SFRZE

2022

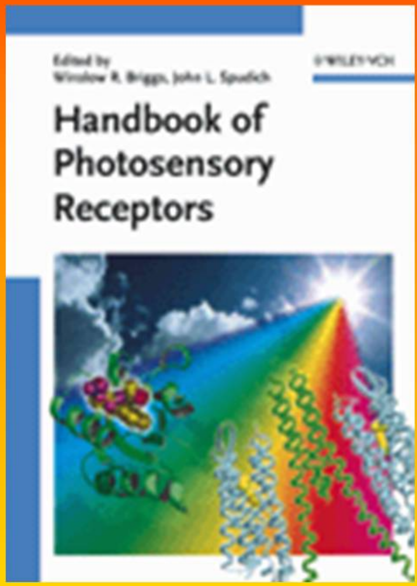
5) Photomorphogenesis

a) Phytochromes

b) Plant responses mediated by phytochromes

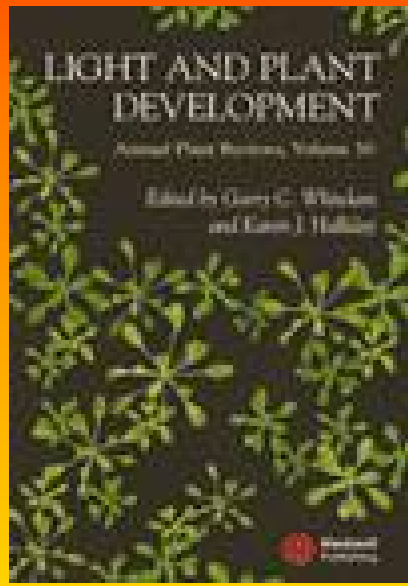
c) Ecological functions of phytochromes

d) Cellular and molecular mechanisms of phytochrome functions



Briggs WR, Spudich 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

Martin Fellner

Growth in the dark
(etiolated plants, skotomorphogenesis)

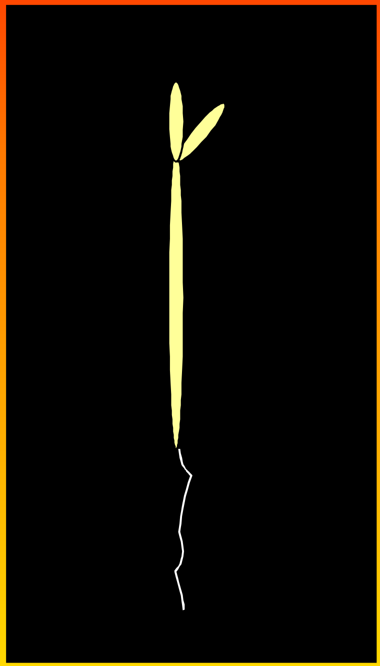
„*Skoto*“ = dark

Growth in light
(photomorphogenesis)

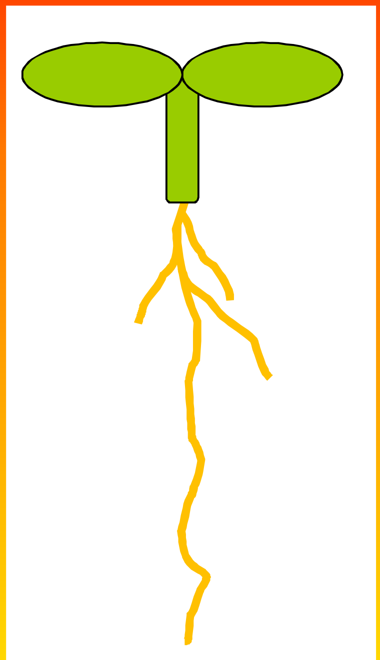
Photomorphogenesis

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 chlorophyl synthesis**
- **Stimulation of leaf growth**

During photomorphogenesis light is capture by pigments that are part of photoreceptors:

- **red light: phytochromes A to E**

- **blie light and UV-A: cryptochromes, phototropins and LOV-domains/F-box proteins:**

  - ZTL (ZEITLUPE, German „slow motion“)
  - FKF1 (FLAVIN BINDING, KELCH REPEAT, F-BOX PROTEIN 1
  - LKP2 (LOV KELCH PROTEIN 2)

- **UV-B: UVR8 (UV RESISTANCE LOCUS 8)**

Update 2020

Liu H et al. (2020) Journal of Integrative Plant Biology 62: 1267-1269

a) Phytochromes

Phytochrome = protein pigment of blue light identified in 1959

Plant responses induced by phytochromes:

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

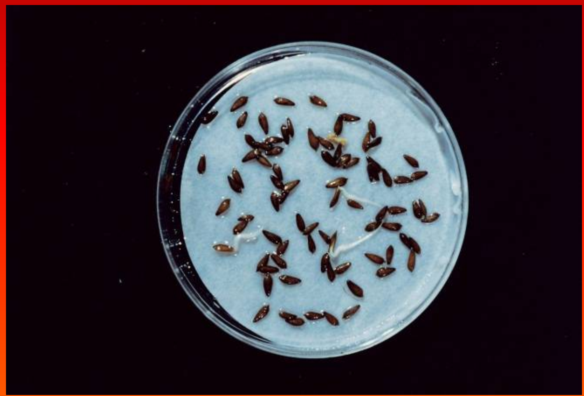
TABLE 17.1
Typical photoreversible responses induced by phytochrome in a variety of higher and lower plants

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

Light perception by receptors and signal transduction differ in various organs

stimulation **inhibition**

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*

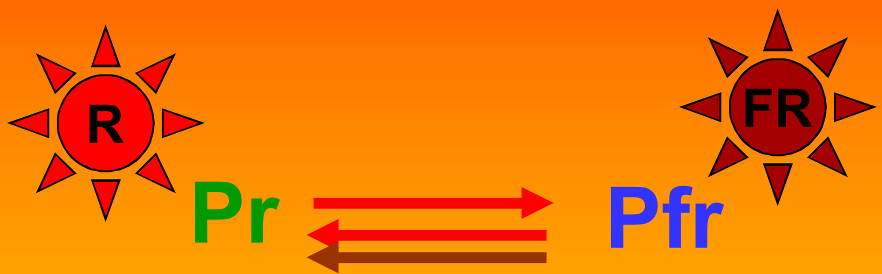
3 following topics

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

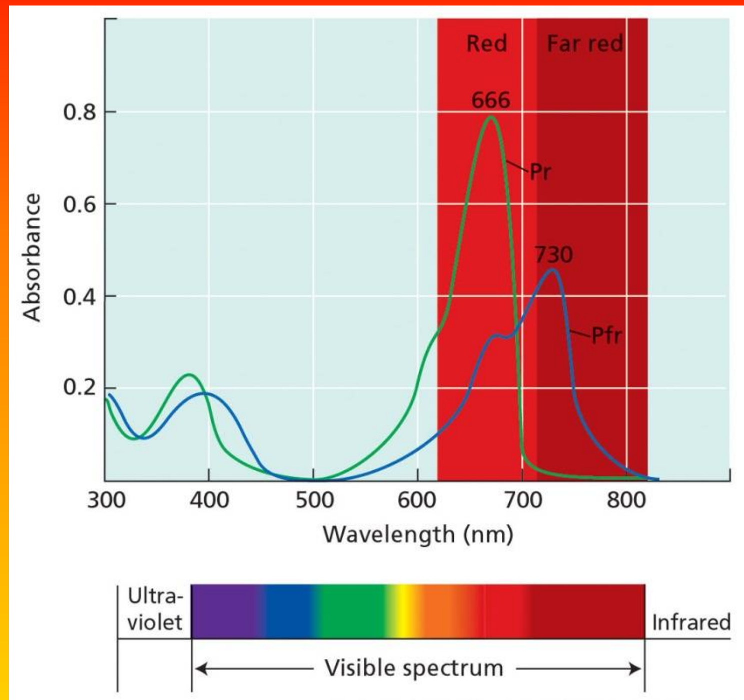
Pr is synthesized in the dark *de novo*



Pr: form of phytochrome absorbing R

Pfr: form of phytochrome absorbing FR and R

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



PLANT PHYSIOLOGY, Third Edition, Figure 17.3 © 2002 Sinauer Associates, Inc.

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

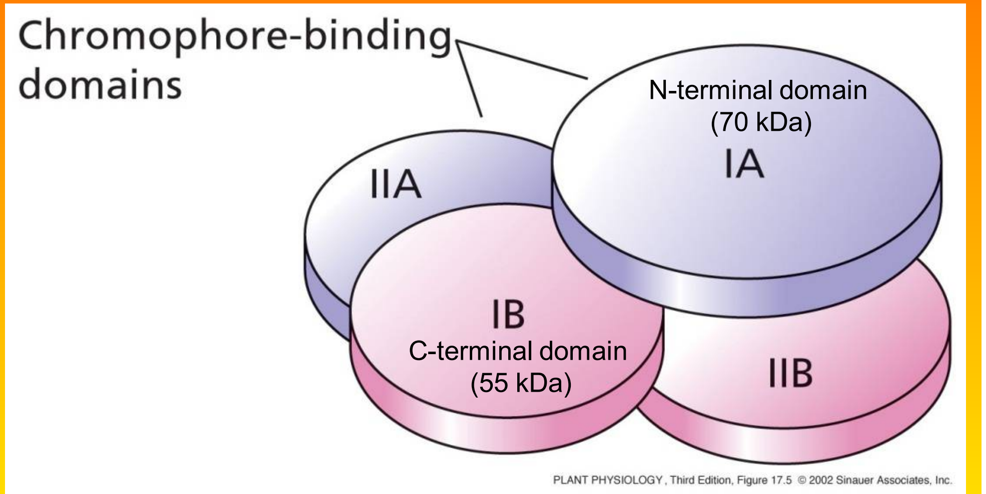
(pigment) (polypeptide, 125 kDa)

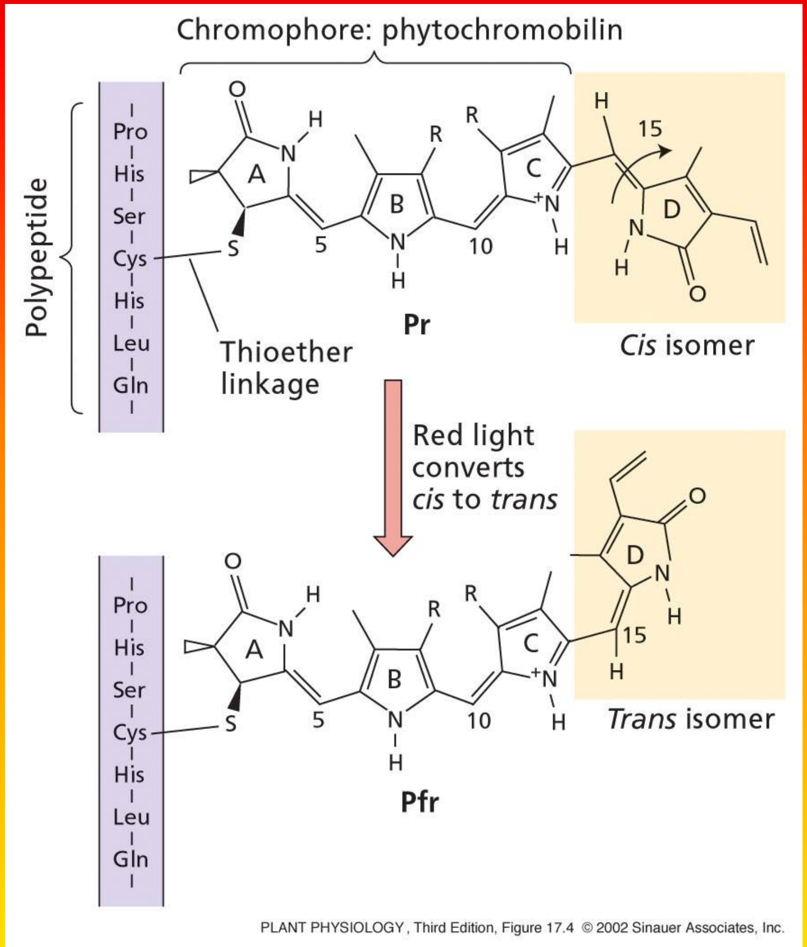
Higher plants:

