SFRZE

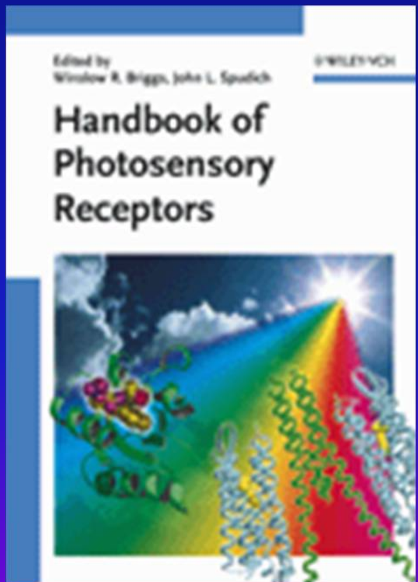
2022

4) Plant responses to blue light and signaling pathways

a) Responses mediated by blue light

b) Photoreceptors of blue light

c) Signal transduction



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

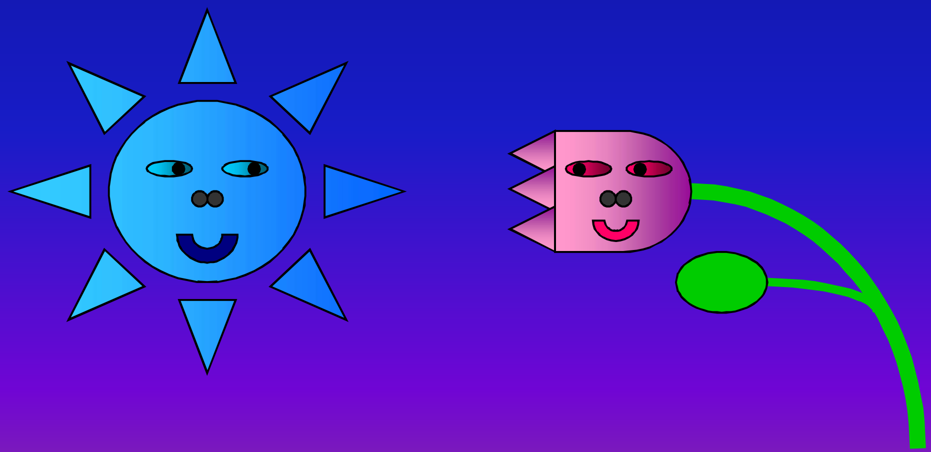
Whitelam GC, Halliday KJ (eds) (2007) Light and Plant Development Blackwell Publishing

Martin Fellner

a) Responses mediated by blue light

Photosynthesis – perceived light serves as a source of chemical energy

Phototropism – light is perceived as a signal; specific response to blue light; growth towards the light source



Plant responses to blue ligh (400 – 500 nm)

1) Phototropism
2) Fast inhibition of elongation
3) Activation of gene expression
4) Stimulation of stomata opening

Stimulation of chlorophyll synthesis and carotenoids

Phototaxis

Nucleus movement

Change of leaf position

Fast responses – seconds (electric events on membrane)

Slow responses – minutes, hours (stimulation of pigment biosynthesis)

Blue light is perceived by specific receptors of blue light but also by phytochromes and by chlorophyll

↓

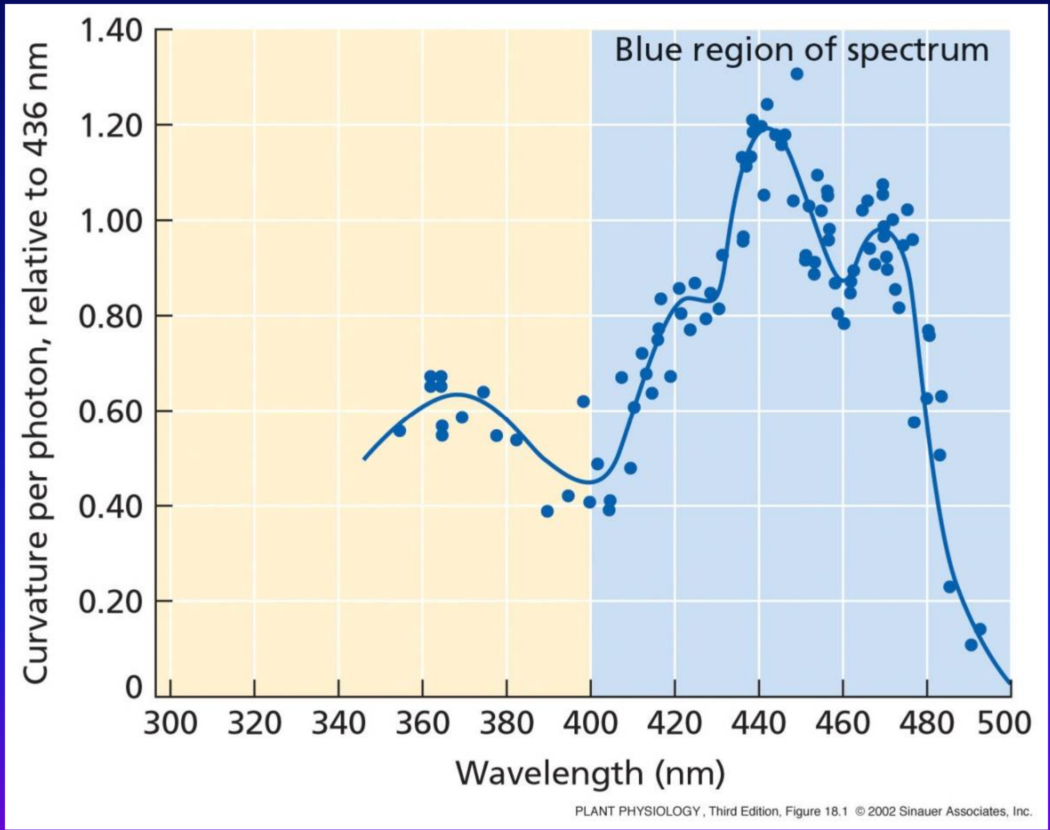
How to distinguish specific responses to blue light?

1) Blue light cannot be replaced by red light

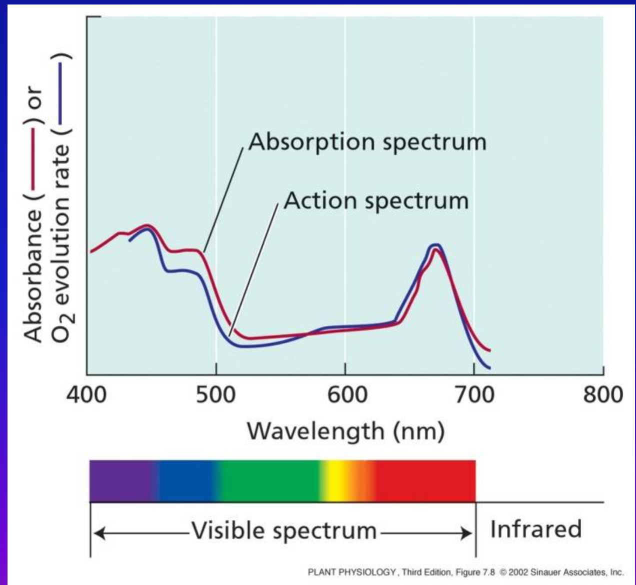
2) Response is not reversible by FR

3) Action spectrum and its comparison with absorption spectrum

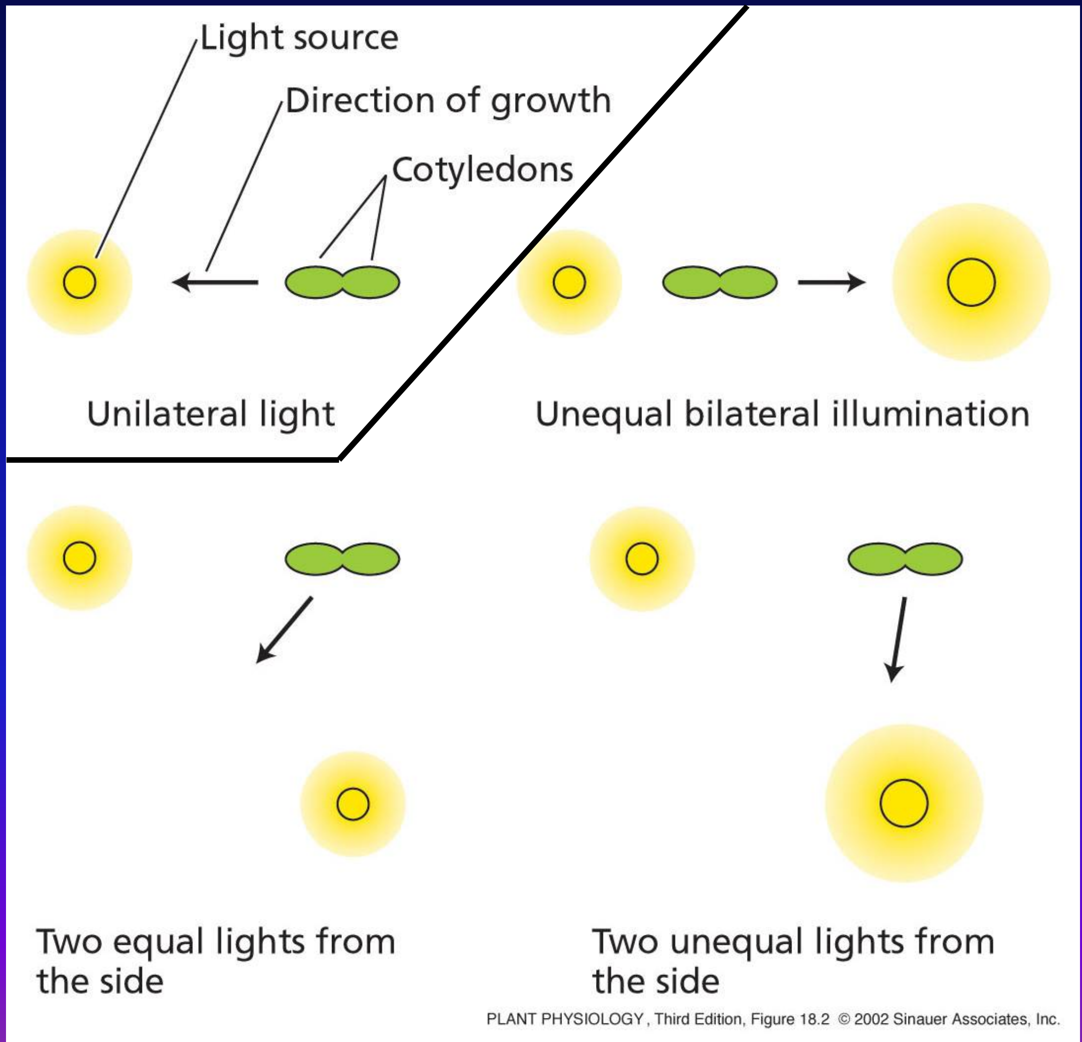
Action spectrum - graph, which shows dependency of observed response on light wavelength



