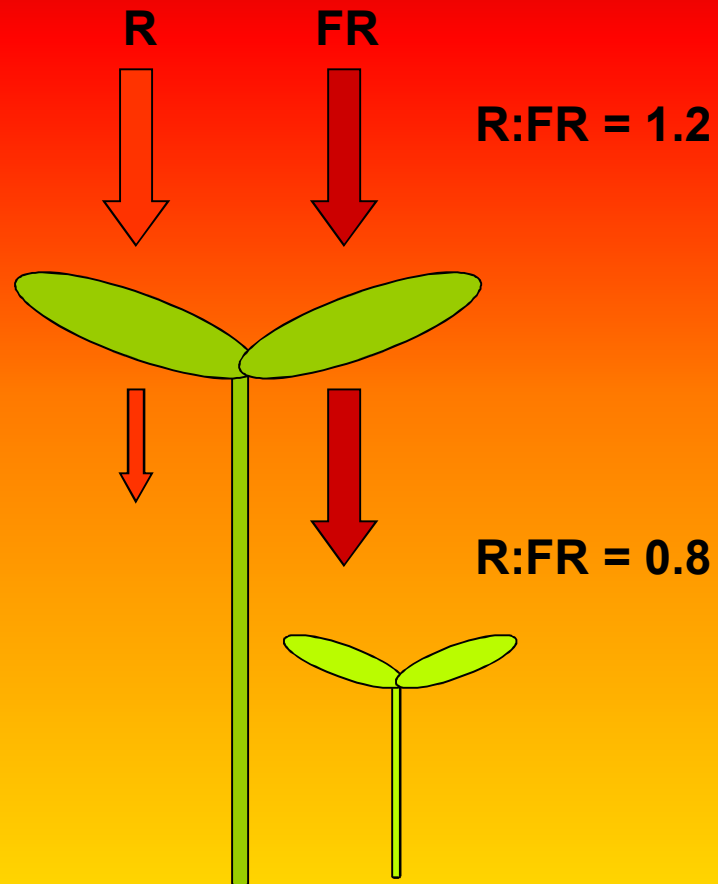
	Photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R/FR ^a
Daylight	1900	1.19
Sunset	26.5	0.96
Moonlight	0.005	0.94
Ivy canopy	17.7	0.13
Lakes, at a depth of 1 m		
Black Loch	680	17.2
Loch Leven	300	3.1
Loch Borrallie	1200	1.2
Soil, at a depth of 5 mm	8.6	0.88

Source: Smith 1982, p. 493.

Note: The light intensity factor (400–800 nm) is given as the photon flux density, and phytochrome-active light is given as the R:FR ratio.

^aAbsolute values taken from spectroradiometer scans; the values should be taken to indicate the relationships between the various natural conditions and not as actual environmental means.

Shade avoidance = plant response to shade



Shade-avoidance response

- elongation
- reduction of leaf size
- decrease in chlorophyll
- reduction of sec. shoot formation

Circadian rhythms

Circadian rhythm = rhythm changes, at which phases of maximum activity alternate with phases of minimum activity

They persist in the absence of exogenous factors

Requirement of endogenous stimuli (pacemakers)



Endogenous oscillator

- plants
- animals

- temperature independent => functional in various climatic conditions
- modulated by light => daily rhythm: 24 hours

Specialization phytochromes

Genes *PHYA* – *PHYE* are very similar, but they differ in their functions

PHYB – identified by analysis of *hy3* mutant (now *phyB*): long hypocotyl in white light; *PHYB* mRNA reduced, protein phyB is not synthesized; normal expression of *PHYA*.

PHYB is responsible for plant sensitivity to R and mediates photoreversible seed germination

PhyA is receptor continuous FR.

Mutant *phyA*:

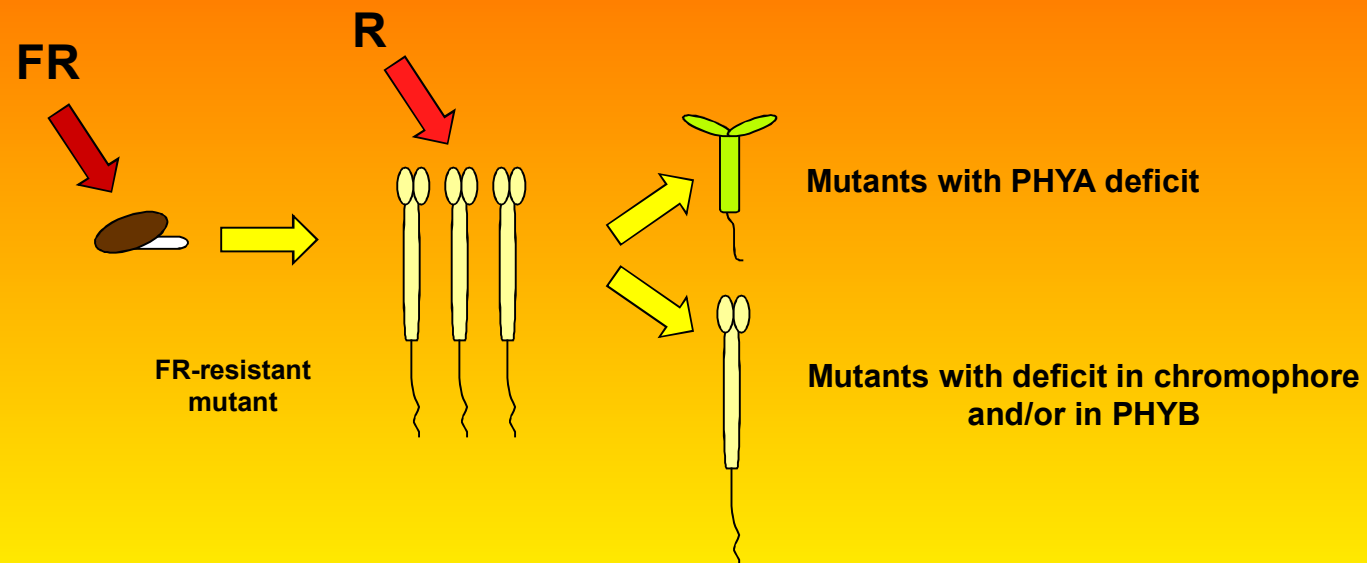
- Does not respond to FR
- Develop tall and thin phenotype