Chromophore = linear tetrapyrrole = phytochromobilin

Phytochromobilin + apoprotein = holoprotein

Phytochrome dimer



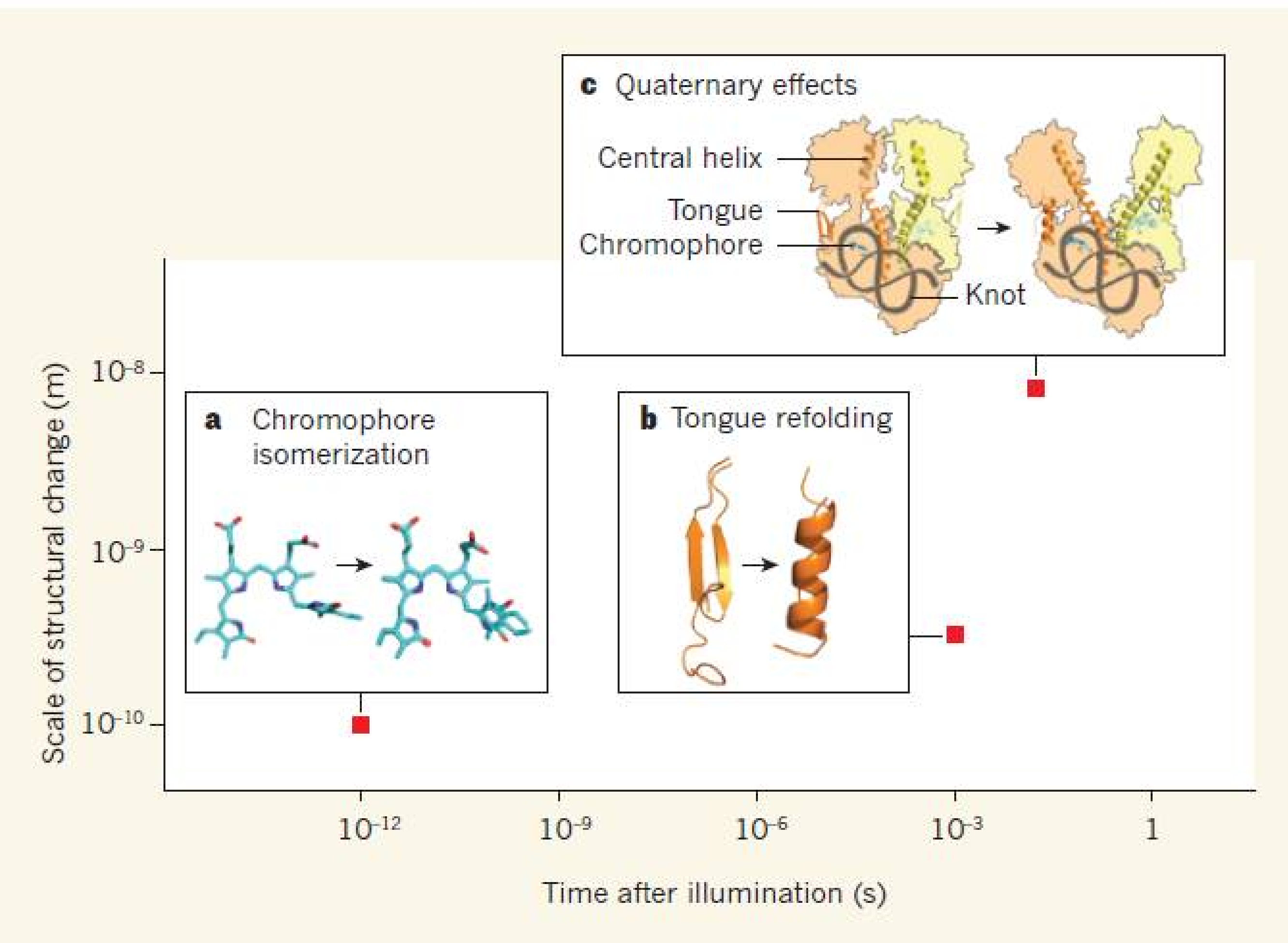


Update 2014

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*



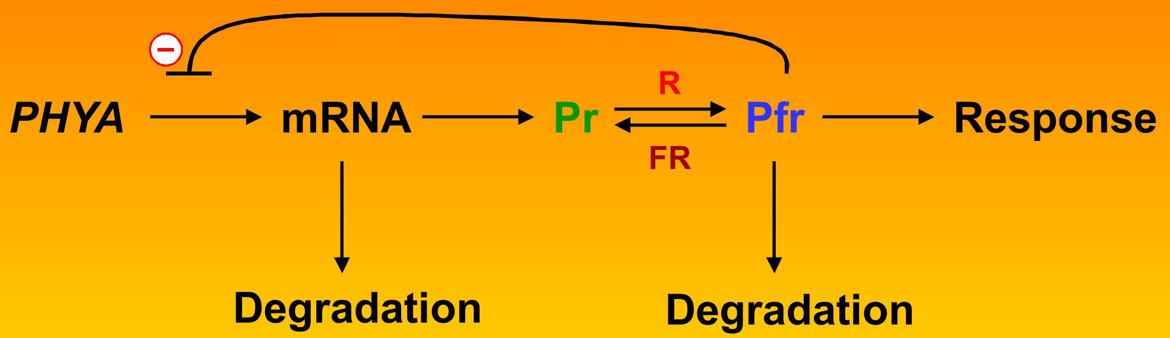
b) Reorganization of key secondary structure „tongue“: structure of β-hairpin changes to the α-helix structure

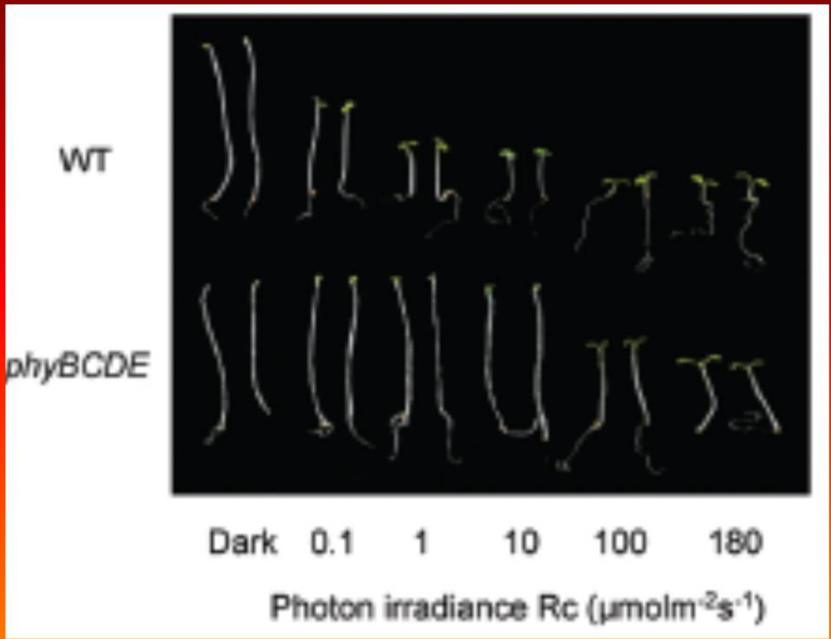
c) Closed quaternary structure of phytochrome (occurring in the dark) open and Y conformation is formed, typical for phytochrome in cells in the light.

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 μmol.m^{-2}.s^{-1}

phyBphyCphyDphyE **– de-etiolation and plant development till flowering**

↓

At high irradiance (over 100 μmol.m^{-2}.s^{-1}):

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

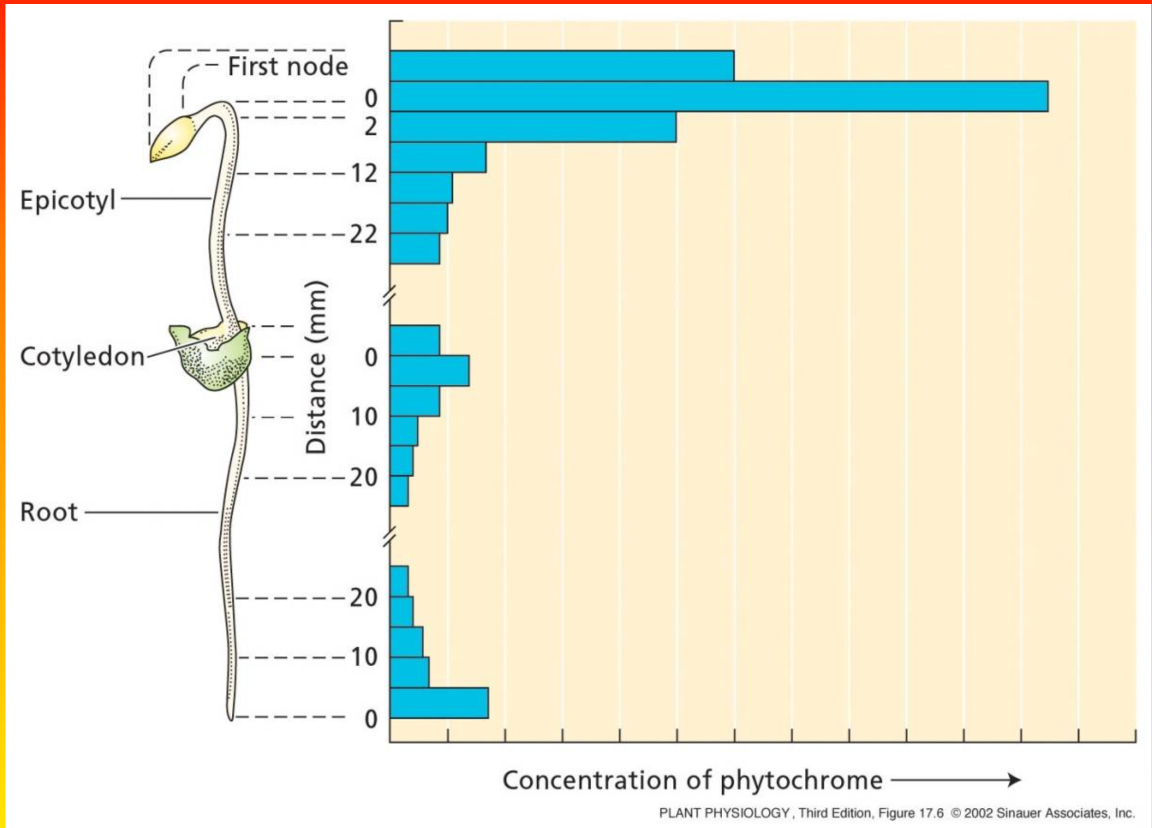
PHYB - E **– expression is not affected by light => transcriptionally active in etiolated and green plants; proteins phyB - E are more stable**

$$\textit{PHYB - E} \longrightarrow \text{mRNA} \longrightarrow \text{Pr} \underset{\text{FR}}{\overset{\text{R}}{\rightleftharpoons}} \text{Pfr} \longrightarrow \text{Response}$$

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)