Action spectrum for phototropism

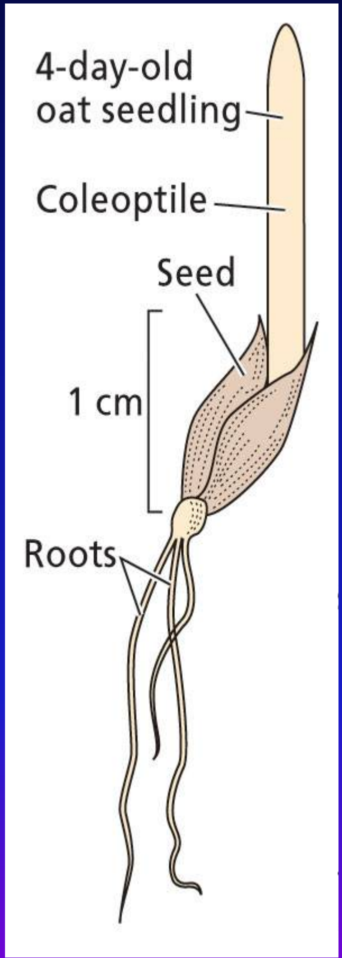


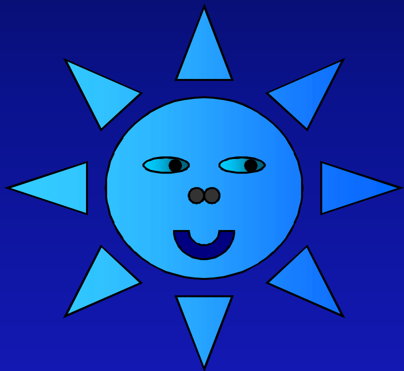
1) Phototropism – asymmetric growth towards the light



- fungi
- ferns
- higher plants

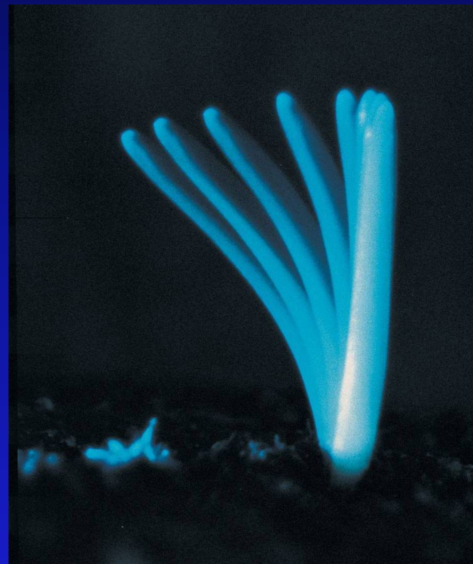
Coleoptile – modified leaves in monocotyledons





Auxin gradient

~ 180 minutes



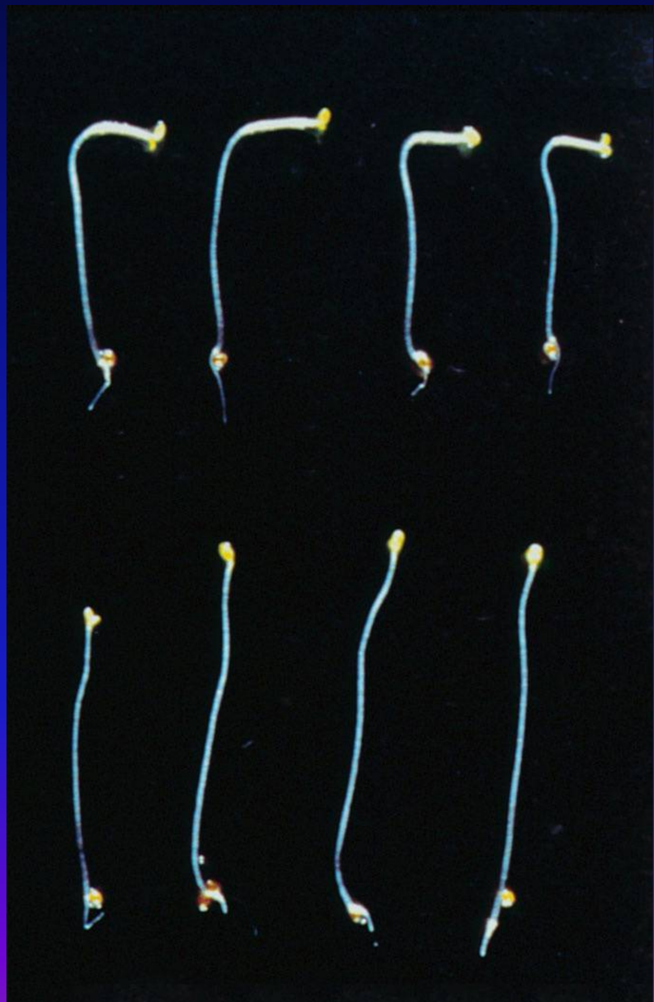
Coleoptile curvature

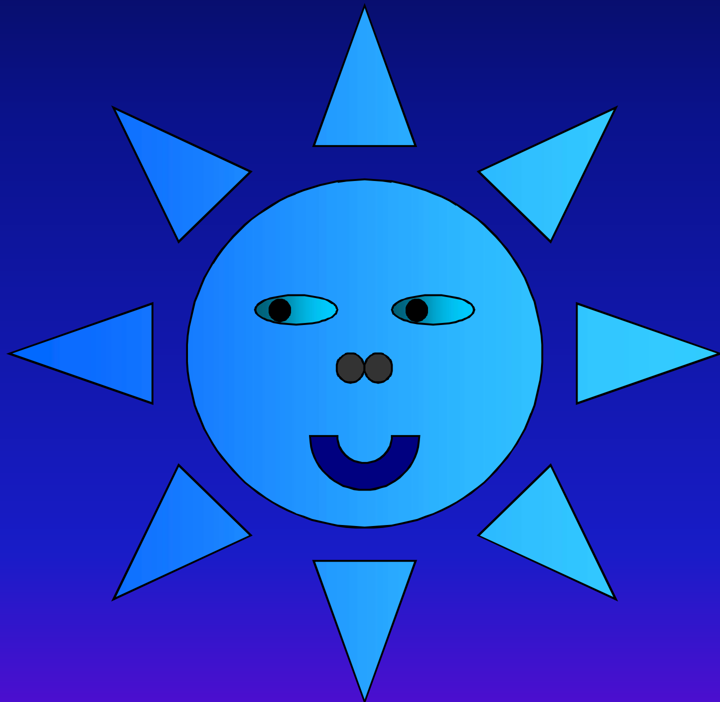
Auxin stimulates cell expansion more in the shaded side than in the lighted side of the coleoptile => asymmetric growth and bending.