=

Phenotype of mutants with defect in chromophore or phyB



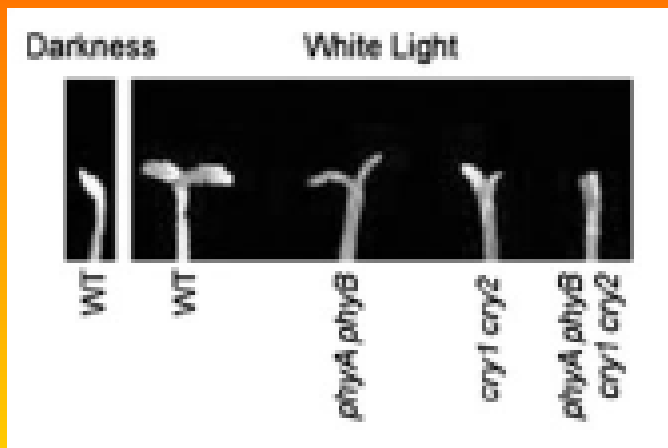
Difficult to select mutants with a defect specifically in protein PHYA



Role of phytochromes C, D and E in plant development

Functions of phyC, D and E overlap with the functions of phyA and phyB.
They play supplementary roles:

Analysis of quadruple mutants *phyAphyBcry1cry2* = phenotype of plants growing in the dark

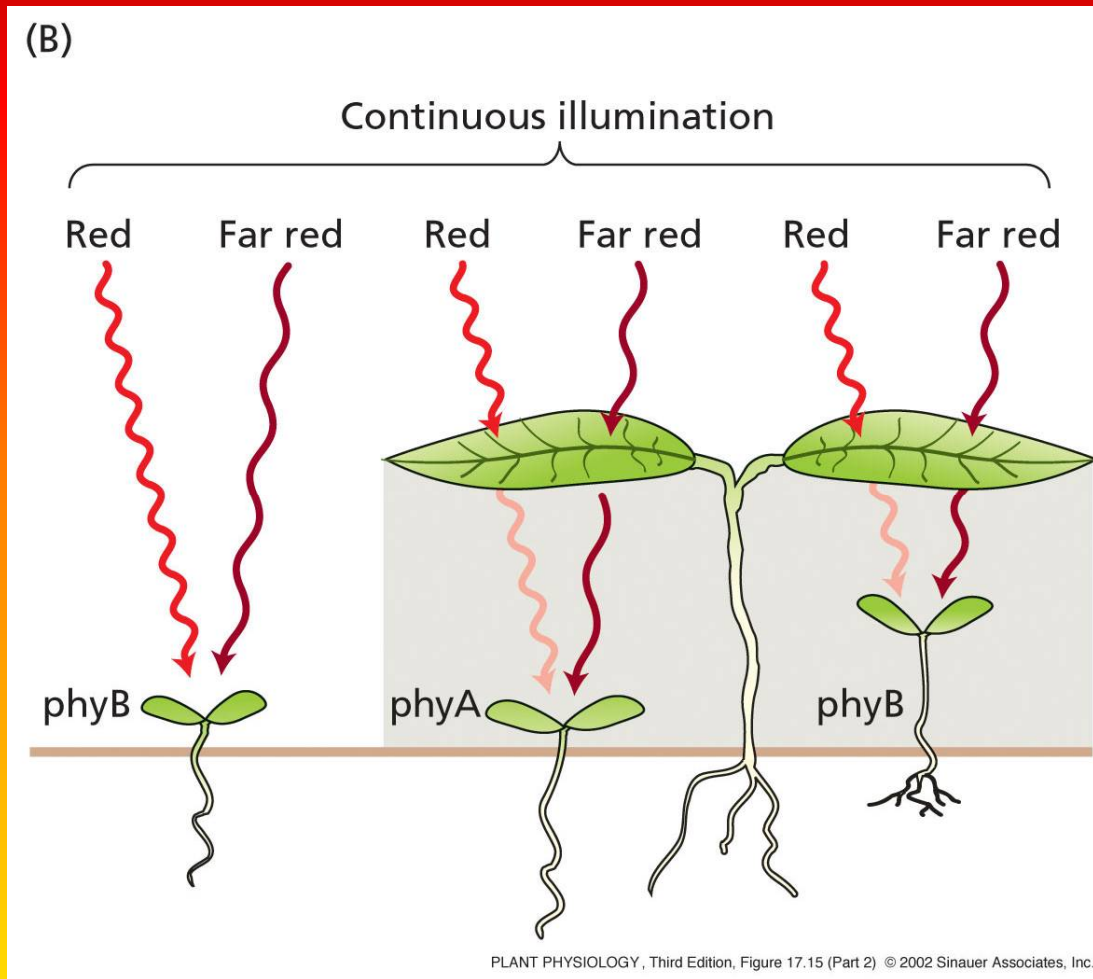


BUT transcription analysis showed expression of light-induced genes!!! Mutant shows responses of circadian rhythm!!!



Photoreceptors phyC, D, E and new receptor ZEITLUPE mediate this expression and responses of circadian rhythm.

Interaction of phyA and phyB in shade-avoidance response



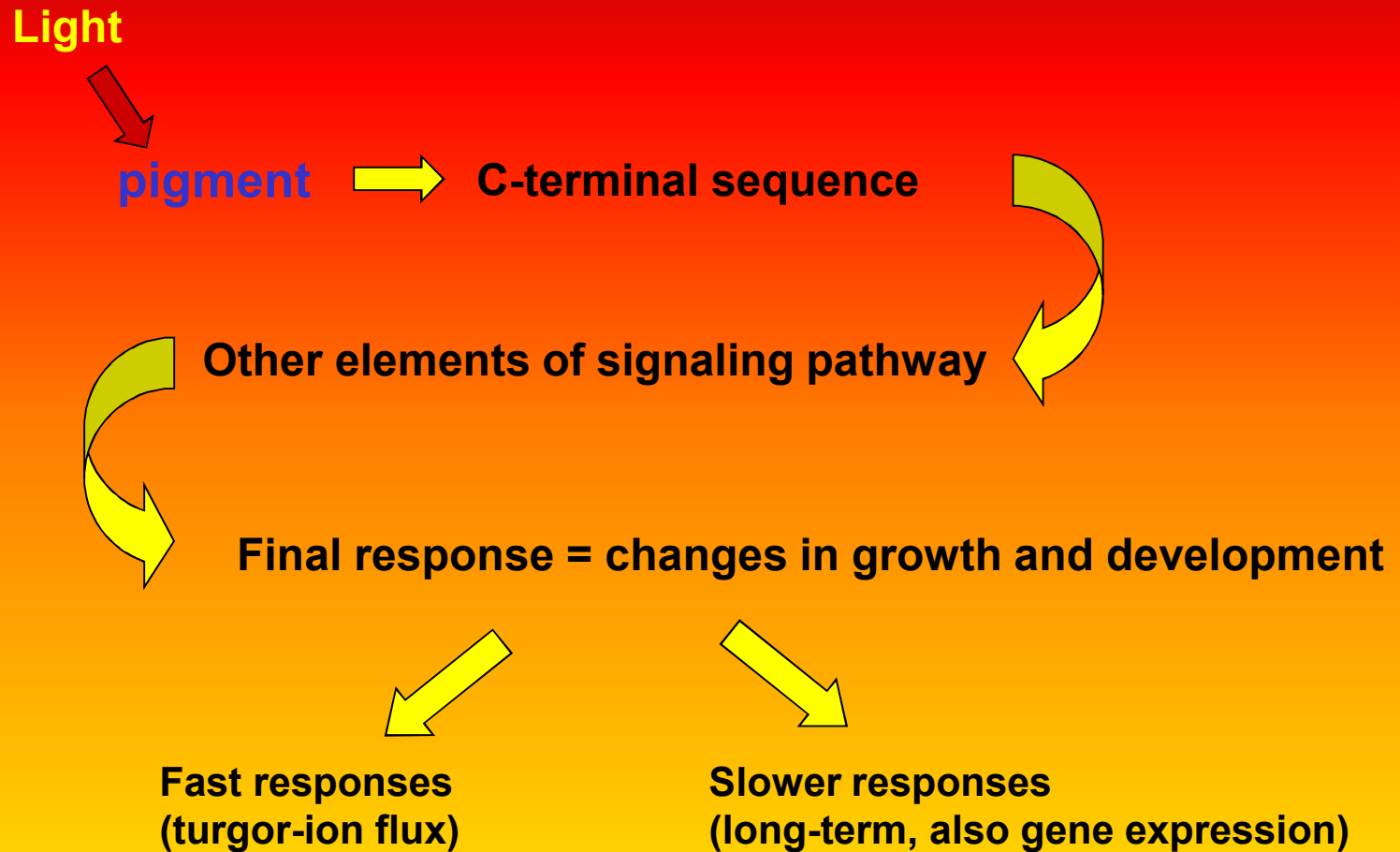
Direct sunlight:

Abundance of R => de-etiolation directed by phyB

Shade:

Abundance of FR => at the beginning de-etiolation mediated by phyA. PhyA is labile => later de-etiolation mediated by phyB.

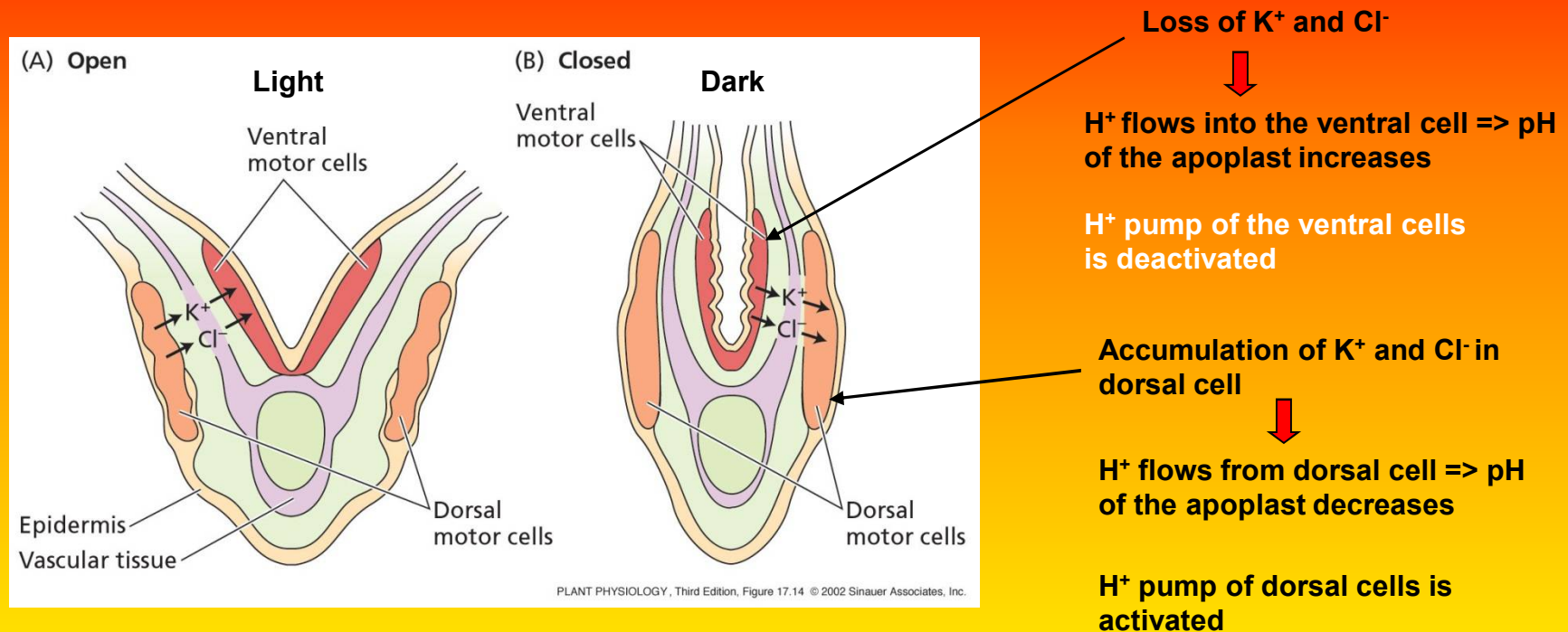
d) Cellular and molecular mechanisms of phytochrome functions



Fast responses

Regulation of membrane potential and ion flux mediated by phytochromes

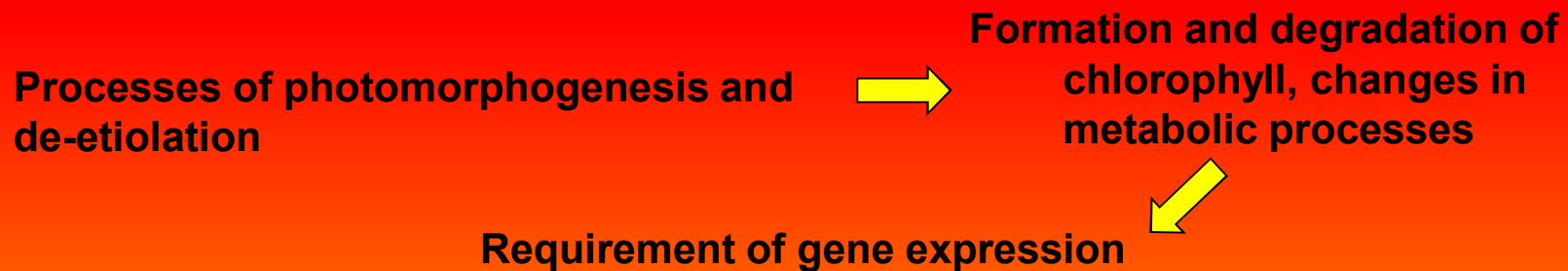
Lag phase of leaf closure ~ 5 min => short time for gene expression => direct changes of membrane permeability mediated by phytochromes