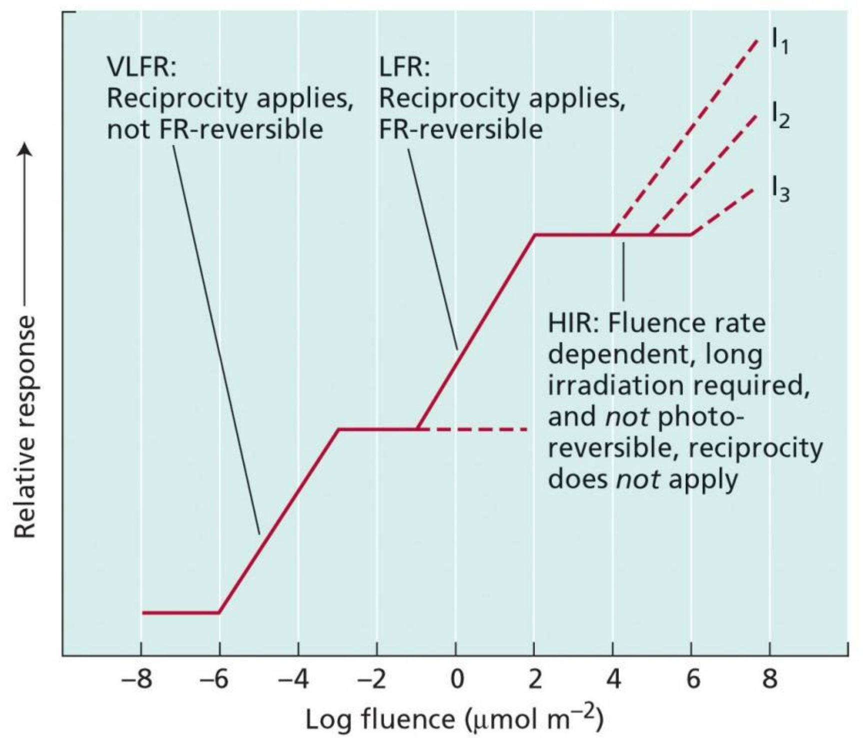
1.0 mmol.m^{-2} to 1000 mmol. m^{-2}

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



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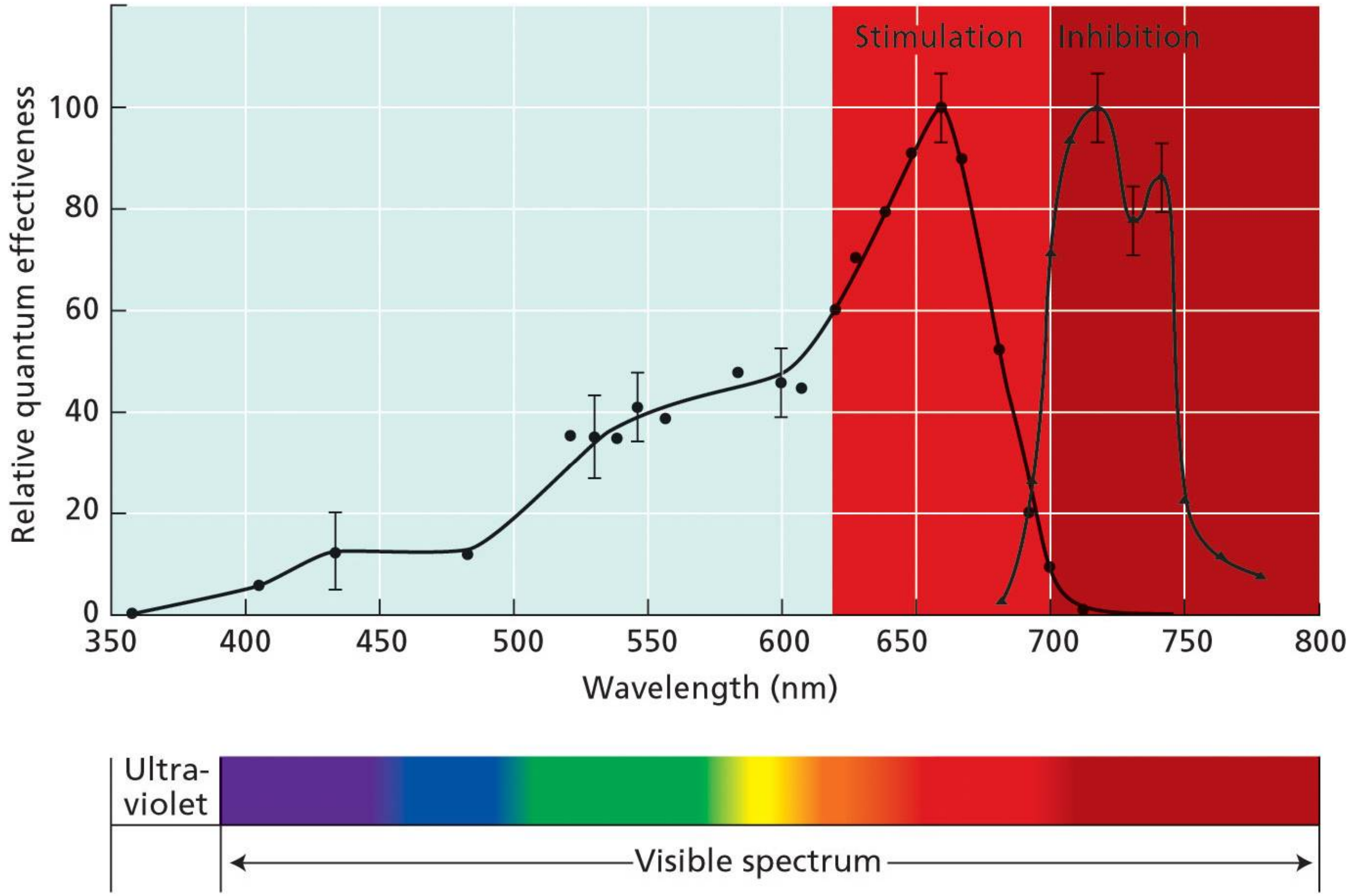
Action spectrum of LFR for photoreversible stimulation and inhibition of *Arabidopsis* seed germination



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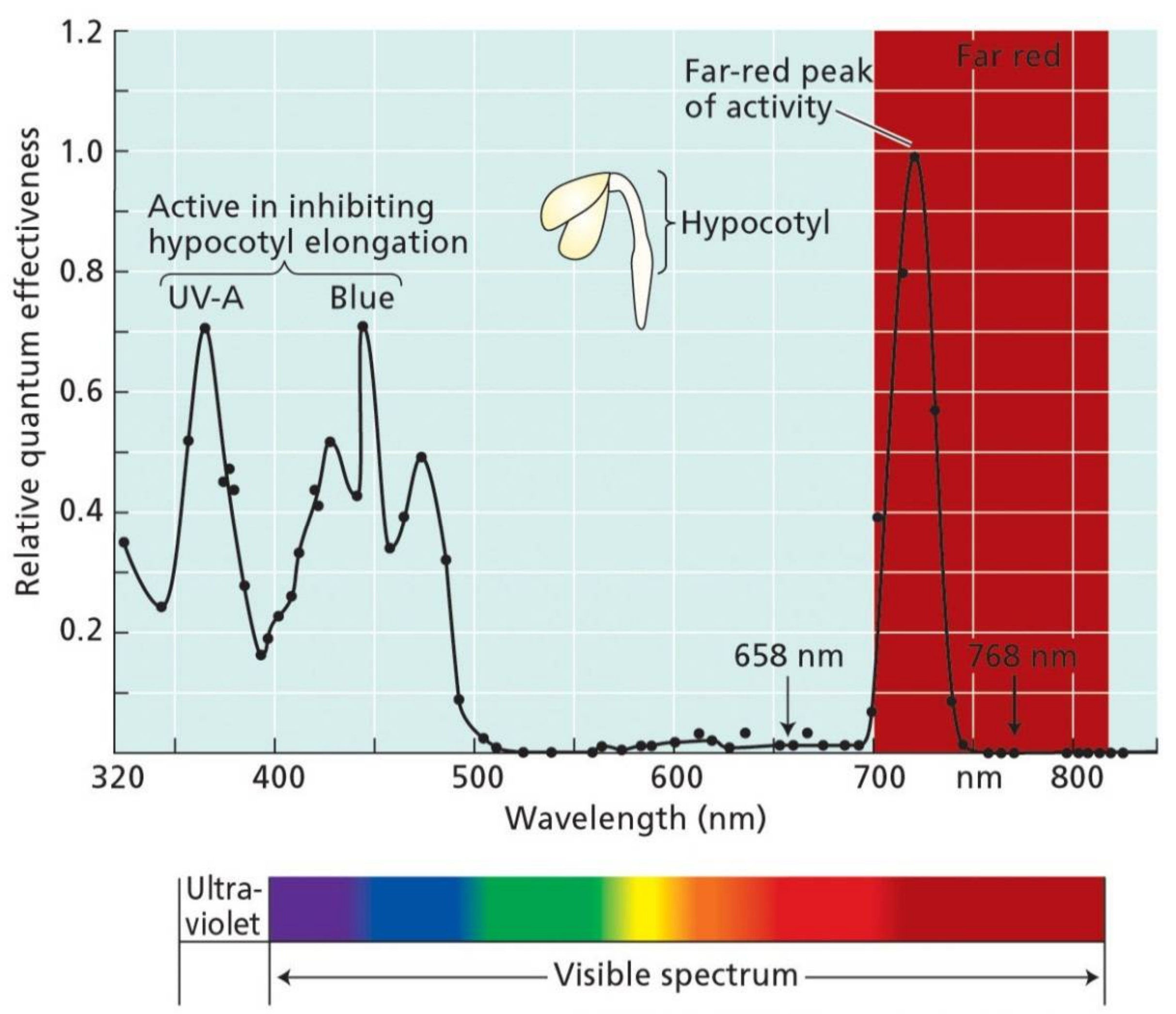
Action spectrum of HIR for inhibition of elongation of etiolated hypocotyl



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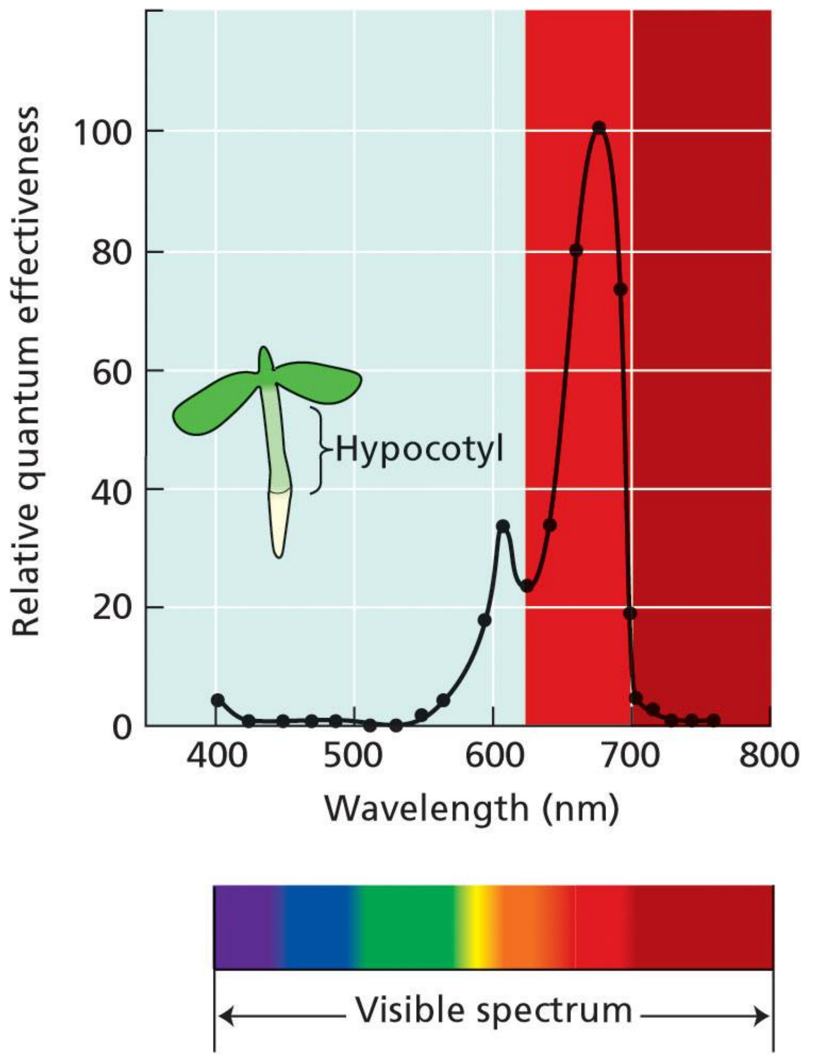
Action spectrum of HIR for inhibition of elongation of green hypocotyl