***Arabidopsis* mutant *phot1* with defect in phototropism**

WT

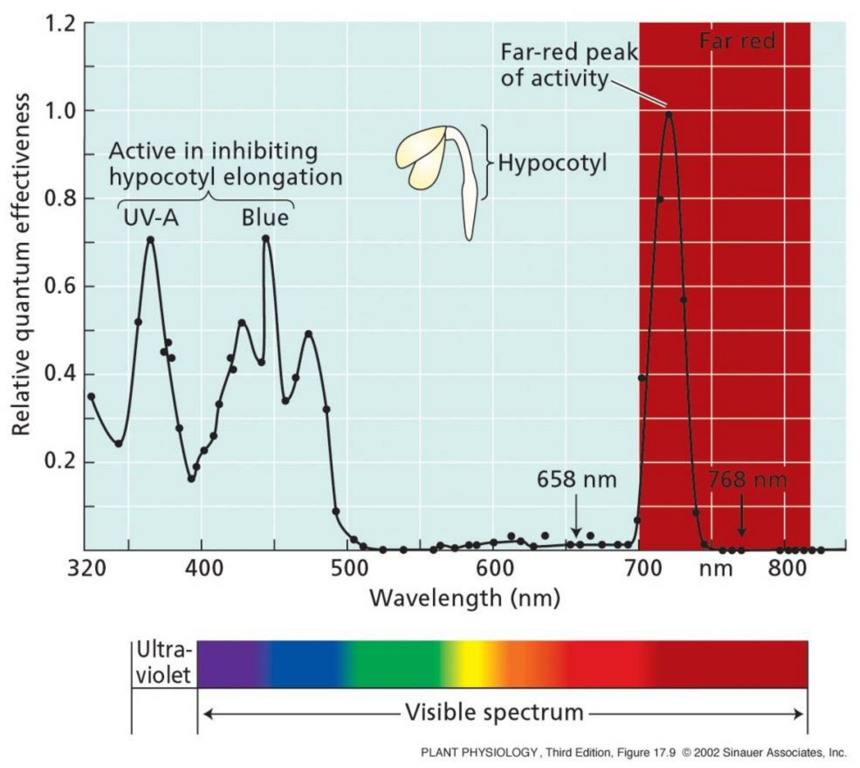
phot1

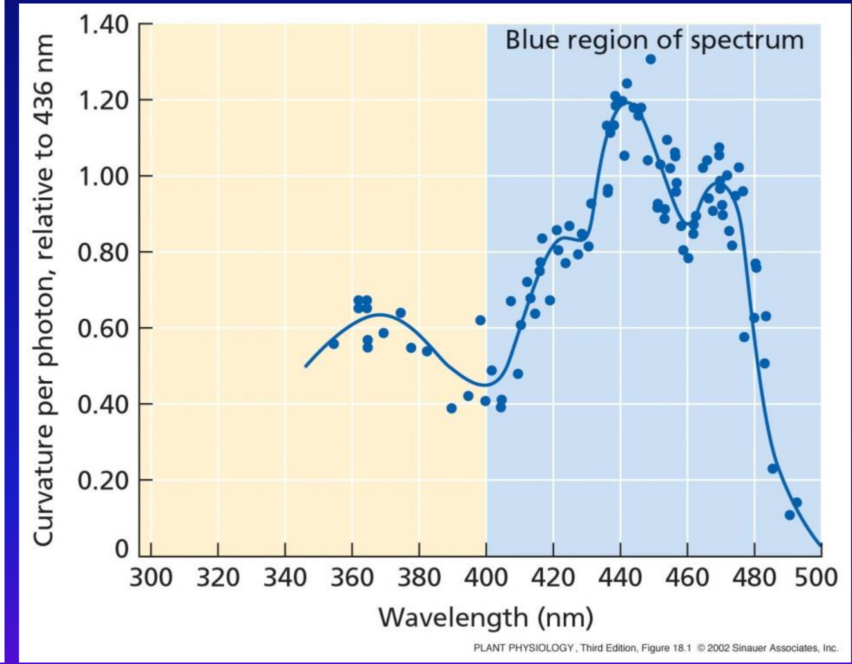




2) Fast inhibition of elongation

Germination → Growth from soil → Photomorphogenic response = growth inhibition





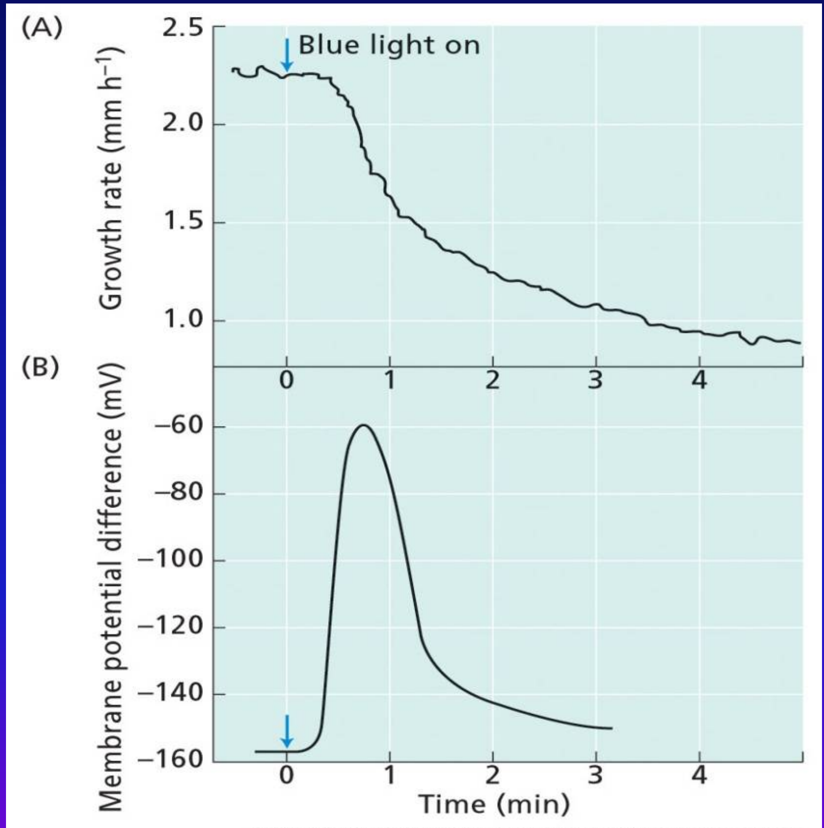
Action spectrum for growth inhibition in etiolated plants

Action spectrum for phototropism

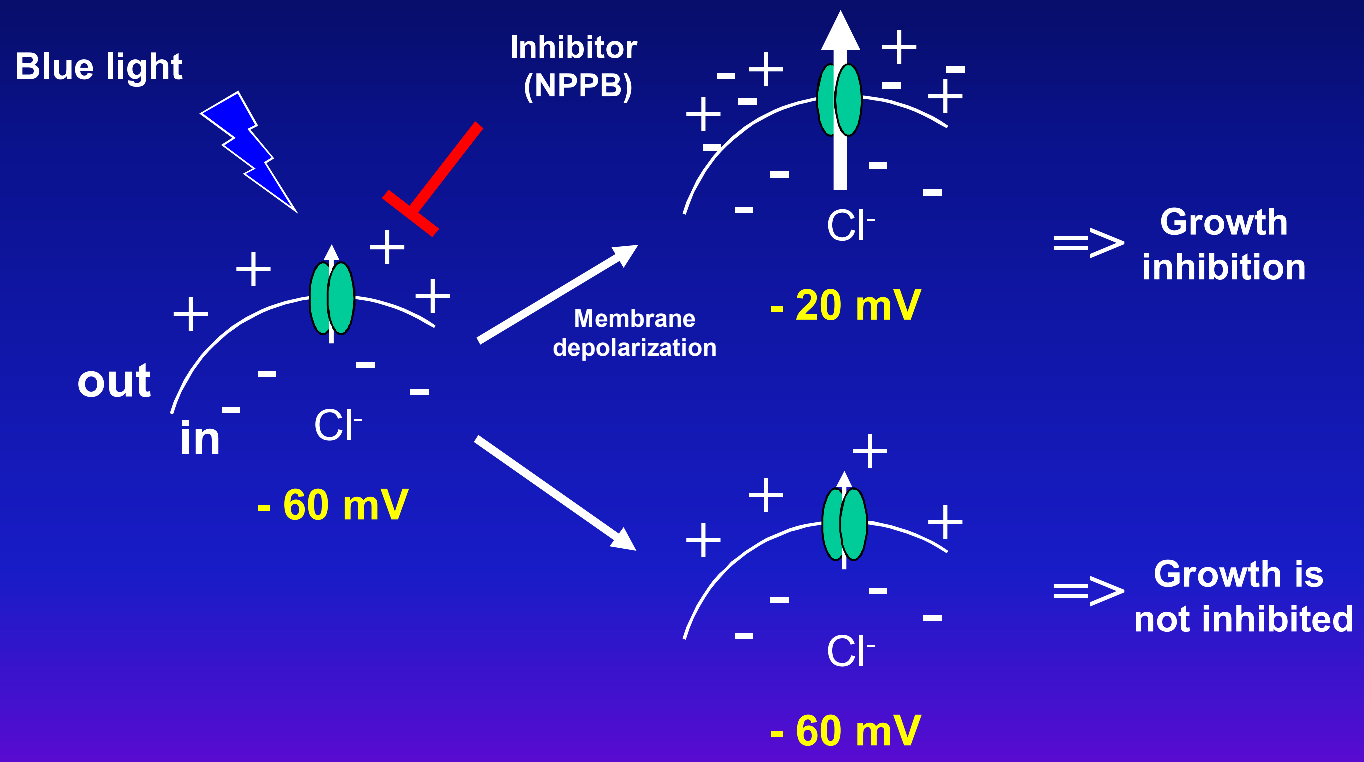
Experimental possibilities to distinguish between inhibition of growth mediated by phytochromes and by blue light-specific receptors.

Blue light induces depolarization of plasma membrane, which precedes growth inhibition.

Depolarization is caused by activation of Cl^- channels.



Anion channels mediate blue light-induced inhibition of elongation growth.