Leaf of *Mimosa*

Slow responses

Phytochrome regulates gene expression

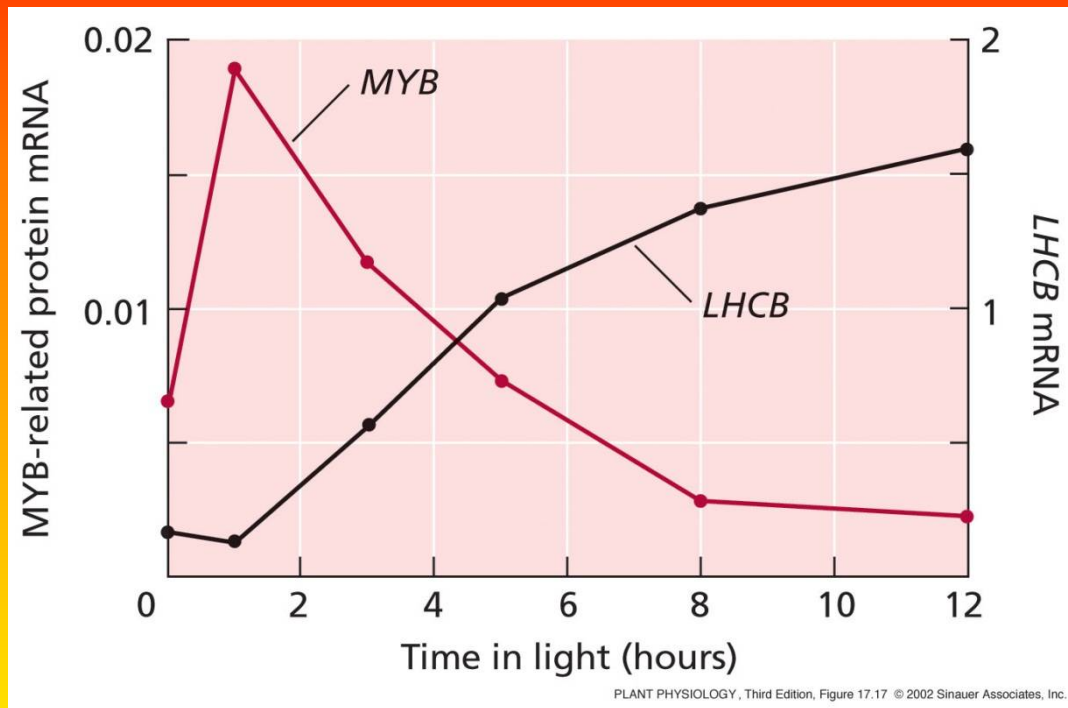
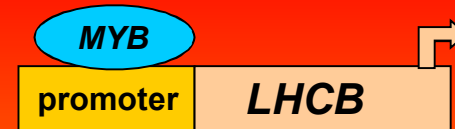
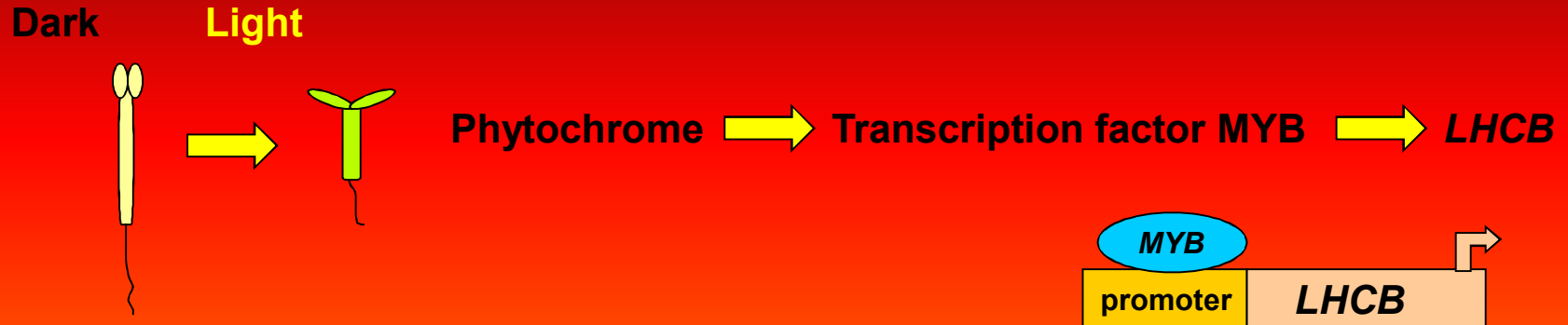


Phytochrome directs activation of transcription factors (TF). TF enter nucleus and stimulate transcription of specific genes.

Expression of early genes = genes of primary response – independent on protein synthesis (*MYB* genes)

Expression of late genes = genes of secondary response – dependent on protein synthesis (*LHCB* genes)

Phytochrome-directed regulation of expression of genes *MYB* and *LHCB*



***MYB* – genes of primary response**

***LHCB* – gene of secondary response**

CCA1 (**c**ircadian **c**lock **a**ssociated1) (belongs to *MYB* genes) – regulates expression of *LHCB* through of circadian rhythm; constitutive expression suppresses circadian rhythm, expression of *LHY* and expression of its own.

Mutation in *CCA1* results in defect of regulation of *LHCB* expression by circadian rhythm and by phytochrome