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

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

R : FR in various environments

TABLE 17.3
Ecologically important light parameters

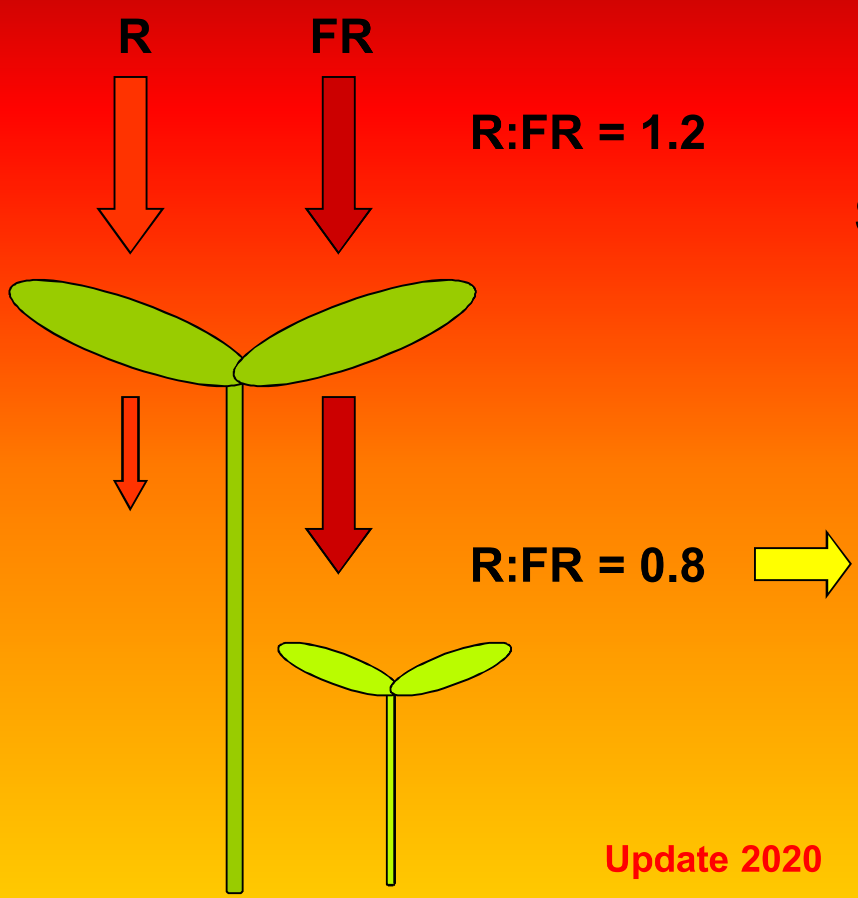
	Photon flux density ($\mu mol\ m^{-2}\ s^{-1}$)	R/FR[a]
Daylight	1900	1.19
Sunset	26.5	0.96
Moonlight	0.005	0.94
Ivy canopy	17.7	0.13
Lakes, at a depth of 1 m		
Black Loch	680	17.2
Loch Leven	300	3.1
Loch Borralie	1200	1.2
Soil, at a depth of 5 mm	8.6	0.88

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.

[a]Absolute 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

Update 2020

Romero-Montepaone S et al. (2020) Plant Cell Environ 43: 1625-1363

Increased environmental temperature increases the response of plants to shading.

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*.

Mutant *phyB*:

- nereaguje na stín
- nereaguje k FR aplikovanému na konci dne
- není schopen reagovat na R/FR reverzibilní indukci klíčení

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

PhyA is receptor continuous FR.

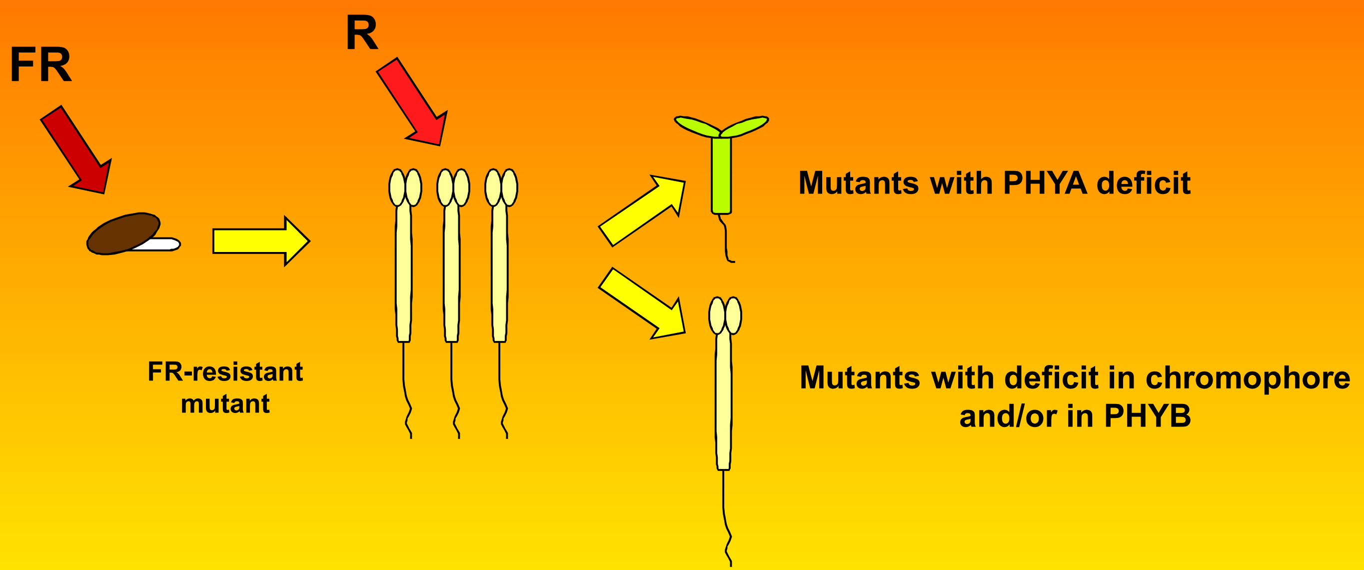
Mutant ***phyA***:

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

=

Phenotype of mutants with defect in chromophore or phyB

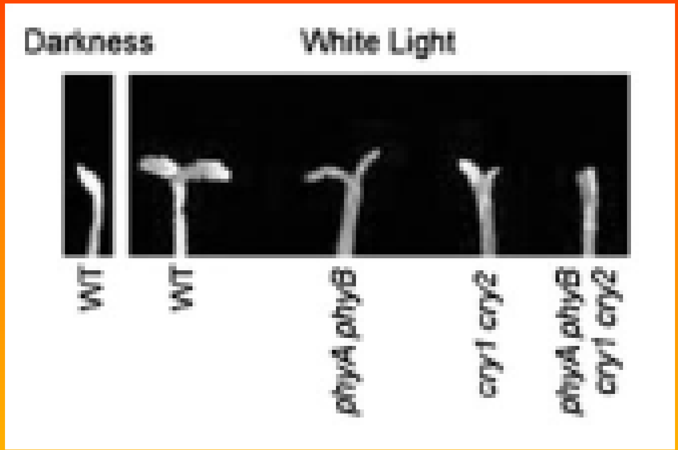
Difficult to select mutants with a defect specifically in protein PHYA



Role of phytochromes C, D a 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



Perelman et al. (2003) Plant Physiol 133: 1717-1725

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

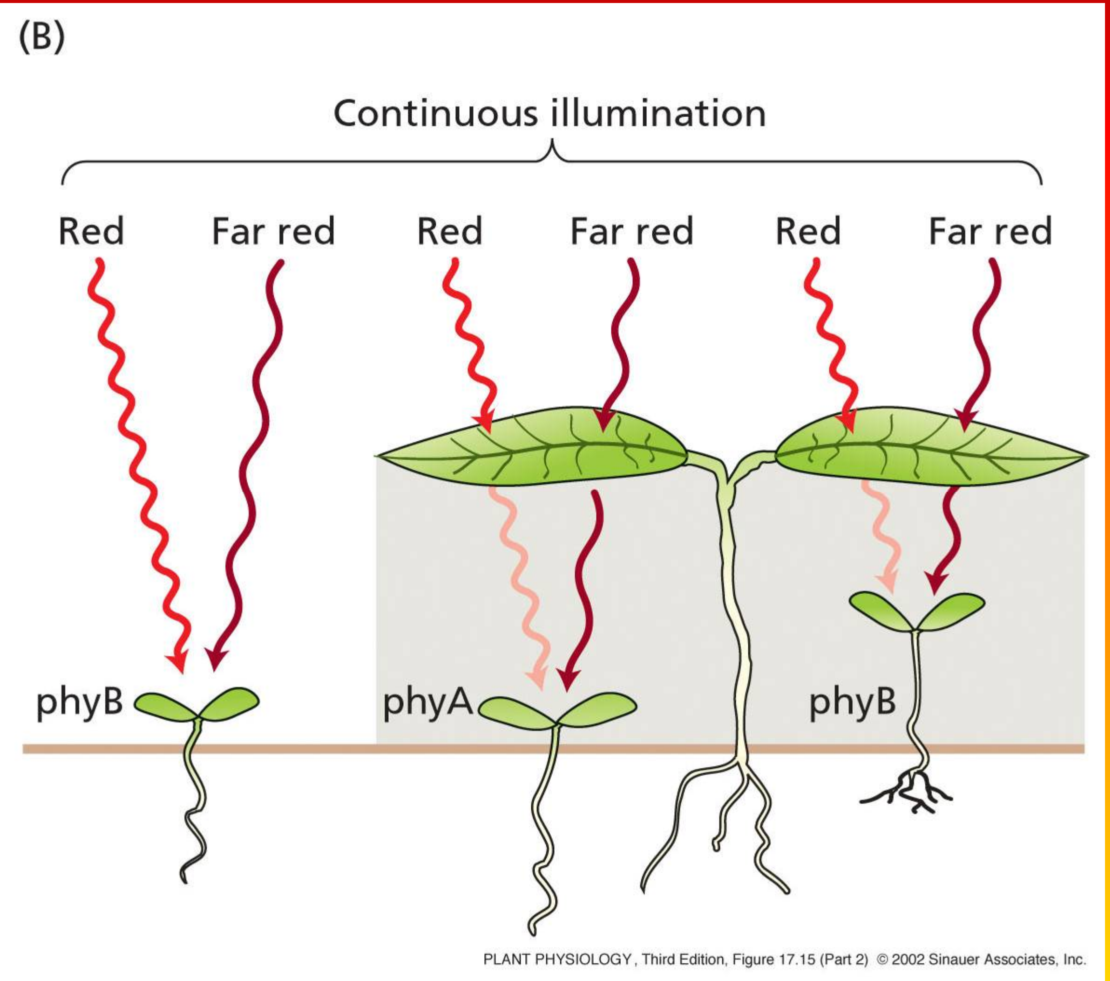
⇓

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

Update 2016

Montgomery BL (2016) Frontiers in Plant Science 7, art. no. 480
Kong S-G, Okajima K (2016) Journal of Plant Research 129: 111-114