3) Activation of gene expression

Blue light induces expression of genes, which codes for proteins involved in various morfological processes.

a) Genes regulated nonspecifically by blue light

- **Gene for enzyme chalcone synthase, involved in flavonoid biosynthesis**
- **Gene coding for proteins binding chlorophyll *a* a *b*.**
- **Gene *AthH2* primarily expressed in expanding and differentiating cells; it codes for membrane protein capable to transport water molecules = aquaporin (water channel); regulated also by ABA**

b) Genes regulated specifically by blue light

Gene *SIG5* – plays regulating role in transcription of chloroplast gene *psbD-BLRP* (*Blue Light Responsive Promoter*), which codes for D2 subunit PSII reaction center.

SIG5 plays a role in plant tolerance to osmotic stress – it induces repairs of PSII

Other 5 genes of *SIG* group is activated nonspecifically by blue and red light

c) Gene for photoreceptor CRY1 is regulated by blue light

Blue light increases amount of mRNA and protein BnCRY1. Promoter of *CRY1* gene contains cis-acting sequence responding to blue light.

4) Stimulation of stomata opening

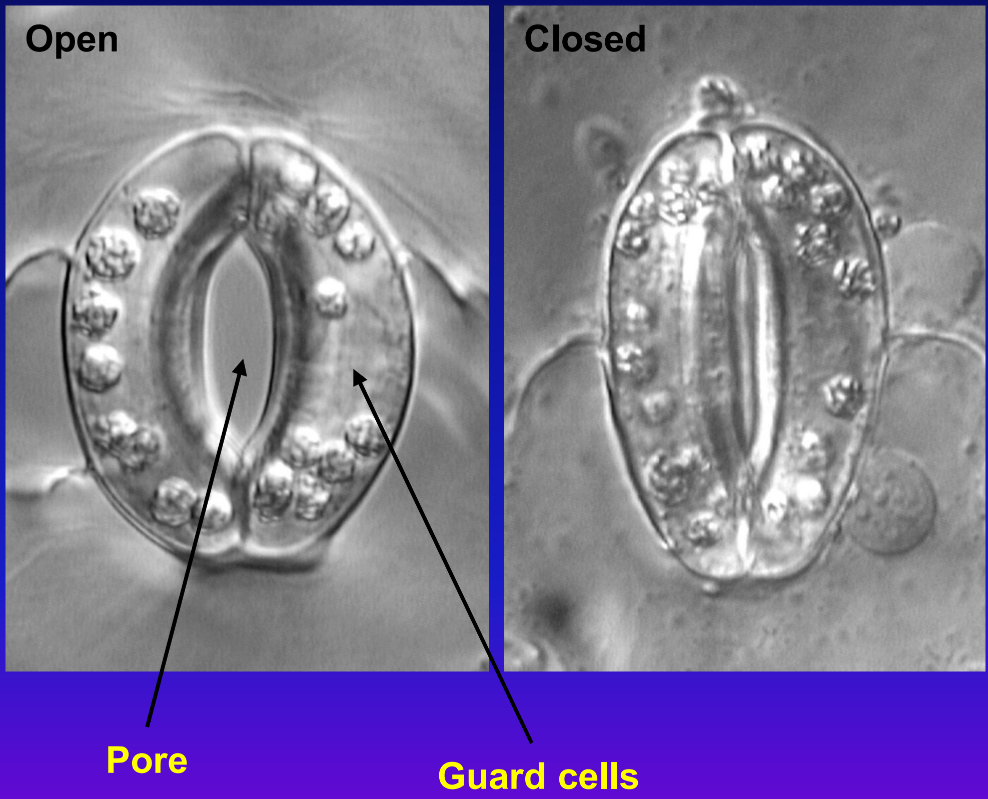
Stomata play main regulatory role in the changes of gases in leaves

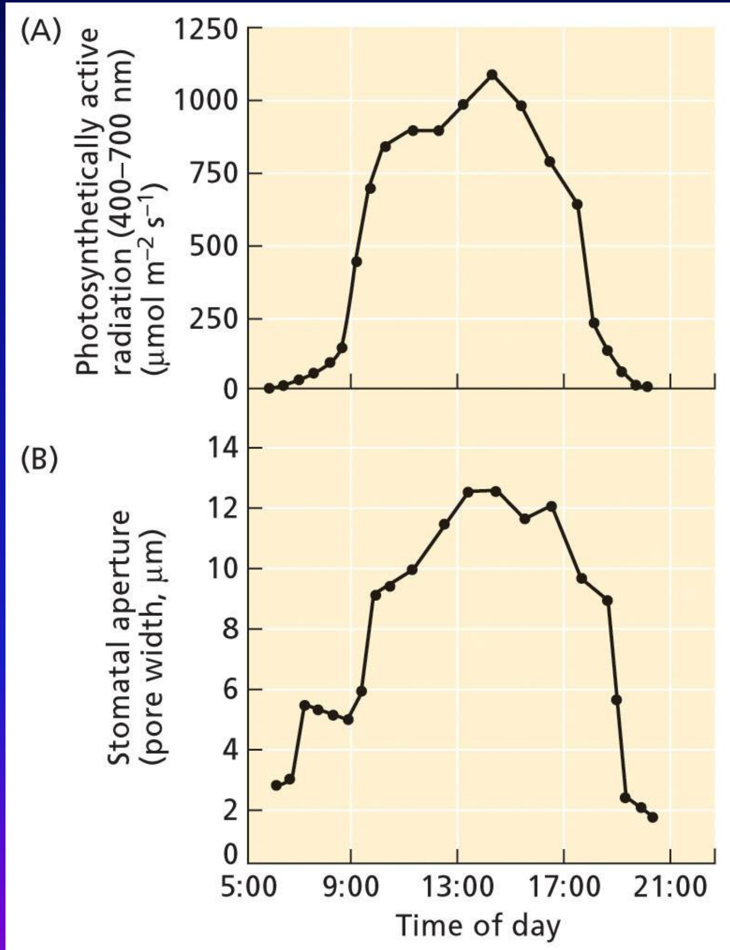
Stomata – model object for study of responses to blue light:

- responses of stomata to blue light is fast and reversible
- responses of stomata to blue light is observable for whole life of plant
- signaling pathway connecting the place of blue light perception with stomata is well studied

Light perceived by epidermal cells of leaves is dominant factor regulating opening and closure of stomata.

Stomata opens at certain level of light intensity and closes when light intensity decreases.





DCMU (dichlorophenyl dimethylurea) – inhibitor of photosynthetic electron transport – partially inhibits opening of stomata induced by blue light

⇓

Photosynthesis in chloroplasts of guard cells plays a role in light-induced stomata opening

+

Nonphotosyntetic part of stomatal response to light

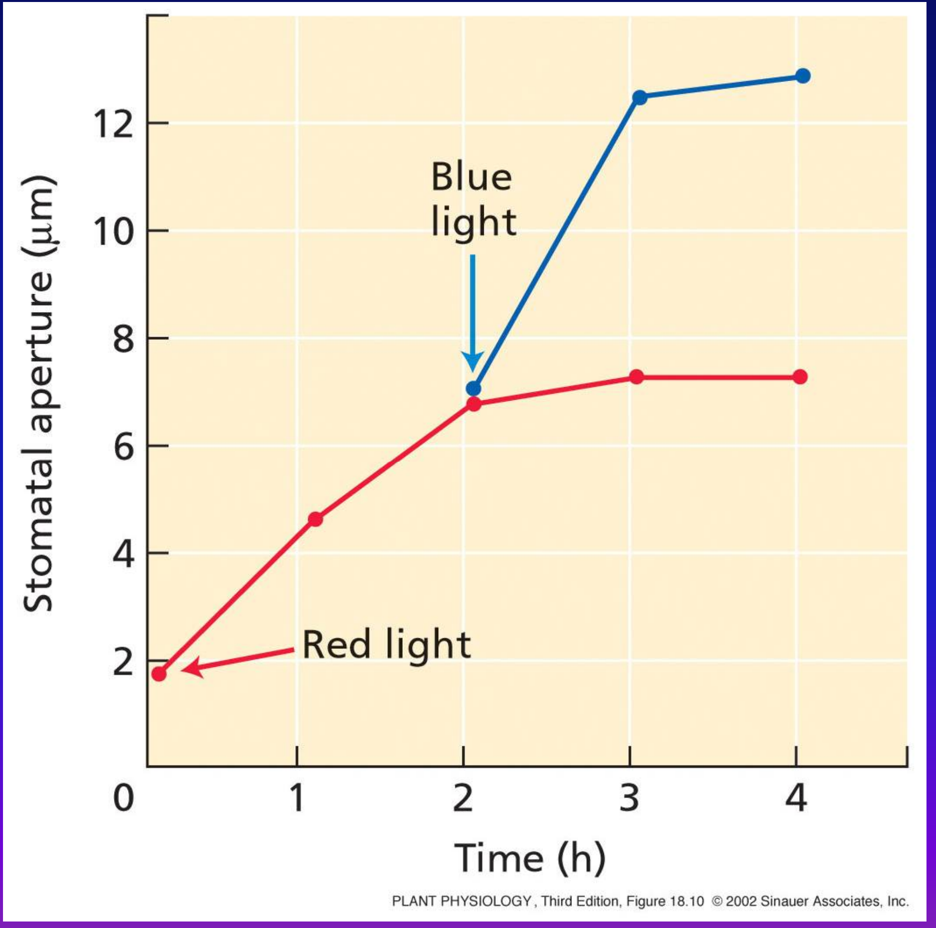
⇓

Light activates two distinct responses of guard cells:

- photosynthesis in chloroplasts in guard cell
- specific response to blue light

Specific stomata responses

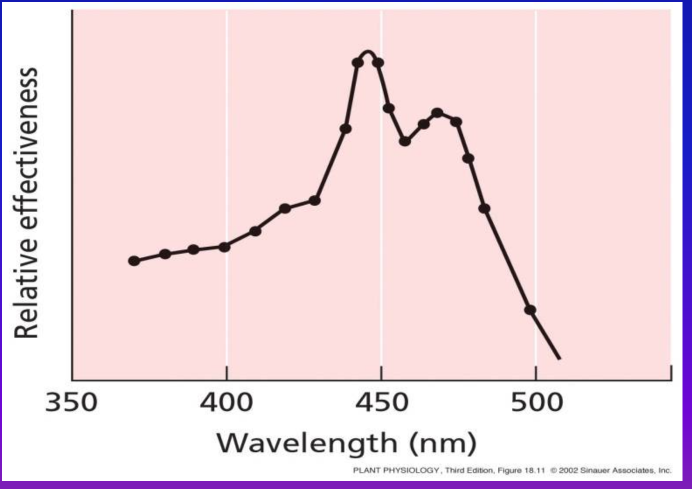
Blue light causes photosynthetic and specific nonphotosynthetic responses



1) Saturation of photosynthetic response by strong red light => partial opening of stomata

2) Application of weak blue light

Additional nonphotosynthetic opening of stomata induced by blue light



Blue light induces swelling of protoplasts isolated from guard cells

↓

Light is really perceived by guard cells

Discovery of mechanisms of light-induced stomata opening and closure

Blue light

↓

K^+ flow into the cell

↓

Increase of osmotic solutes in the cell

↓

Water uptake

↓

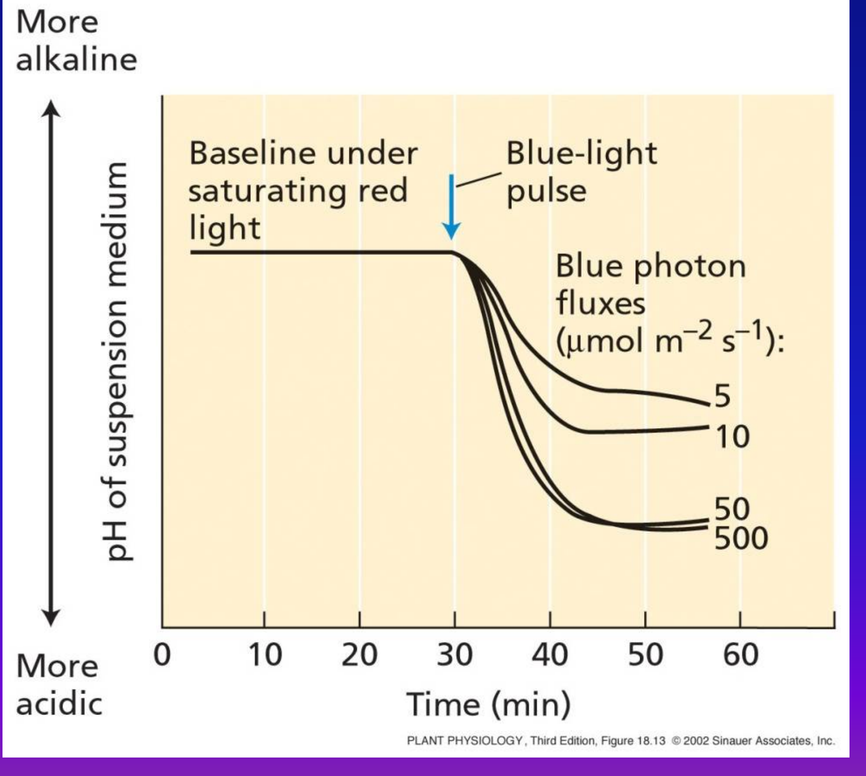
Guard cell swelling

Blue light →

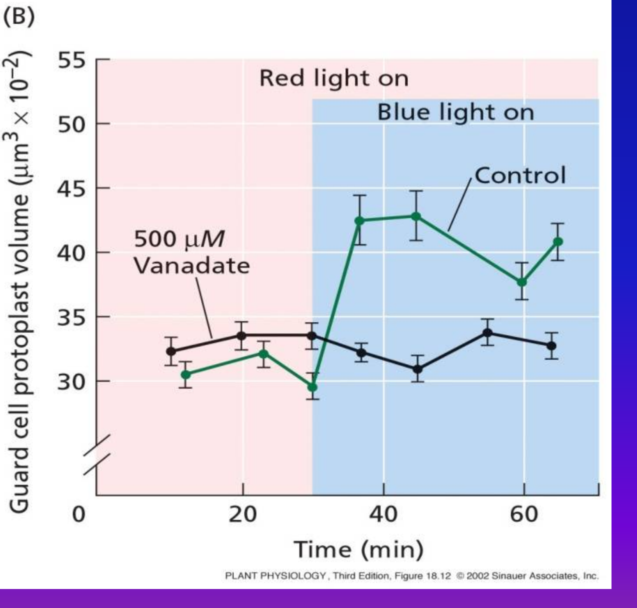
Blue light activates proton pump (H^+- ATPase)

H^+- ATPase pumps proton from the cell to apoplast => acidification of apoplast

Acidification can be blocked by CCCP (inhibitor of pH gradient formation) or by vanadate ($\text{inhibitor of } H^+$- ATPase)

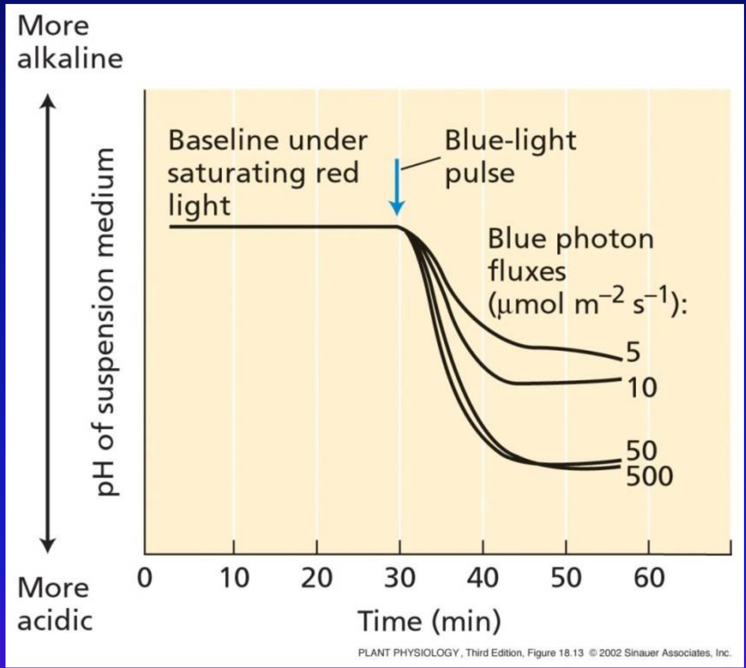


Acidification is caused by activation of proton pump by blue light



Increasing of proton pumping and size of stomata aperture are proportional to the amount of photons of blue light captured by leaf

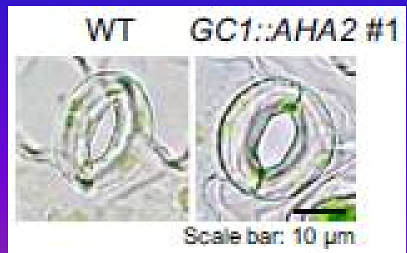
Stomata function as photon sensor



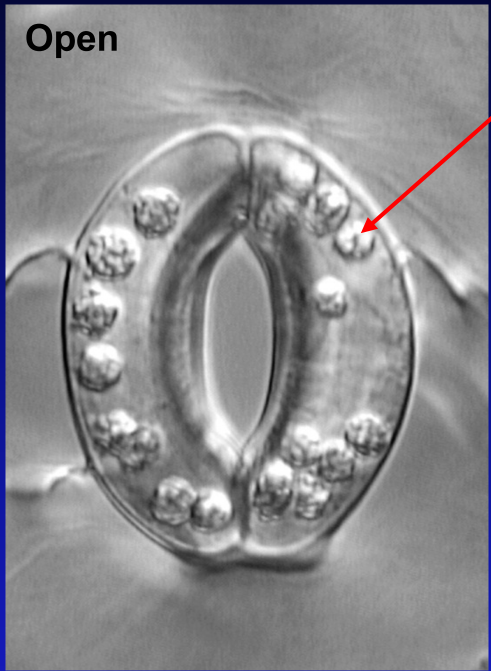
UPDATE 2014

Wang Y et al. (2014) PNAS 111: 533-538

Transgenic *Arabidopsis* plants with overexpressed H^+-ATPase show increased light-induced opening of stomata



Chloroplasts contain starch grains



Starch is insoluble high molecular polymer of glucose – not osmotically active

⇓

When stomata are opening, starch hydrolysis begins. Starch - soluble sugars rise – osmotically active