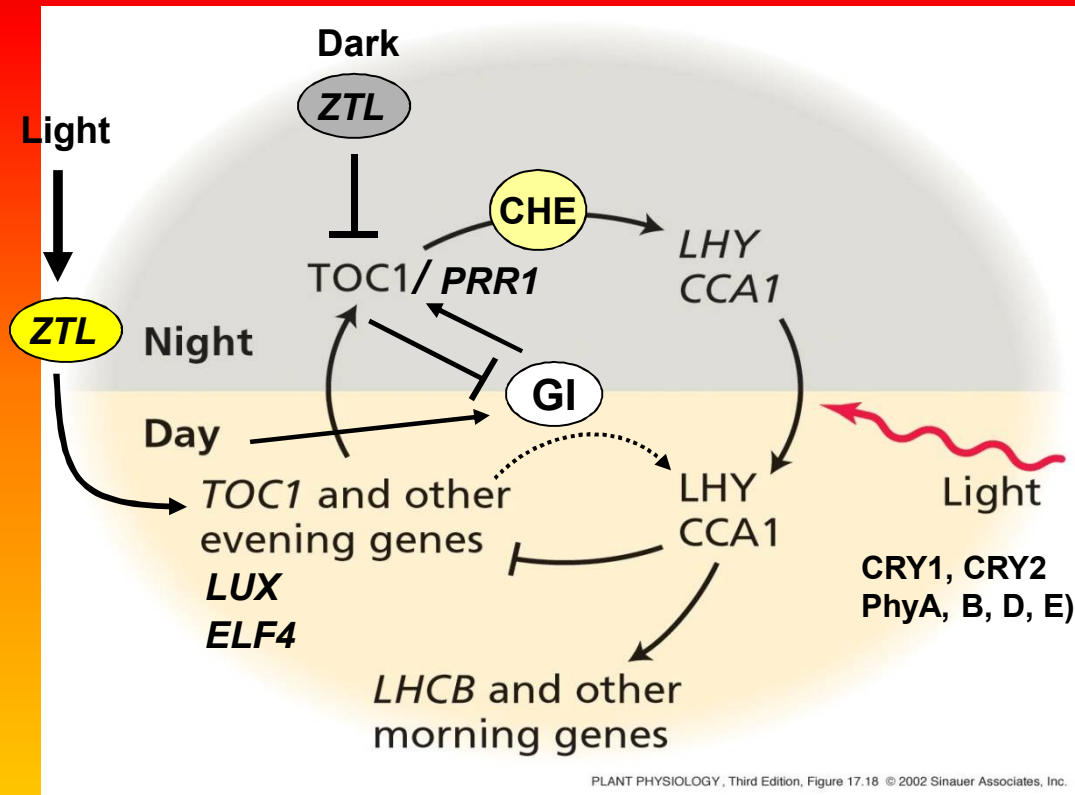
LHY (**l**ate **e**longated **h**ypocotyl) (belongs to *MYB* genes) – transcript oscillates with circadian rhythm



CCA1 and **LHY** play a role in circadian rhythm

Circadian oscillator - transcriptional-translational negative feedback – found in bacteria, fungi, insect and mammals; synchronizes physiological and developmental events of plant with daily and annual changes in surrounding environment

Circadian oscillator in *Arabidopsis*

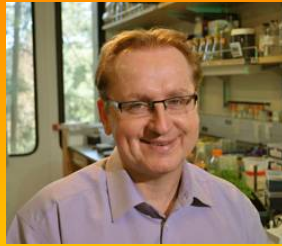


PLANT PHYSIOLOGY, Third Edition, Figure 17.18 © 2002 Sinauer Associates, Inc.

Alabadí D et al. (2001) Science 293: 880-883

Model of interaction of genes *LHY* and *CCA1*, plus gene *TOC1*, proposed in 2001.

Light and *TOC1* activate expression of *LHY* and *CCA1* – light functions as amplifier of *TOC1*



Steve Kay



C. Robertson McClung

CHE (CCA1 Hiking Expedition) - TF, blocks expression of *CCA1* by binding to its promoter. *TOC1* binds to CHE, blocks CHE and releases expression of *CCA1*.

**Phytochrome functions in the nucleus – activates transcription factors.
However, it is localized in cytoplasm => must be moved to the nucleus**

Sharma R (2001) Current Science 80: 178-188

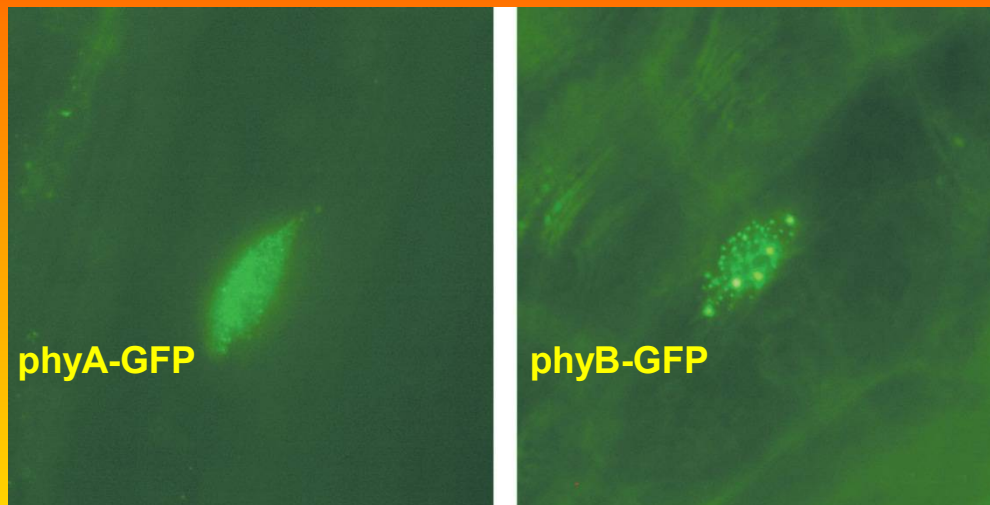


Phytochrome is moved to the nucleus by influence of light



- Movement of phyB – induced by R, inhibited by FR; only Pfr is transported to the nucleus, motion is slow
- Movement of phyA – induced by FR; transported in both forms; motion is fast.

Visualization by means of GFP (green fluorescent protein; GFP activated by light emits fluorescent radiation)



Construct



Plant transformation

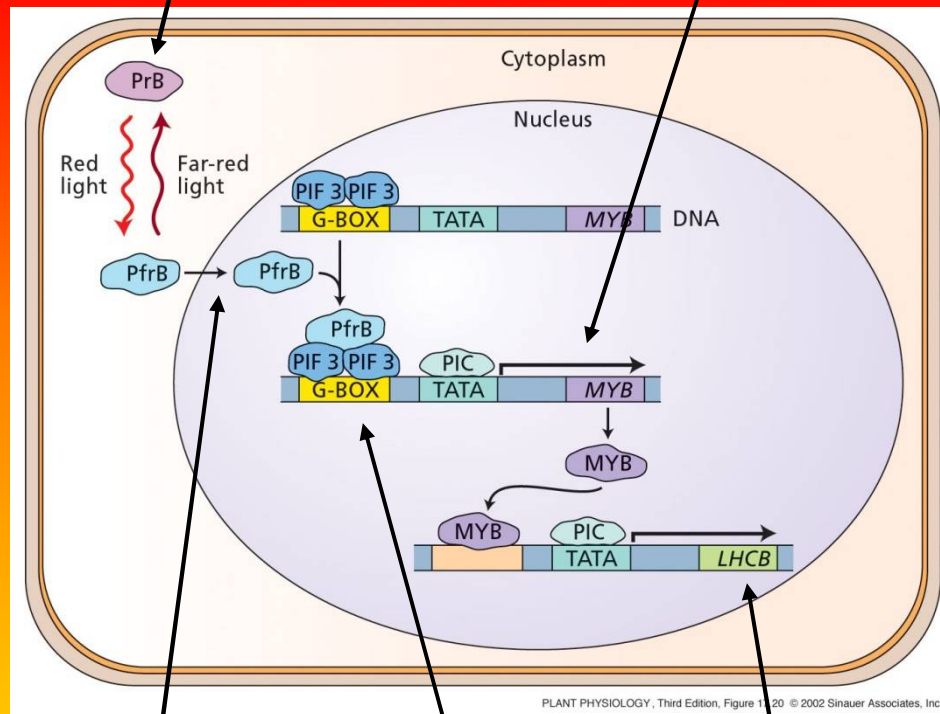


Observation of *PHYB* expression in cells and tissues

Regulation of gene expression by phytochrome B

PhyB is synthesized in the cytoplasm
in Pr form

PIC initiates transcription of *MYB* genes
(*CCA1*, *LHY*)