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

Light

pigment → C-terminal sequence

Other elements of signaling pathway

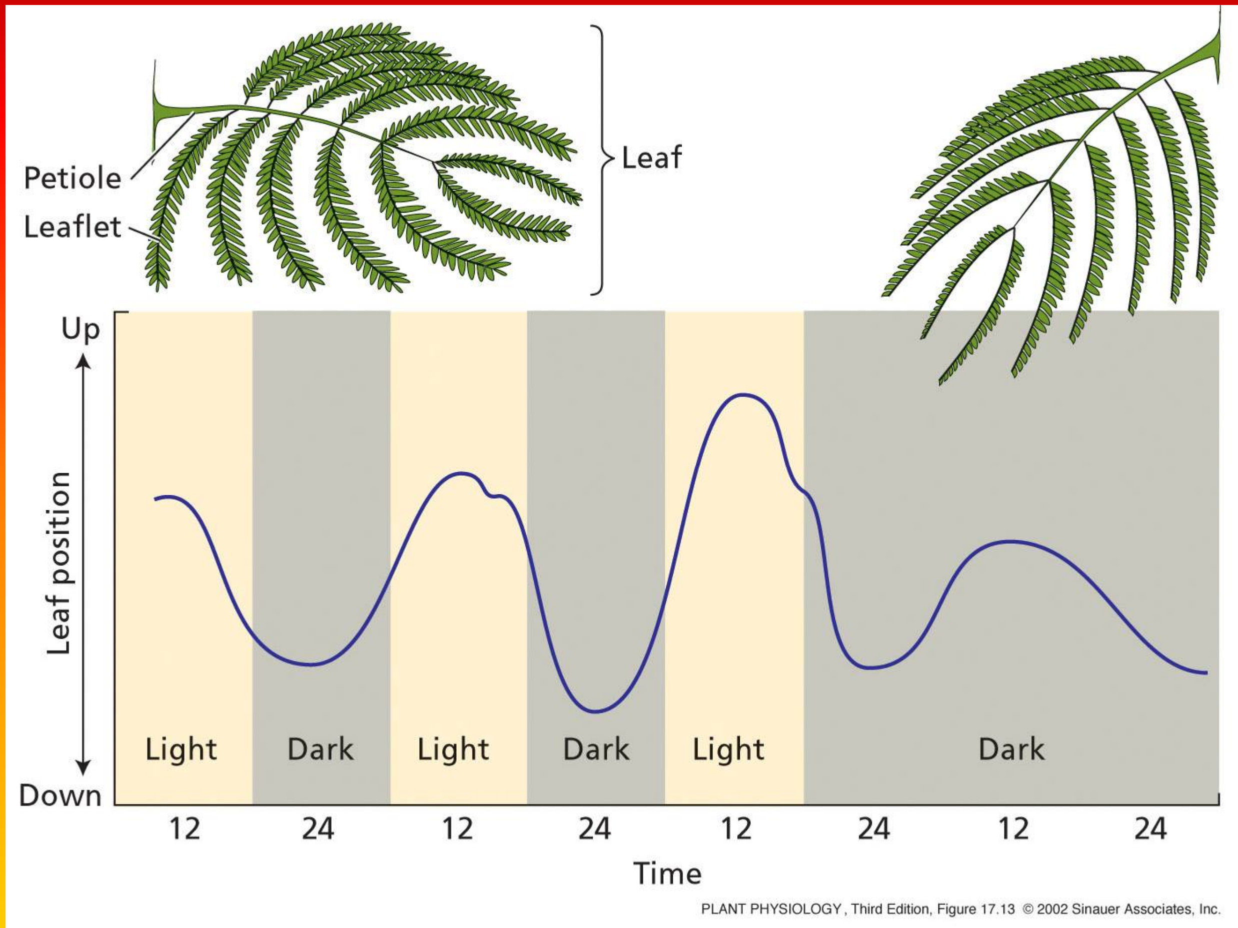
Final response = changes in growth and development

Fast responses
(turgor-ion flux)

Slower responses
(long-term, also gene expression)

Fast responses

Nyctinastia of the leaves and petals of the shy mimosa (*Mimosa)* – sleeping movement, e.g. circadian movement (alternation of maximum and minimum during 24 hrs)

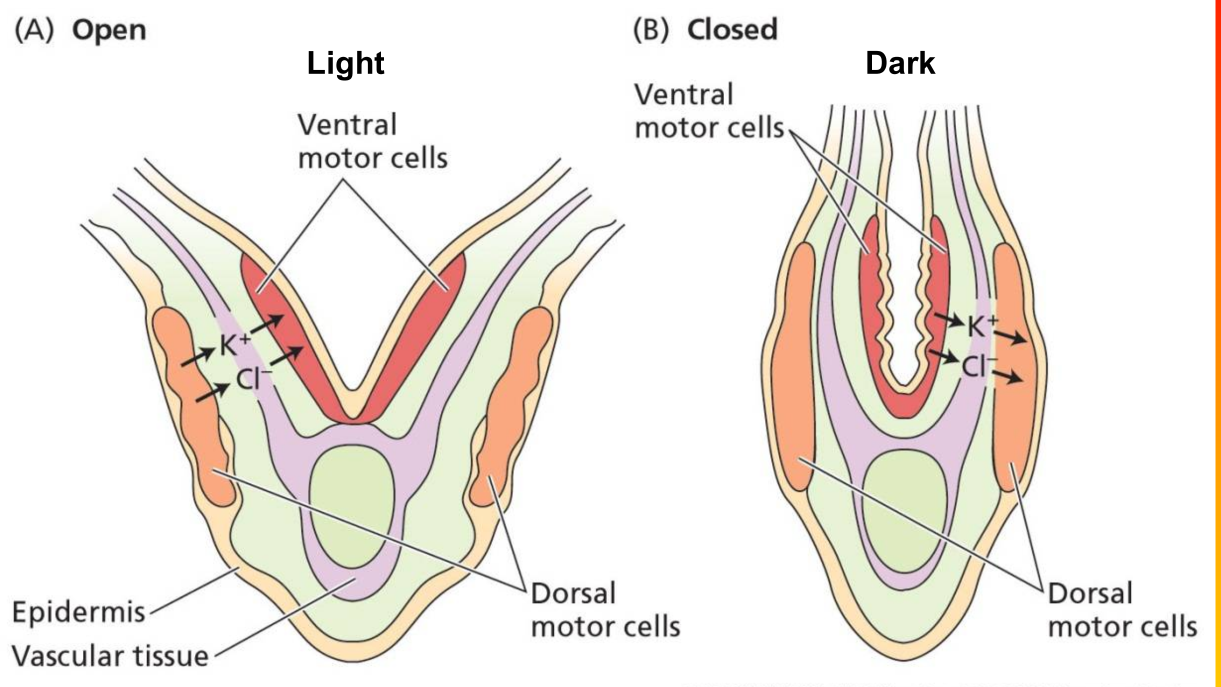


R (red) and B (blue) light stimulates leaf opening; FR cancels the R effect **=> Involvement of phytochromes**

Physiological mechanism of leaf movement – changes in pulvinia cell turgor

Changes in turgor of the dorsal and ventral cells = changes in flow of K^+ and Cl^-

Acumulation of K^+ and Cl^- in ventral cells => cell enlargement => leaf opening
Ventral cells are losing K^+ and Cl^- => cell shrinkage => leaf closure



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Phytochrome-mediated of membrane potential and ion flux

Lag phase of lef closure ~ 5 min => short time for gene expression => direct induction of membrane permeability change via pytochromes

Slow responses

Phytochrome regulates gene expression

Processes of photomorphogenesis and de-etiolation ⇨ **Formation and degradation of chlorophyll, changes in metabolic processes**

⇩

Requirement of gene expression

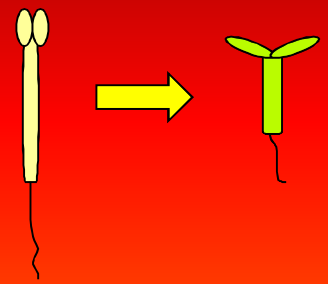
Phytochrome directs activation of transcription factors (TF). TF enter nucleus and stimulate transcription of specific genes.

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

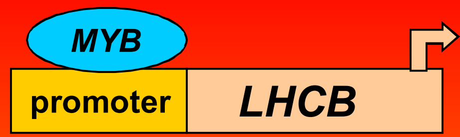
Expression of late genes = genes of secondary response – dependent on protein synthesis (*LHCB* genes)

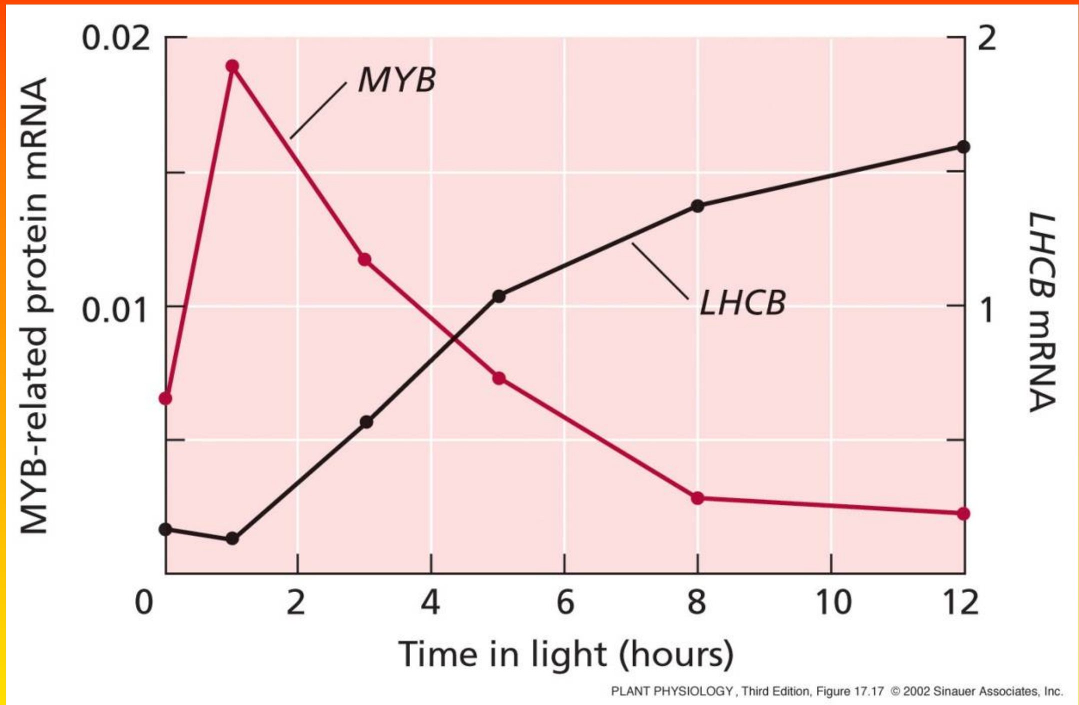
Phytochrome-directed regulation of expression of genes *MYB* and *LHCB*

Dark **Light**



Phytochrome → Transcription factor MYB → *LHCB*





***MYB* – genes of primary response**

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

CCA1 (*circadian clock associated1*) (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

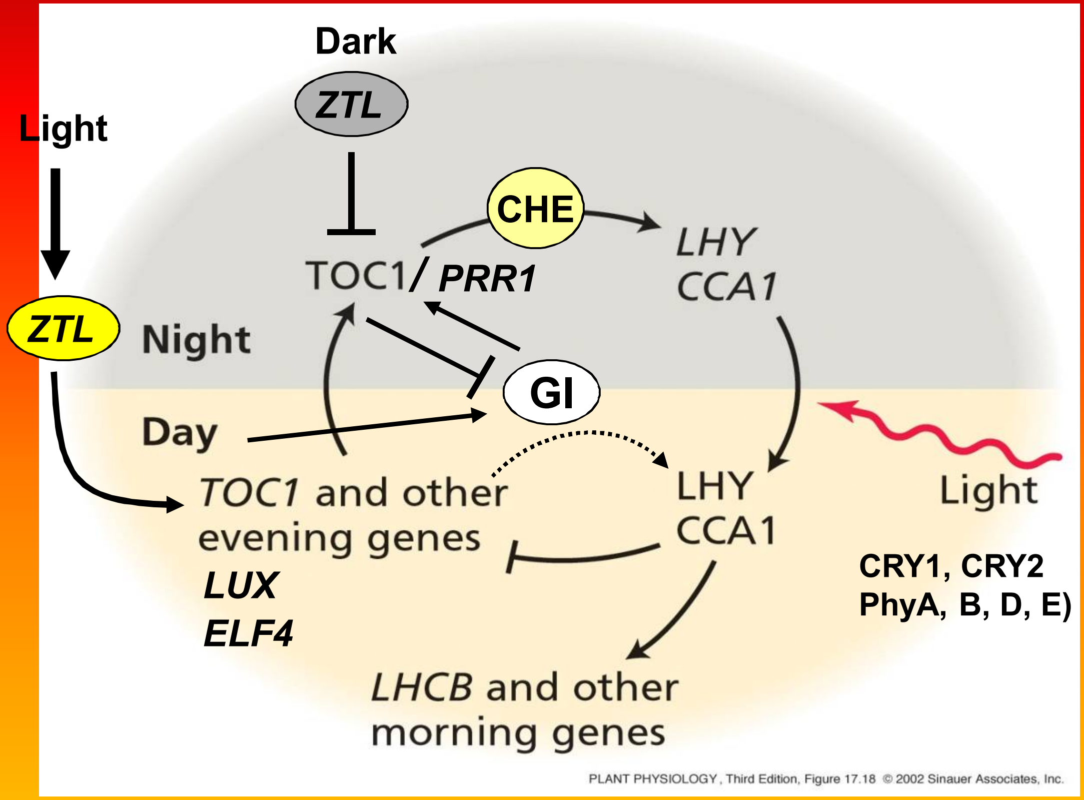
LHY (*late elongated hypocotyl*) (belongs to *MYB* genes) – transcript oscillates with circadian rhythm

↓

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

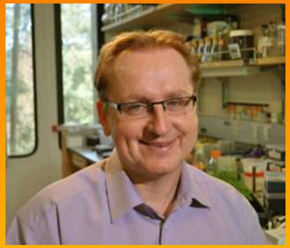
Circadian oscillator in *Arabidopsis*



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.