⇓

Osmotic pressure ↑ (Osmotic potential) ↓

⇓

Further stomata opening

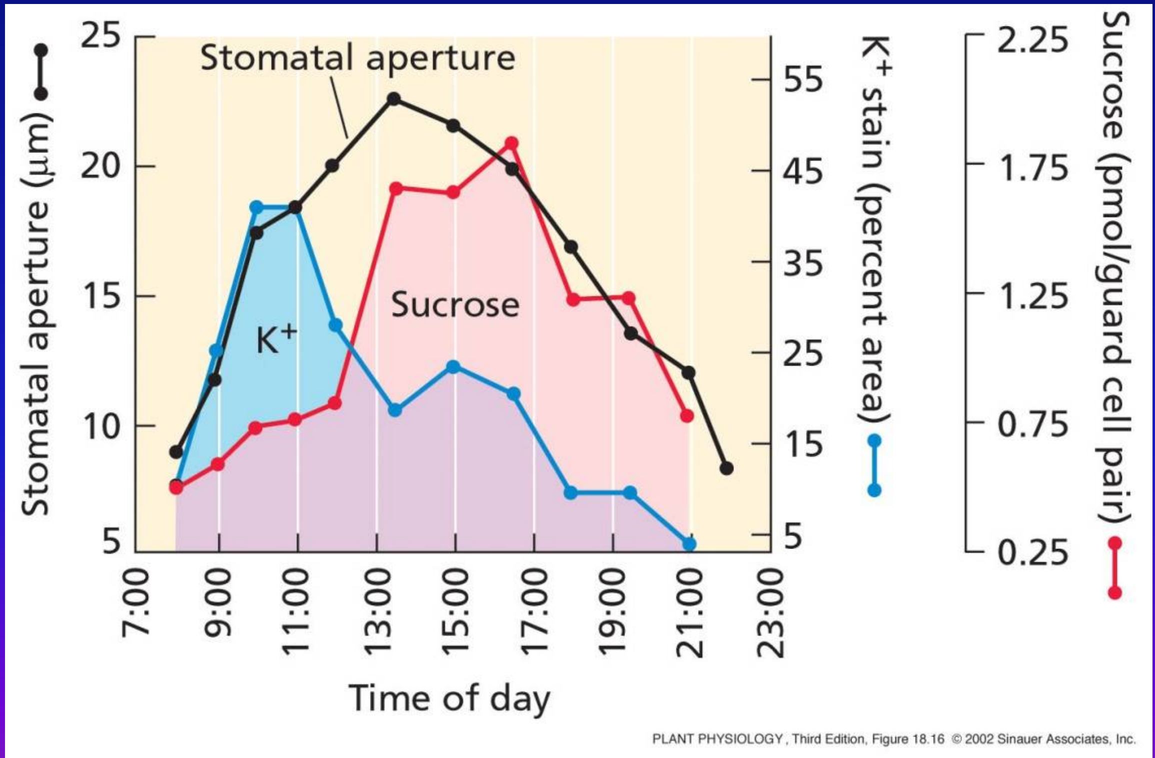
Stomata closure: starch synthesis ⇒ osmotic pressure ↓ (osmotic potential) ↑

Current model of osmoregulation in guard cells

K^+ increases in the mornings and stomata open; content of sugar slowly increases

K^+ decreases afternoon, but stomata openig continues by increasing content of sugar

Late afternoon content of sugar decreases – it corresponds with starting of stomata closure



K^+ : stomata opening

Sugar: stomata closure

19th century

Charles and Francis Darwin → Study of coleoptile phototropism

Early 90th → Identification of photoreceptors

Identification of genes regulating phototropism and inhibition of elongation

Protein characterization

b) Photoreceptors of blue light

Cryptochromes – growth inhibition

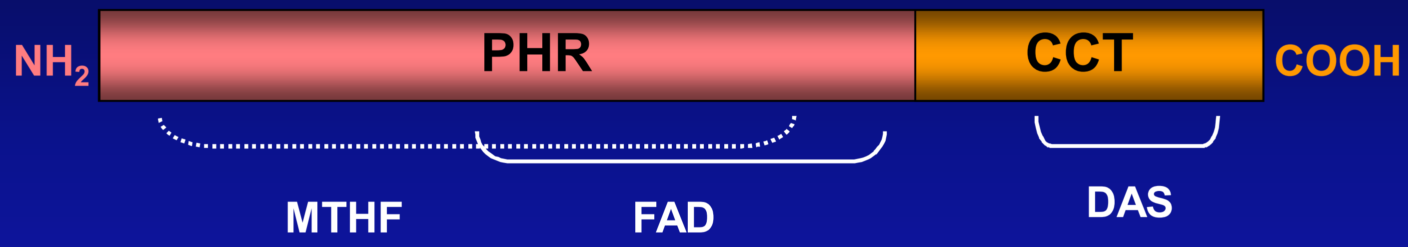
Phototropins – phototropism, chloroplast movement, stomata movement

Zeaxanthin – stomata opening

Cryptochromes

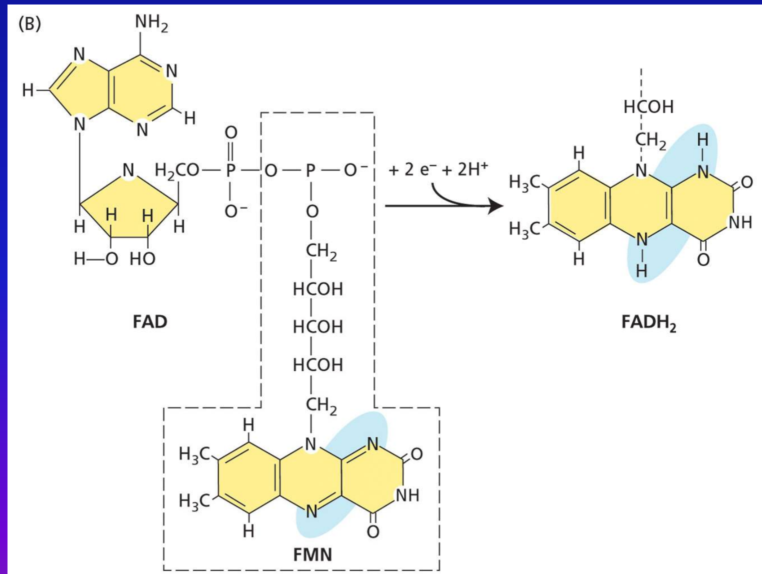
***Arabidopsis* mutant *hy4* – hypocotyl is not inhibited by blue light**

Gene *HY4* => protein, monomer 75 kDa



PHR = Photolyase-related domain; N- terminal domain; homologous to DNA photolyase; binds two types of chromophore

- **Flavin = flavin adenine dinucleotide, FAD**
- **Pterin = methenyltetrahydrofolate, MTHF**



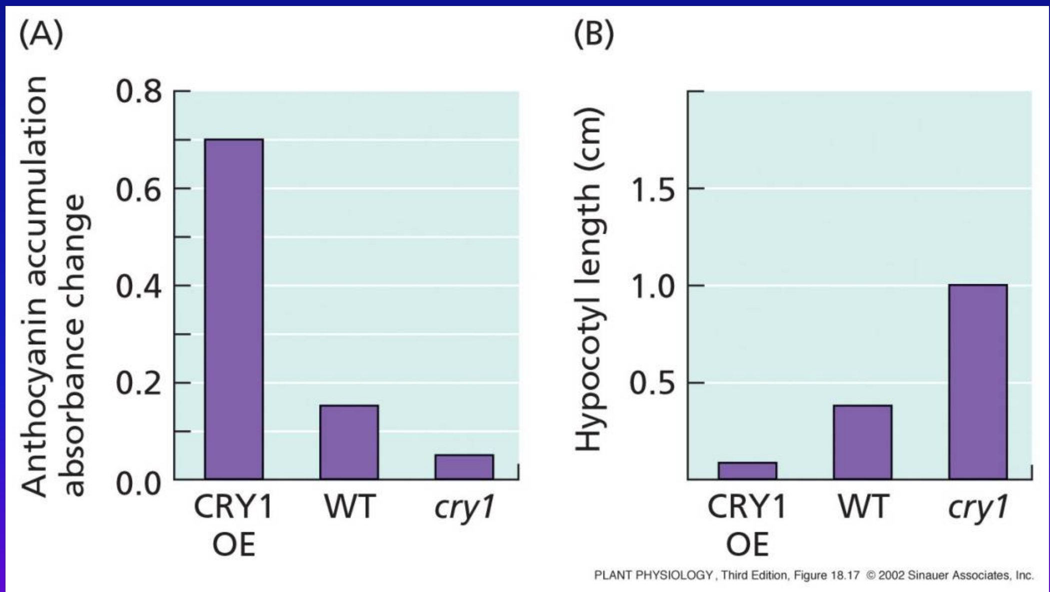
PLANT PHYSIOLOGY, Third Edition, Figure 11.2 (Part 2) © 2002 Sinauer Associates, Inc.