In Pfr form, phyB is
transported into the nucleus

PfrB binds to PIF3
dimer

Transcription factor MYB
activates transcription of LHCb

1) Regulation of gene expression
directly by PfrB

2) Regulation of gene expression
through PIF3

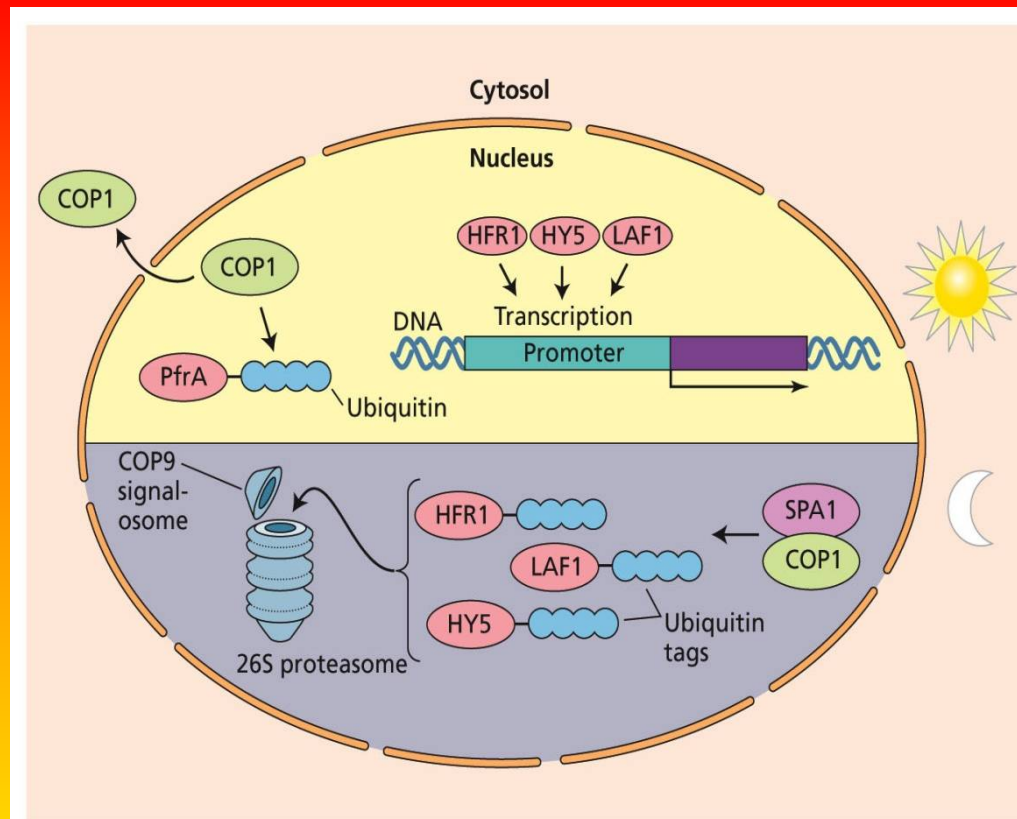
PIF3 (phytochrome interacting factor3)

- Transcription factor bHLH interacting
with G-box (= part of promoter of *MYB*
gene); necessary for skotomorphogenesis

- Interacting with C-terminal end of
PfrB => PIF3 and PfrB form a complex

Regulation of gene expression by phytochrome A

- 1) Directly by PfrA
- 2) Through PIF3
- 3) Through COP1



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Dark:

Accumulation of COP1 in the nucleus



Repression of expression of photomorphogenic genes – transcription factors (HY5, HFR1, LAF1,...) are ubiquitinated.

Light:

Transport of COP1 from the nucleus to cytoplasm by ubiquitination of protein PfrA



Restoration of expression of photomorphogenic genes by release of transcription factors (HY5, HFR1, LAF1,...)