TOC1=Timing of CAB expression

Update 2015 a 2016

Romanovski A, Yanovsky MJ (2015) Frontiers in Plant Science 6: 1-11
Nohales MA, Kay SA (2016) Nature Structural & Molecular Biology 23: 1061-1069

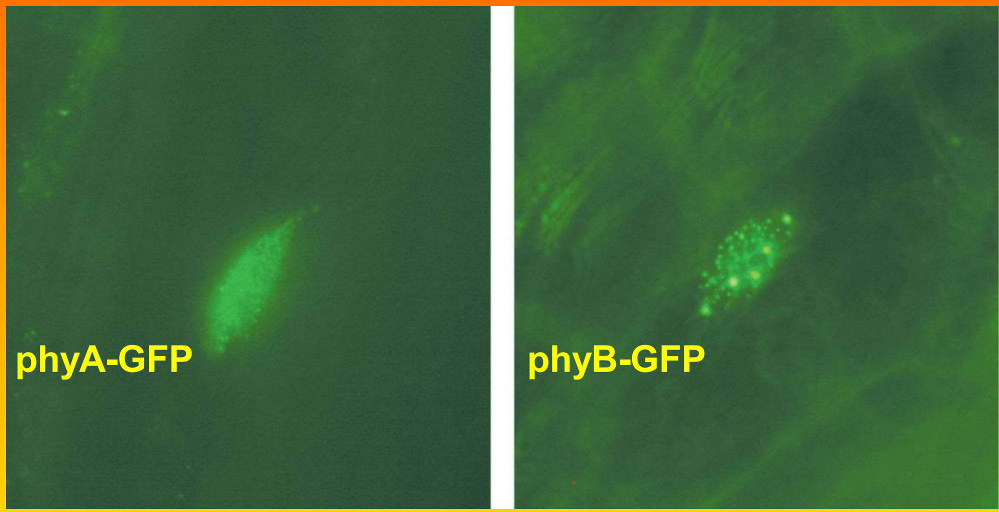
Phytochrome functions in the nucleus – activates transcription factors. However, it is localized in cytoplasm => must be moved to the nucleus

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

Promoter *PHYB*	*GFP*

⇩

Plant transformation

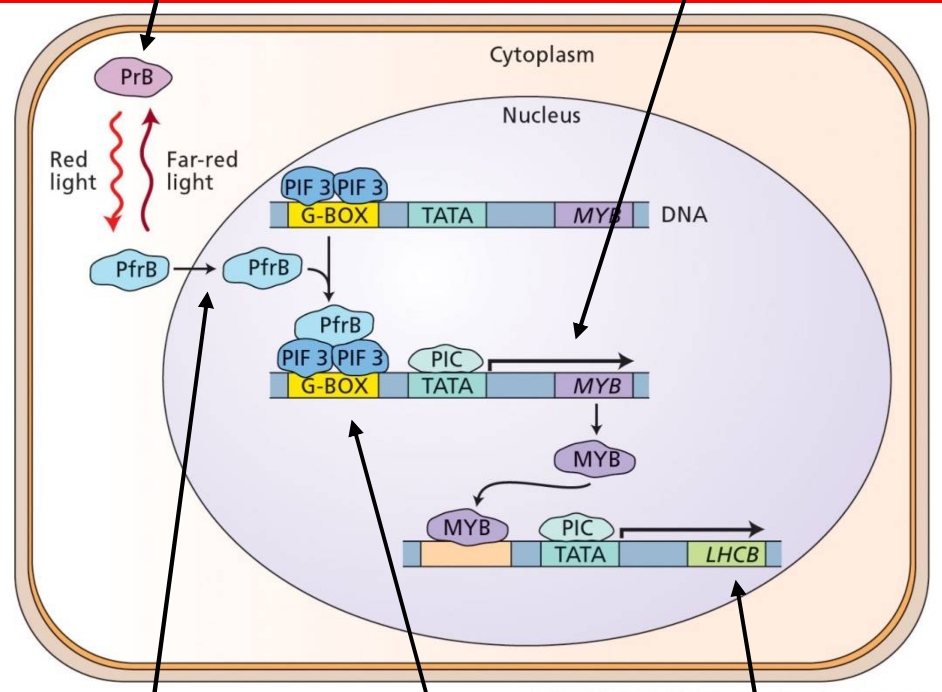
⇩

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)

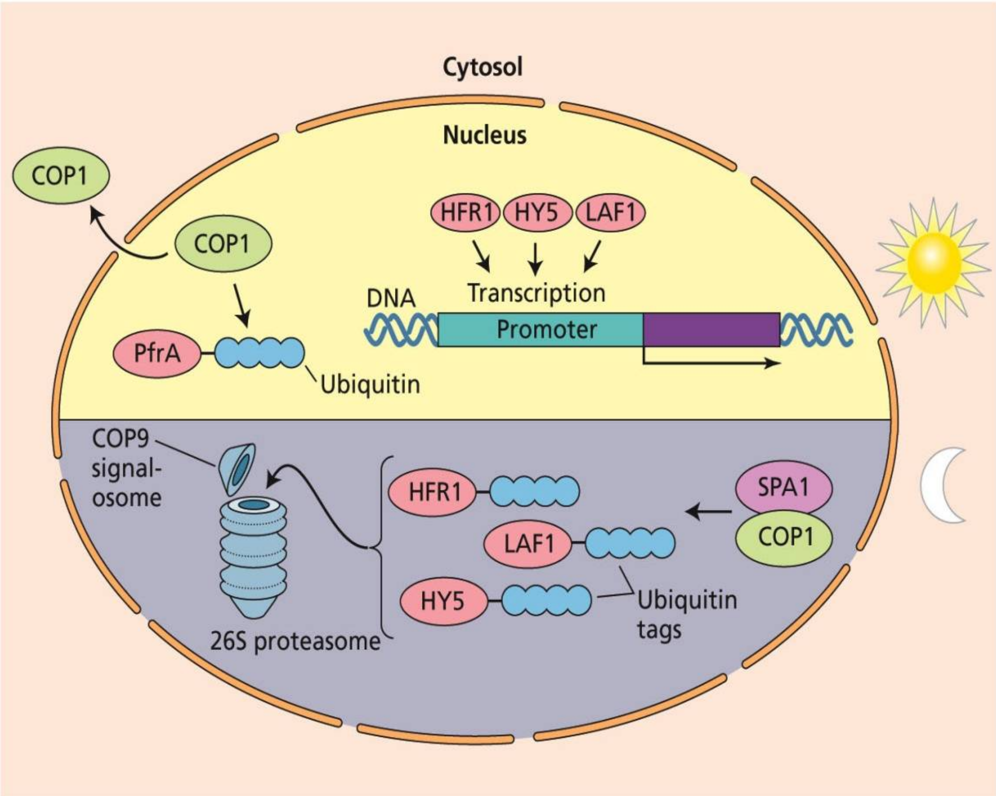
- Transcription factor bHLH interacting with G-box (= part of promoter of *MYB* gene); necessary for skotomorphogenesis

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

PIC – Pre-Initiation transcription Complex

Regulation of gene expression by phytochrome A

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



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 aktivity in the dark is enhanced by SUMOylation by the E2 sumo-conjugating enzyme SCE1 and E3 ligase SIZ1.