***cop1* (constitutive photomorphogenesis 1)** – etiolated plants show phenotype of plants growing in light



Xing-Wang Deng
Yale University, New Haven



Nonmutated plant

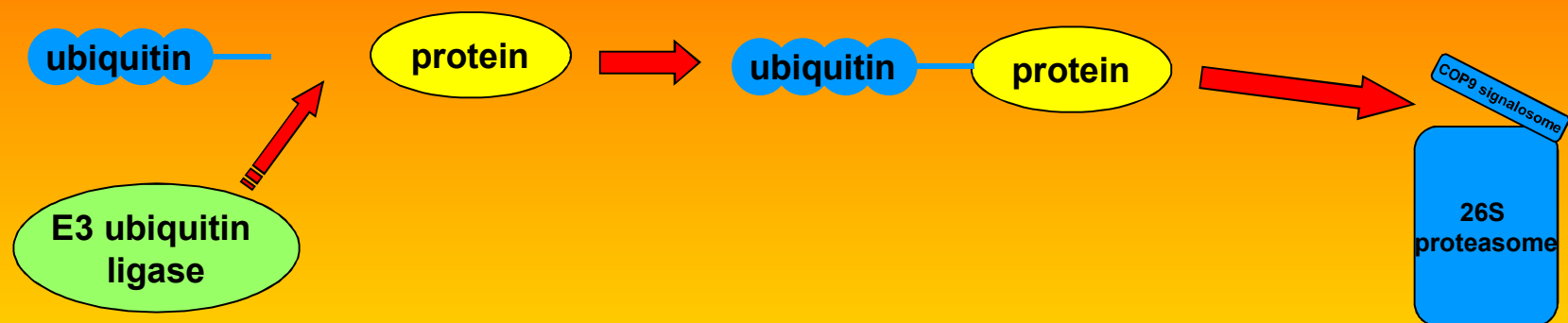
Mutant *cop1*

Nonmutated (= functional) gene *COP1* – negative regulator of photomorphogenesis

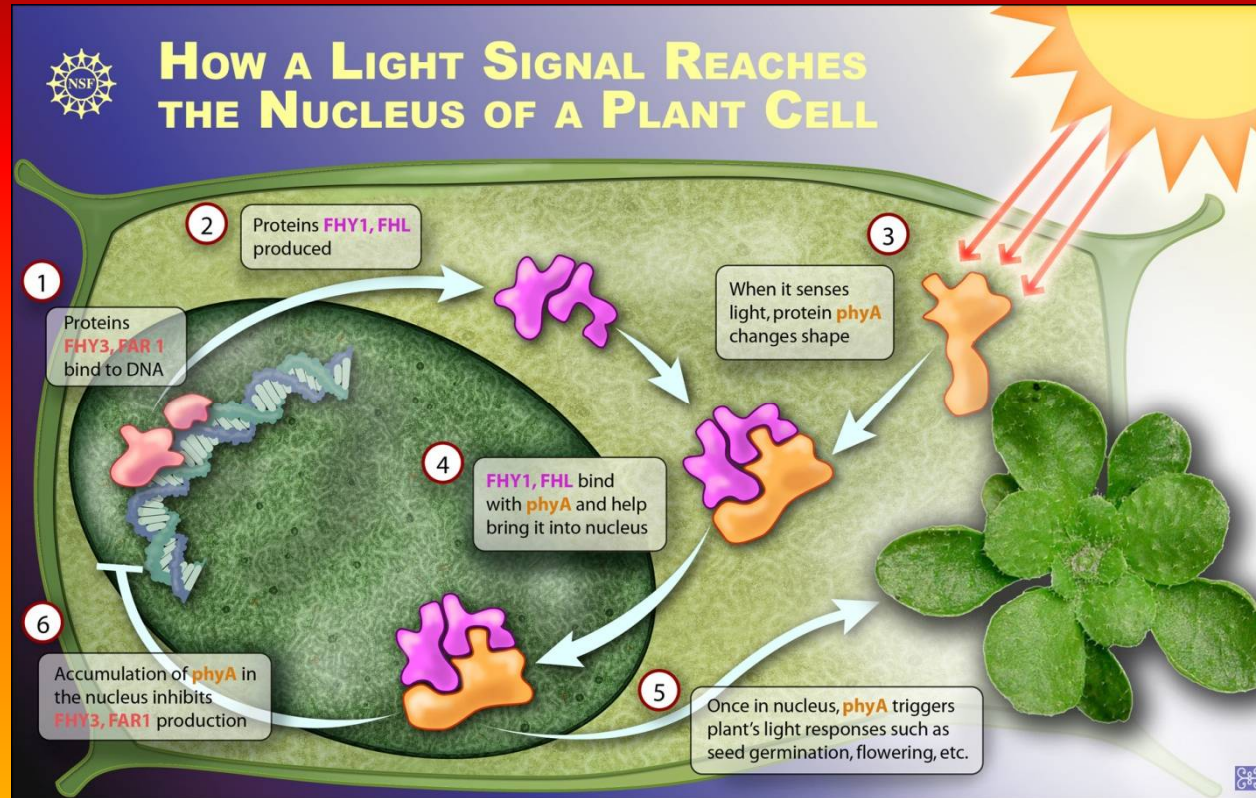
COP1 functions as E3 ubiquitin ligase – enzyme ensuring protein degradation in cell (proteolysis)

Proteolysis mediated by proteasome requires protein **ubiquitin**.

Ubiquitination – general mechanism of protein degradation in organisms



Regulation of transport of phyA into the nucleus



Transcription factors: **FHY3** and **FAR1** – direct (trigger) production of proteins **FHY1** and **FHL**

Proteins: **FHY1** and **FHL** – binding to phyA – regulation of phyA transport into the nucleus

Transport of phyA into the nucleus – triggering of light responses (germination, flowering, etc.) + regulation of production of transcription factors **FHY3** and **FAR1** => feedback: phyA influences its own transport to the nucleus

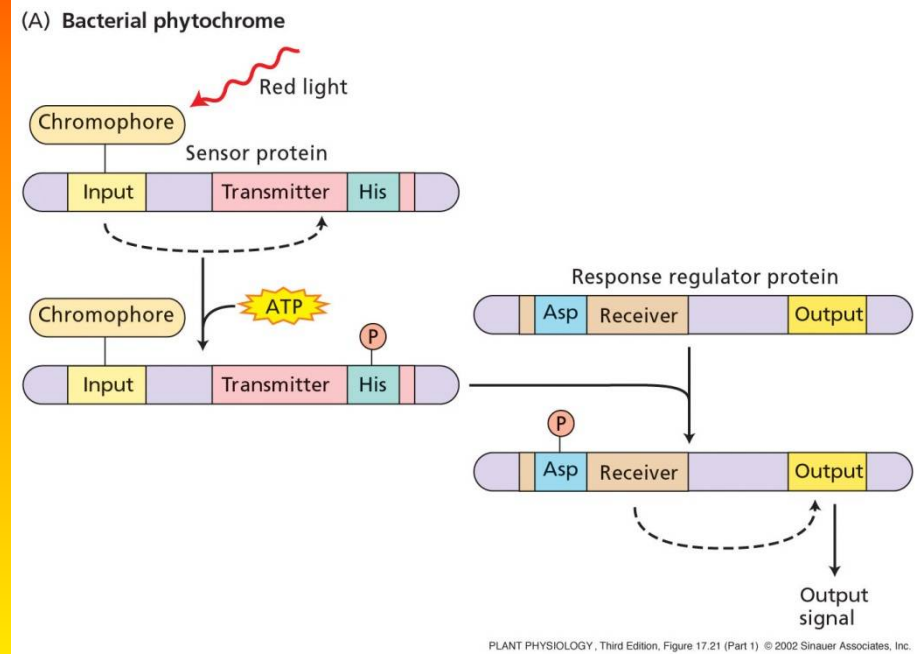
Phosphorylation – important mechanism working in various signaling pathways, including phytochromes

Phosphorylation regulates activity of transcription factors (and other enzymes)

Phosphorylation = attachment of phosphate group to amino acid residue of a protein