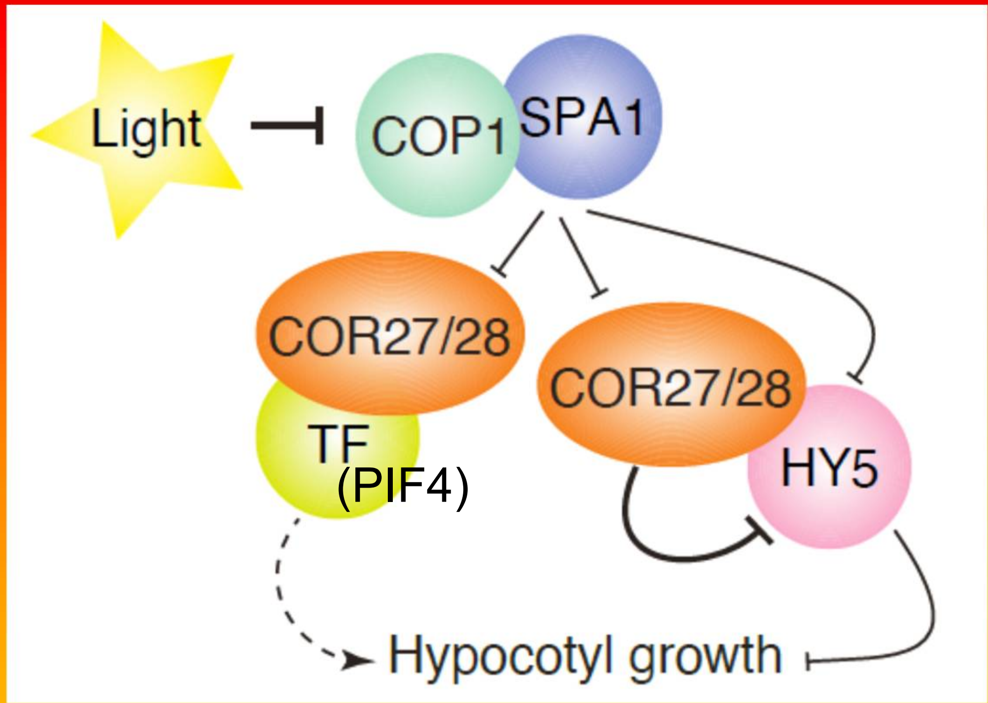
Update 2020

Li X et al. (2020) Plant Cell 32: 3139-3154

Zhu W et al. (2020) Plant Cell 32: 3155-3169

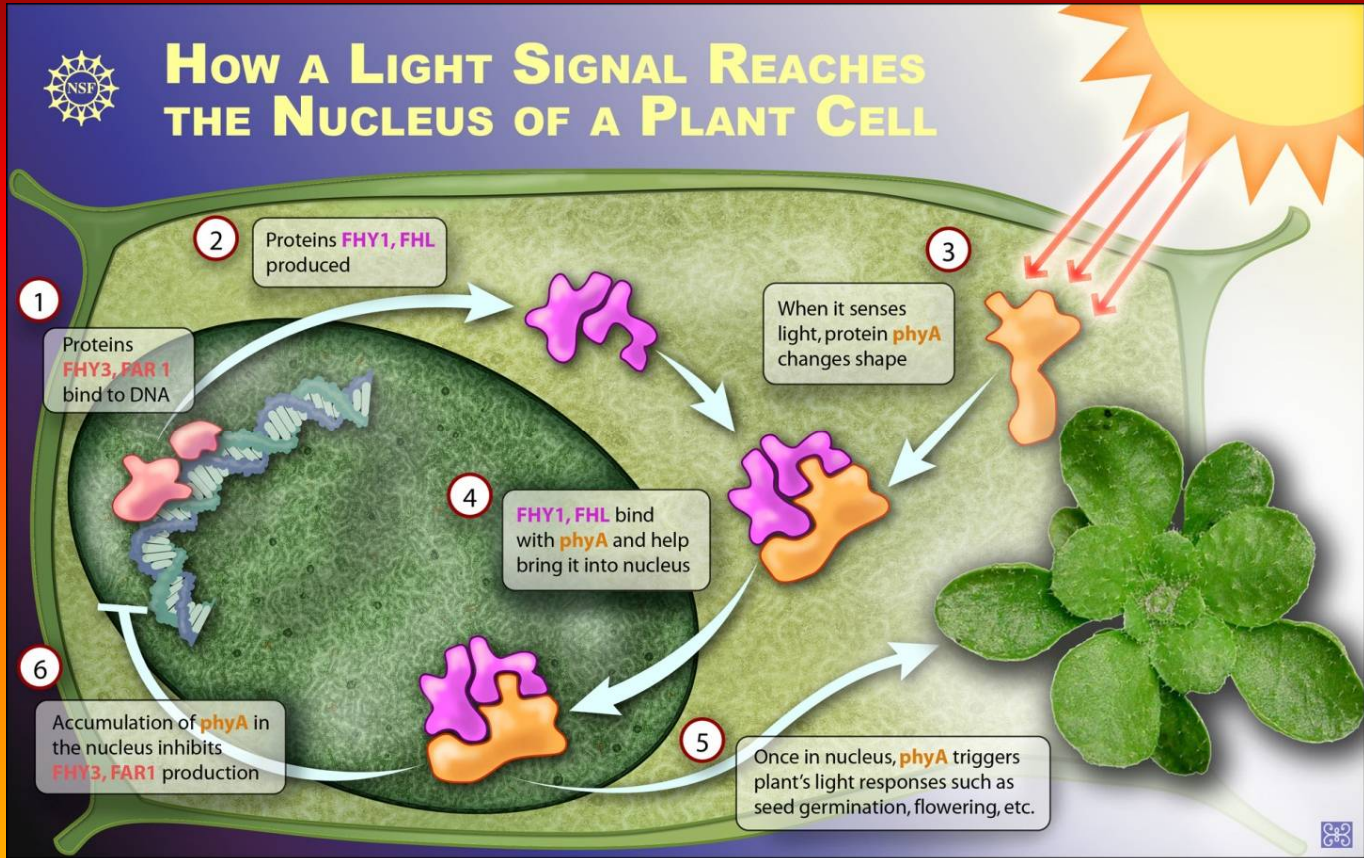
Negative regulators of photomorphogenesis: proteins COR27 and COR28

COR – COLD REGULATED – proteiny regulované chladem, cirkadiálními rytmy a světlem



During photomorphogenesis, elongation growth is inhibited. However, the elongation is simultaneously stimulated by the influence of COR27 and COR28 because the activity of the HY5 proteins (positive regulator of photomorphogenesis) is deactivated by the influence of both COR proteins. At the same time, the transcriptional activity of PIF4 (a negative regulator of photomorphogenesis) is stimulated and thus the hypocotyl elongates.

Regulation of phytochrome A transport into the nucleus by influence of light



Transcription factors: FHY3 and FAR1 – control (trigger) protein production FHY1 and FHL

Proteins: FHY1 and FHL – binding to phyA – regulation of phyA transport into the nucleus; FR simultaneously stimulates SUMOylation of FHY1 and causes its degradation => fine tuning (fine regulation) of FR signaling (Qu et al. 2020).

Transport of phyA to the nucleus – triggering of light reactions (germination, flowering, etc.) + regulation of the production of transcription factors FHY3 and FAR1 => a feedback: phyA affects its own transport into the nucleus *cop1* (*constitutive photomorphogenesis 1*) – etiolated plants show phenotype of plants growing in light

Xing-Wang Deng
Yale University, New Haven

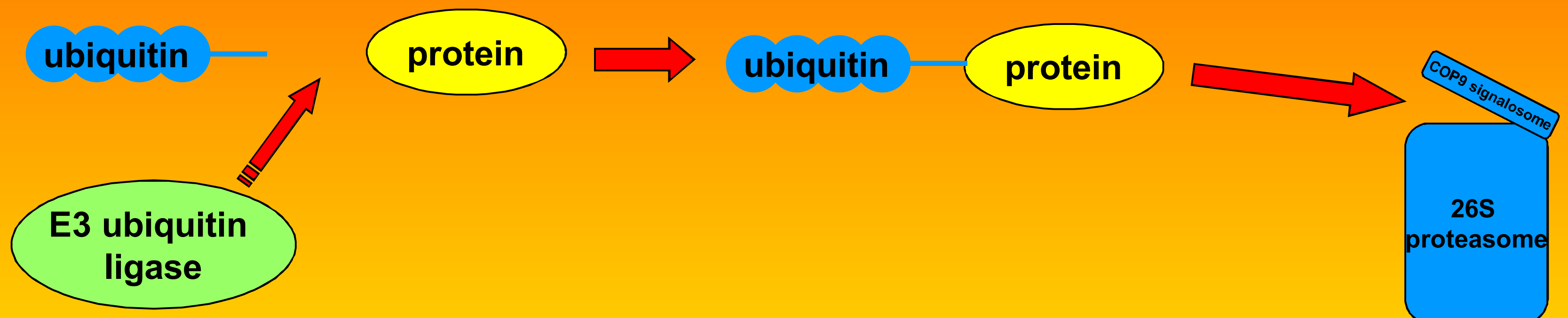
Nonmutated plant   Mutant *cop1*

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

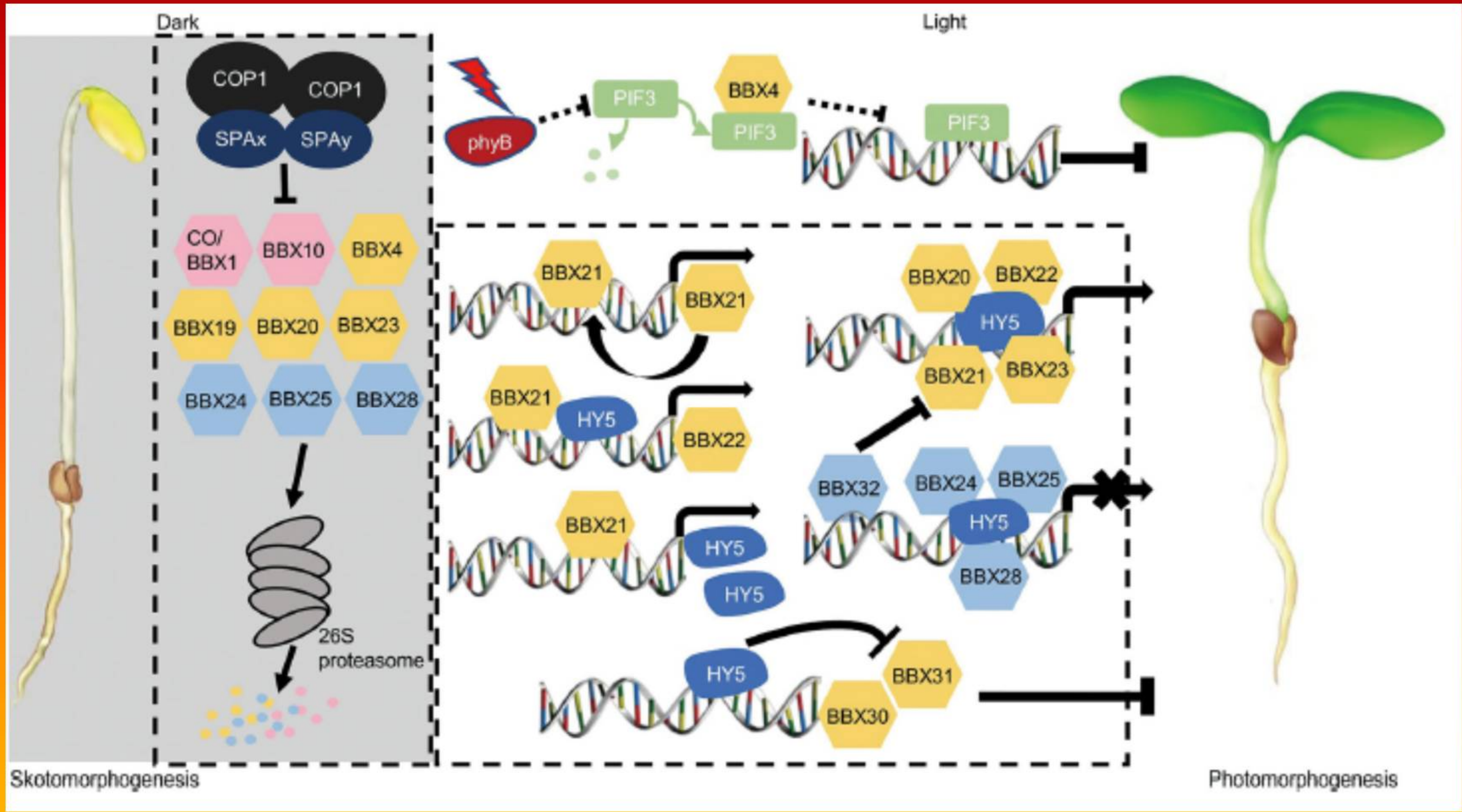


B-box proteins (BBX) – key elements in process of photomorphogenesis

Zinc-coordinated transcription factors contanin at least one B-box domain

Update 2020

Song Z et al. (2020)
J Int Plant Biology 62: 1293-1309



PIF3 – negative regulator of photomorphogenesis

BBX4, BBX20 to BBX23 – positive regulator of photomorphogenesis

BBX24, BBX25, BBX28, BBX 30 to BBX32 – negative regulator of photomorphogenesis

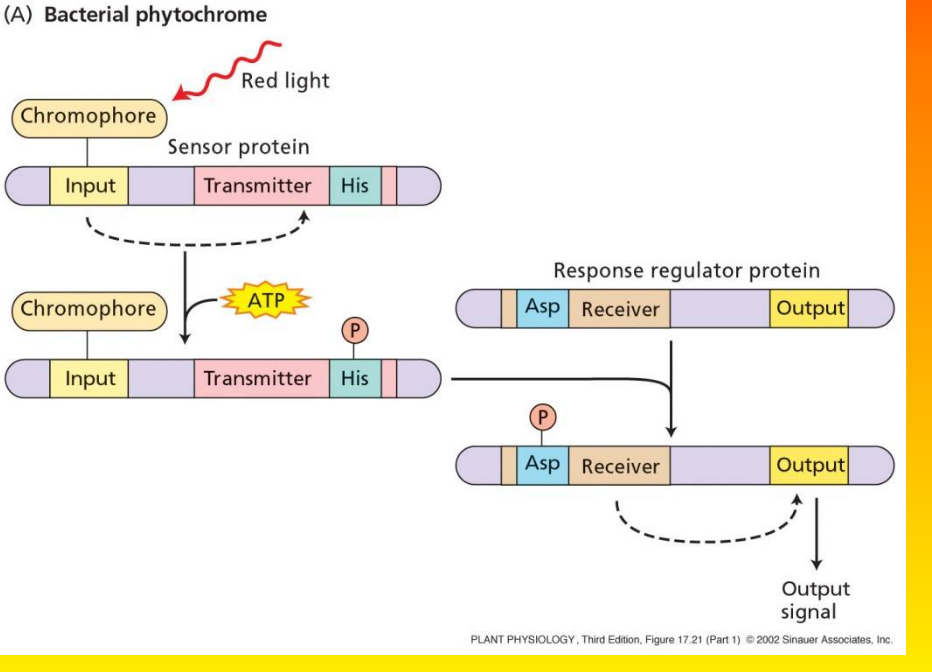
Phosphorylation – important mechanism working in various signaling pathways, including phytochromes

Phosphorylation regulates activity of transcription factors (and other enzymes)

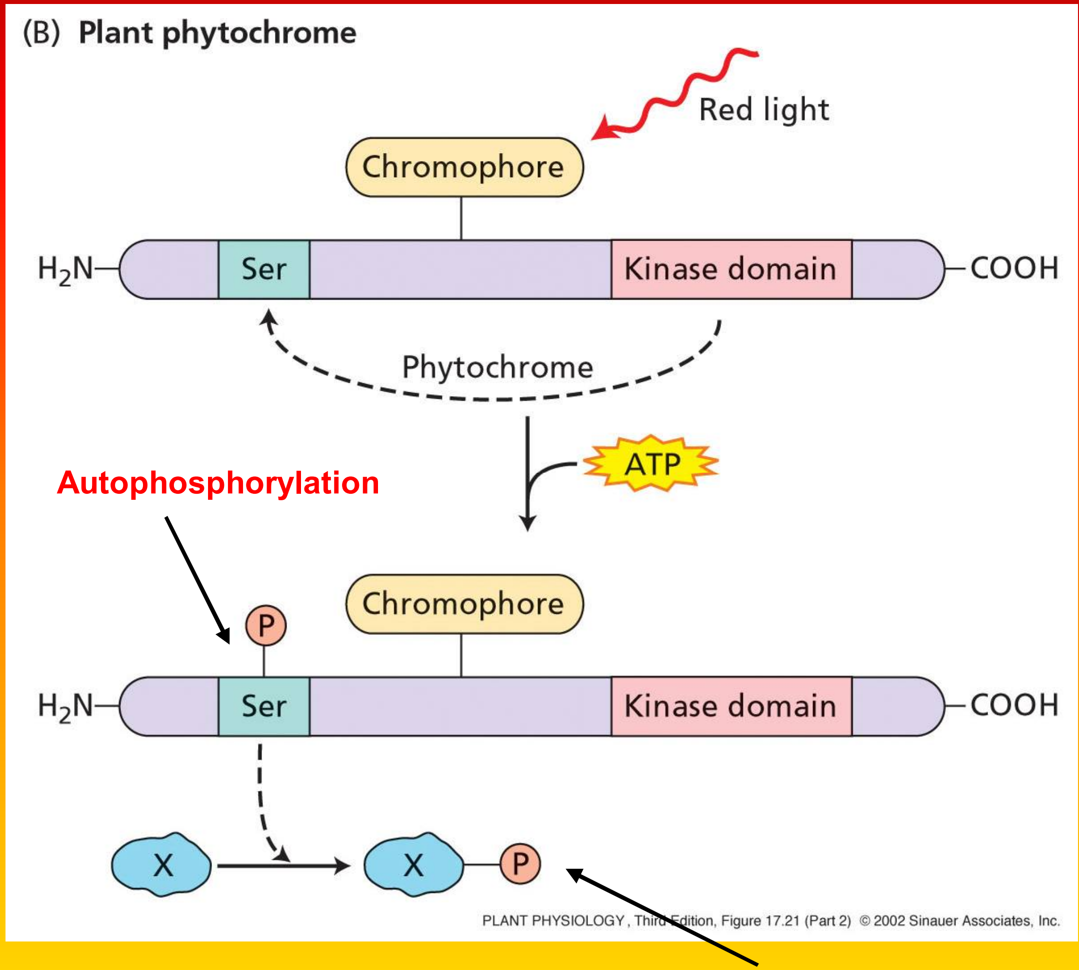
Phosphorylation = attachment of phosphate group to amino acid residue of a protein

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



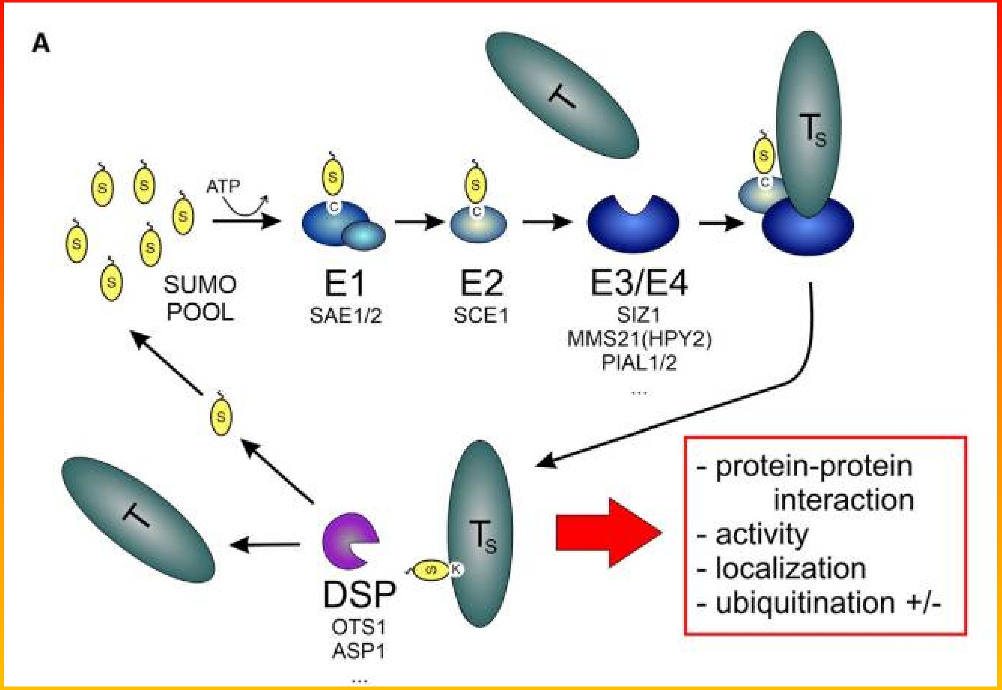
PKS1 (phytochrome kinase substrate) – protein fosforylován fytochromem A v cytoplazmě

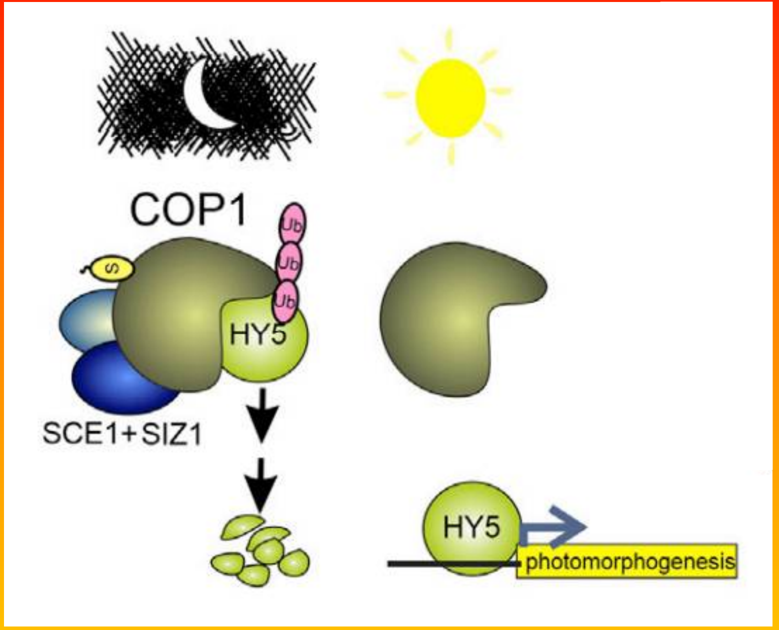
NDPK2 (nukleotid disphosphate kinase2) – protein fosforylován fytochromem B, kinázová aktivita se zvyšuje v případě Pfr; lokalizace není známa

The role of SUMOylation in light signaling

SUMO (Small Ubiquitin-like MOdifier) - is a small signaling protein that shows spatial similarity to ubiquitin. By binding the SUMO protein, the proteins usually become more stable.

The SUMO protein attaches to a lysine side chain on the target protein in the process of sumoylation.

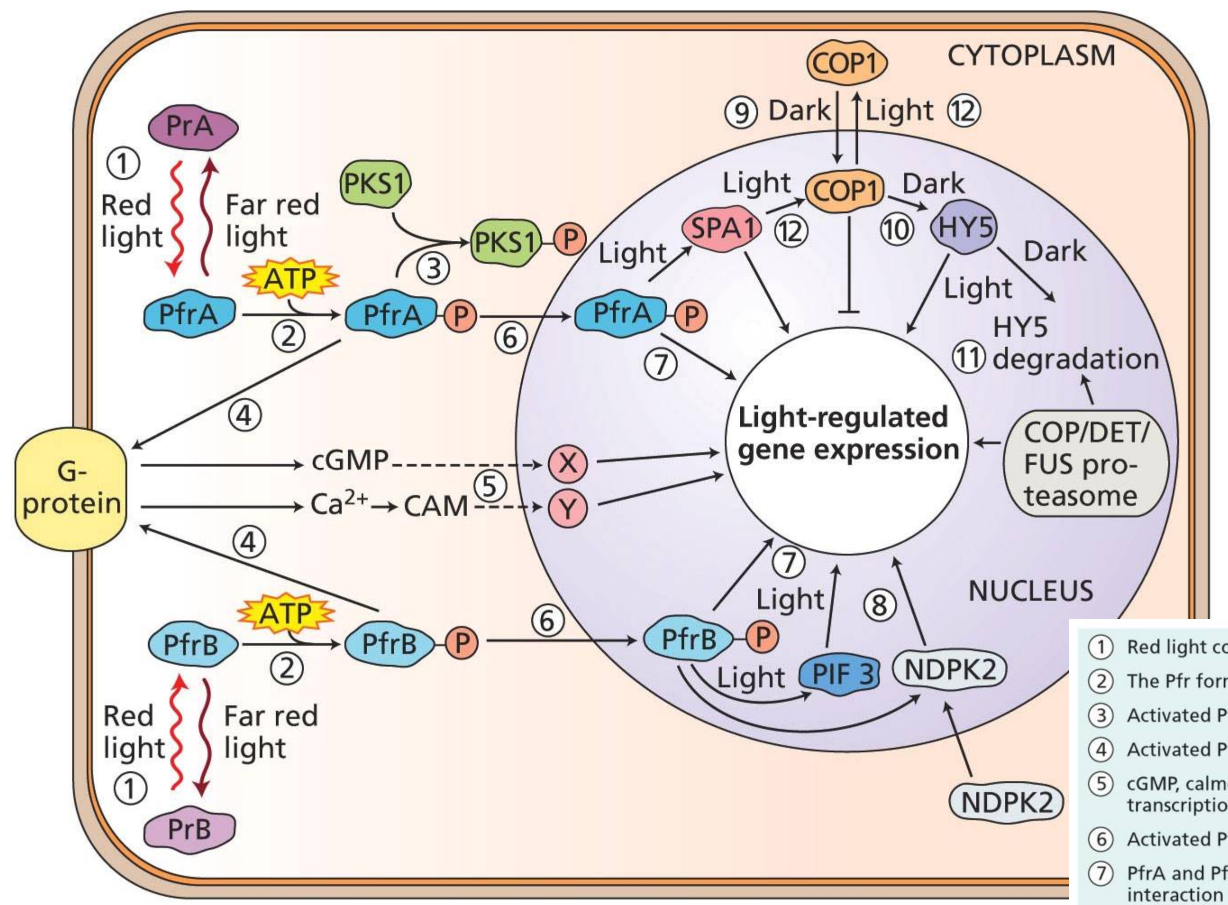




Update 2020
Zeidler M (2021) Molecular Plant 13: 943-945

E1 - S-activatong enzymes
E2 - S-conjugation enzymes
E3 - S-ligating enzymes

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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