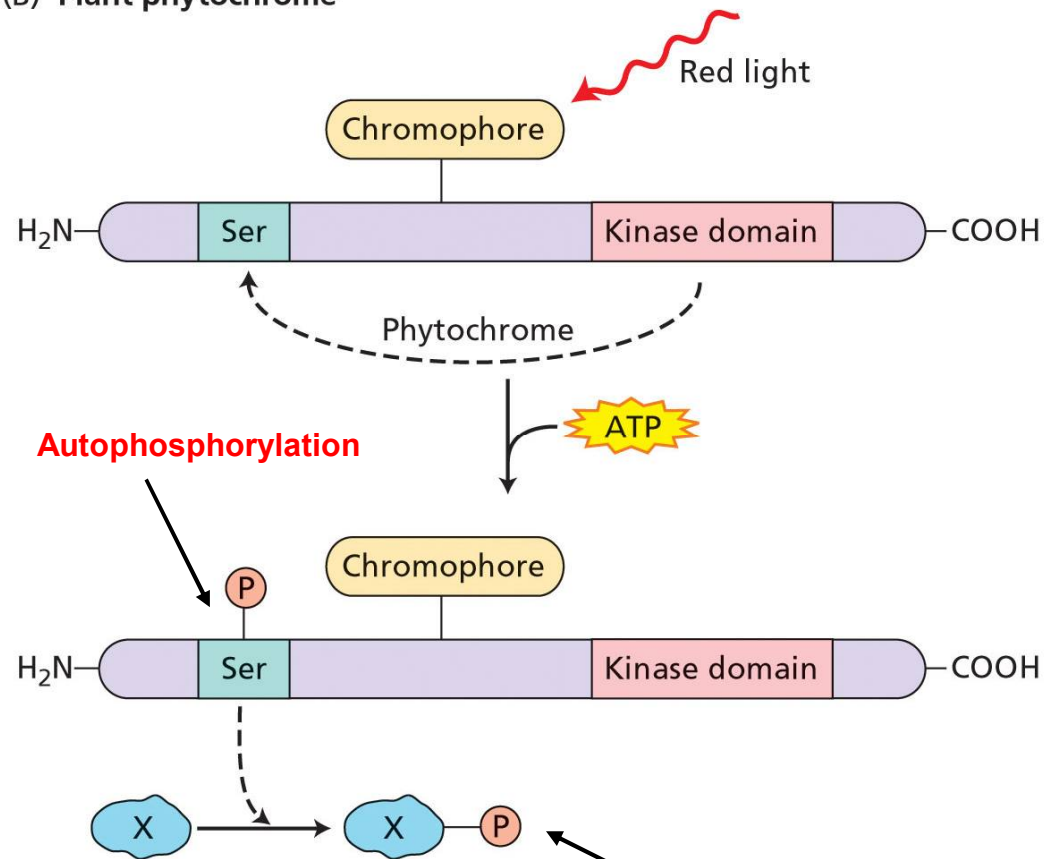
Protein kinase = ATP-dependent enzyme, which attaches phosphate group to protein. Protein becomes phosphorylated and thus is activated.

Bacterial phytochrome = histidine kinase, light-dependent, acts as a sensor protein, phosphorylates regulatory protein



Plant phytochrome = serine/threonine kinase

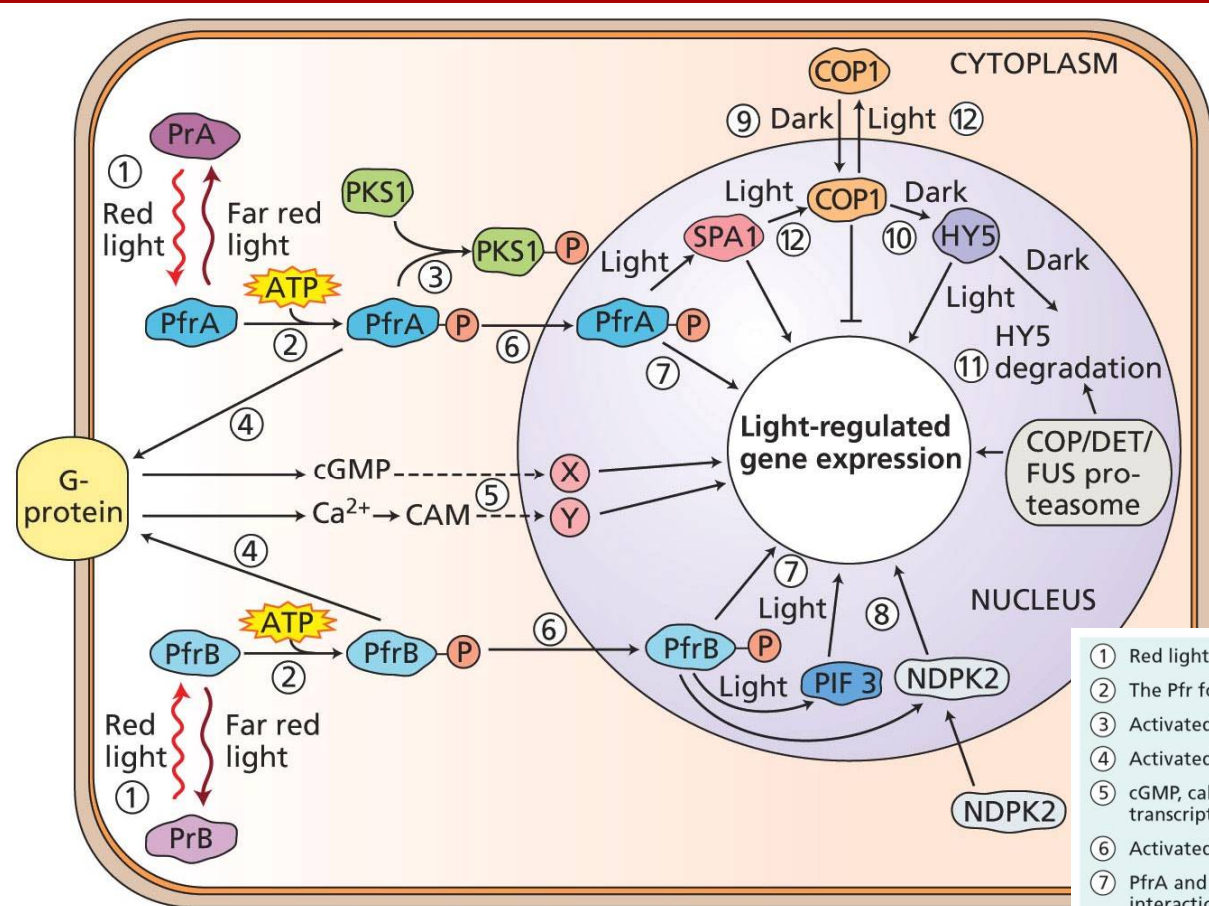
(B) Plant phytochrome



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Phosphorylation of another protein

Factors involved in gene expression regulated by phytochromes



PLANT PHYSIOLOGY, Third Edition, Figure 17.22 (Part 1) © 2002 S

- 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.
- ④ Activated PfrA and PfrB may interact with G-proteins.
- ⑤ cGMP, calmodulin, and calcium 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.
- ⑧ Nucleoside diphosphate kinase 2 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.

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