CCT = CRY C-Terminus; C-terminal domain – contains 3 motives: D, A, S – important for cell localization and intermolecular interaction (e.g. COP1)

***HY4* = *CRY1* (*CRYPTOCHROME 1*) – codes for photoreceptor of blue light; mediates inhibition of elongation induced by blue light**

Evidence:

- Overexpression of *CRY1* in transgenic plants => strong inhibition o hypocotyl growth and overproduction of anthocyanins



***CRY1* plays a role in inhibition of elongation growth.**

CRY2 (*CRYPTOCHROME 2*) – homologous to *CRY1;* light unstable

Transgenic plants overexpressing *CRY2*

– weak inhibition of elongation by blue light
– increased growth of cotyledons induced by blue light

CRY1 and *CRY2* – play role in flowering induction and circadian rhythm

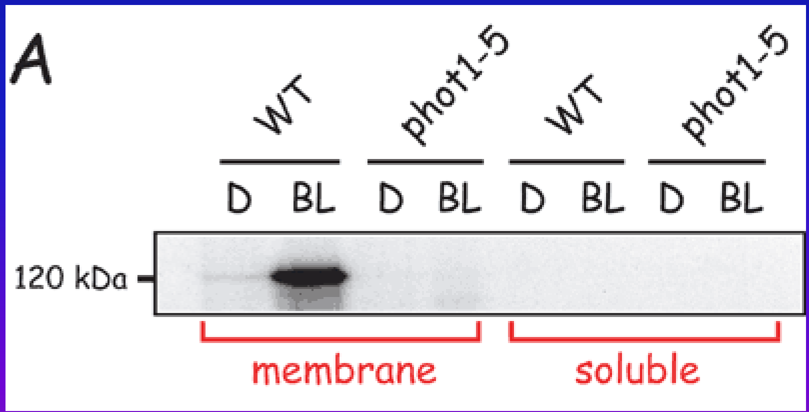
2003 – identification of gene *CRY3* → Function of CRY3 ?

CRY3 belongs to CRY-DASH enzymes with photolyase activity.

Phototropins

Arabidopsis mutant *nph1 (nonphototropic hypocotyl1)* – genetically independent of *cry1*

nph1 – inhibited by blue light; lack phototropic response; membrane protein 120 kDa is not phosphorylated by blue light



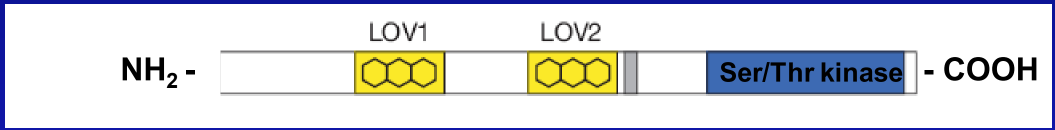
NPH1 protein – receptor for phototropism; autophosphorylation induced by blue light

NPH1 protein (PHOT1)

Structure

Structure of PHOT1

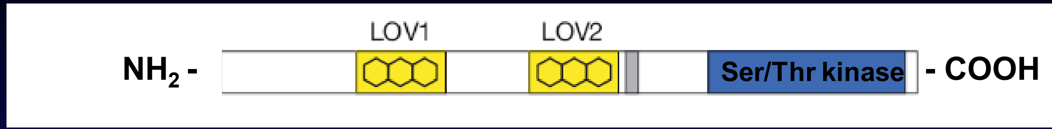
- 966 amino acids
- Hydrophilic protein; ability to be attached to membrane
- C-terminal part – 11 typical domains in serine/threonine kinase
- N-terminal part – 2 domains LOV1, LOV2; each 110 amino acids



LOV – similar to domain PAS in proteins regulated by Light, Oxygen (*Escherichia coli*), Voltage (*Drosophila*, verterbrates)

Phototropin expressed in insect cells: N-terminal domain binds chromofore FMN (flavin mononucleotide) in spots of LOV1 and LOV2; autophosphorylation after blue light exposure.

PHOT1 – spectral characteristics of receptor for phototropism => PHOT1 proposed as light receptor kinase inducing phototropism



PHOT2

- similar to PHOT1
- binds FMN and undergoes by autophosporylation after blue light exposure

Mutant *phot1*:

- does not respond phototropically to blue light 0.01 – 1 $\mu mol.m^{-2}.s^{-1}$
- respond phototropically to blue light 1 – 10 $\mu mol.m^{-2}.s^{-1}$

Mutant *phot2*:

- normal phototropic response

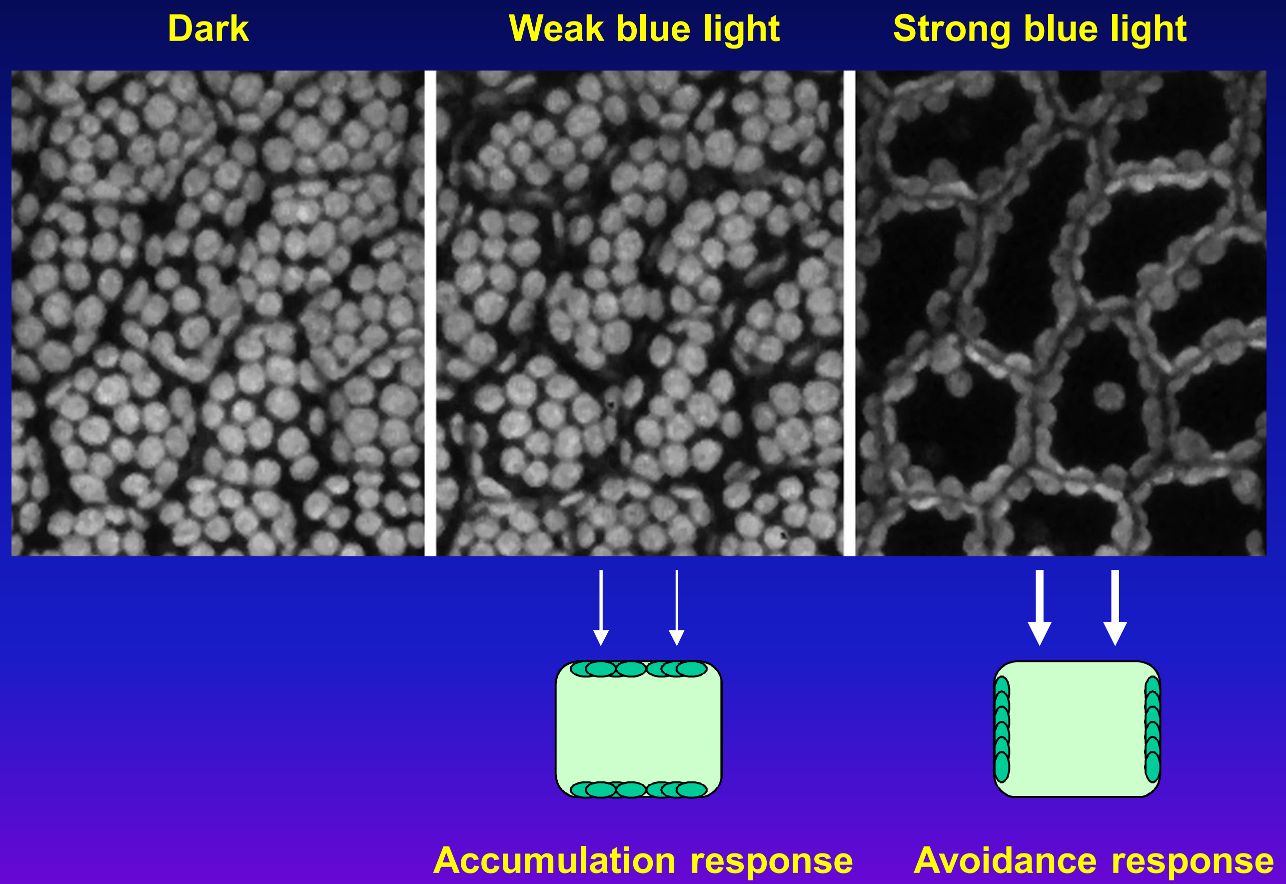
Mutant *phot1/phot2*:

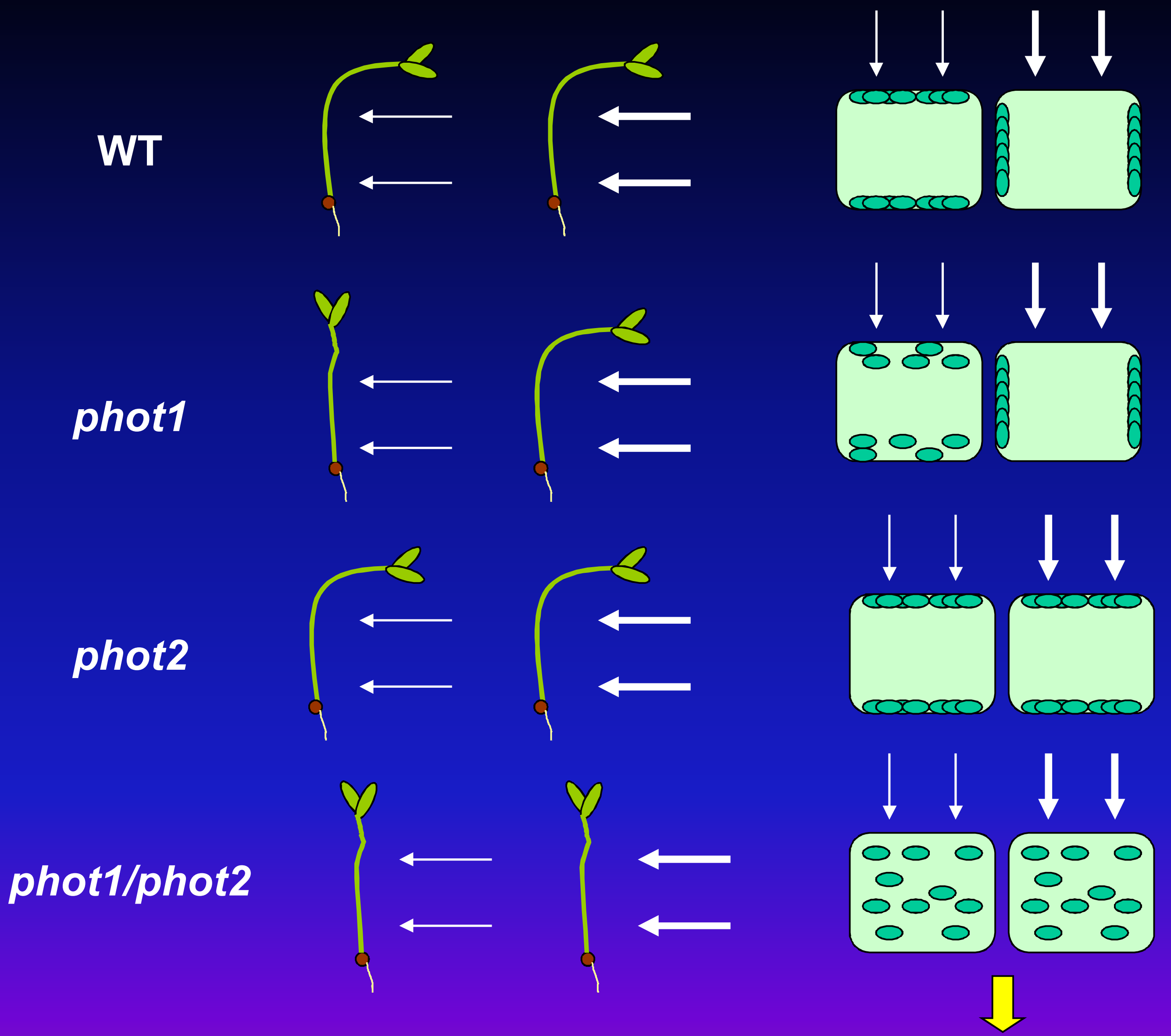
- does not respond phototropically to blue light of both intensities

⇩

PHOT1, PHOT2 play role in phototropism; PHOT2 functions at high irradiance of blue light

Phototropins play role in chloroplast movement





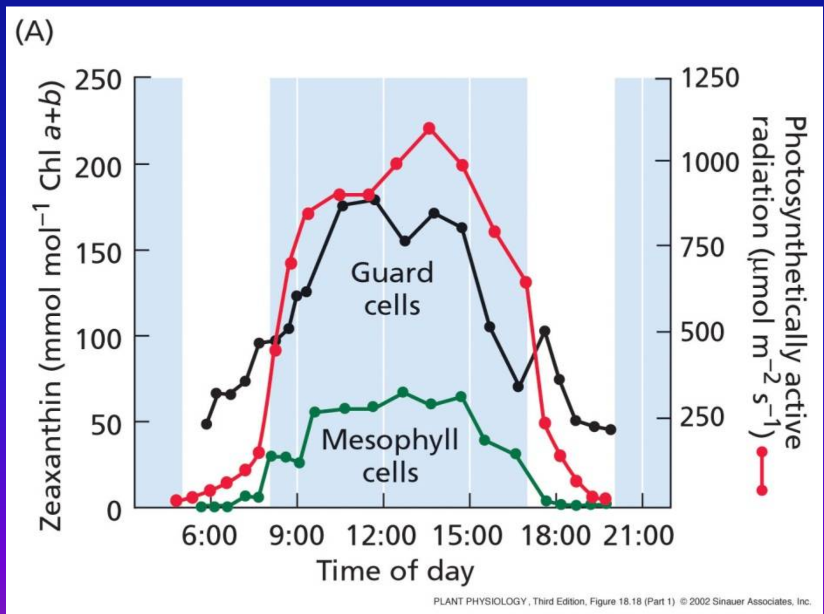
PHOT2 play role in avoidance response

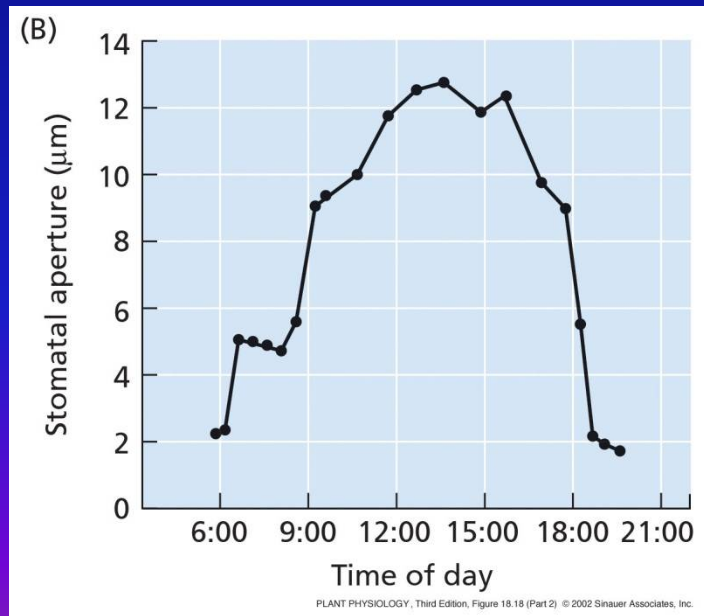
Both genes, *PHOT1* and *PHOT2* play role in accumulation response

Zeaxanthin

Zeaxanthin – carotenoid; component of xanthophyll cycle in the chloroplasts of mesophyll cells – protects photosynthetic pigments against light overdoses

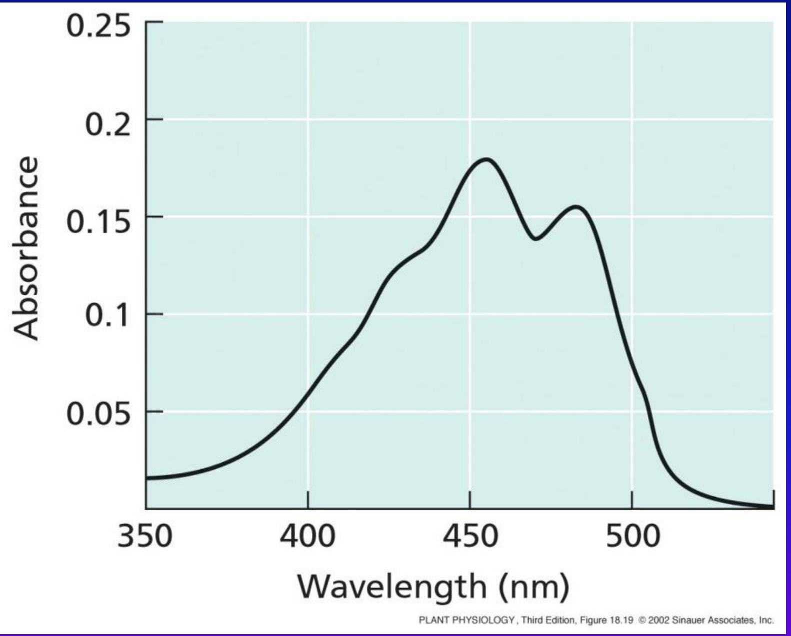
Zeaxanthin in guard cells acts as receptor mediating opening stomata



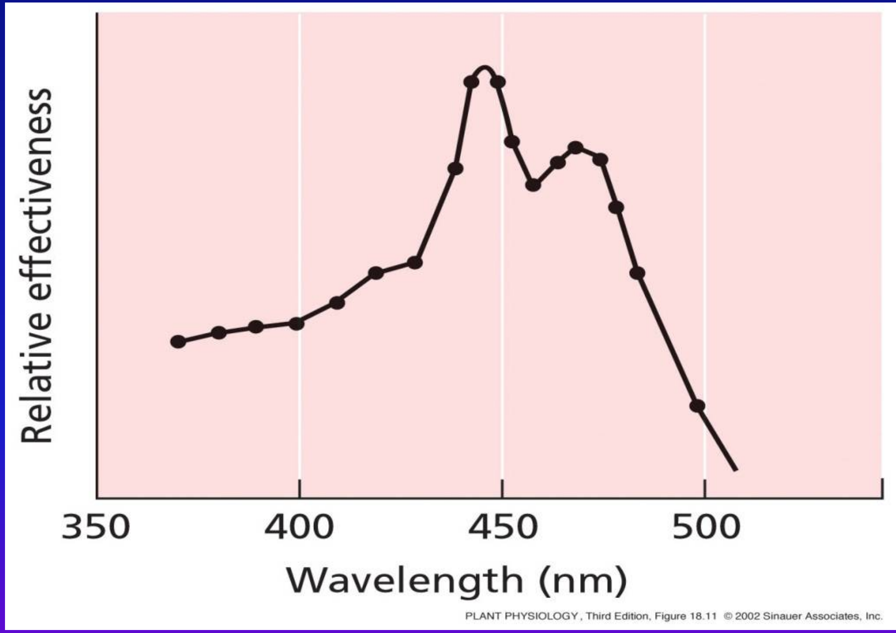


Evidence confirming the role of zeaxanthin as a photoreceptor In stomata:

- Absorption spectrum of zeaxanthin corresponds with action spectrum of stomata opening induced by blue light



Absorption spectrum of zeaxanthin

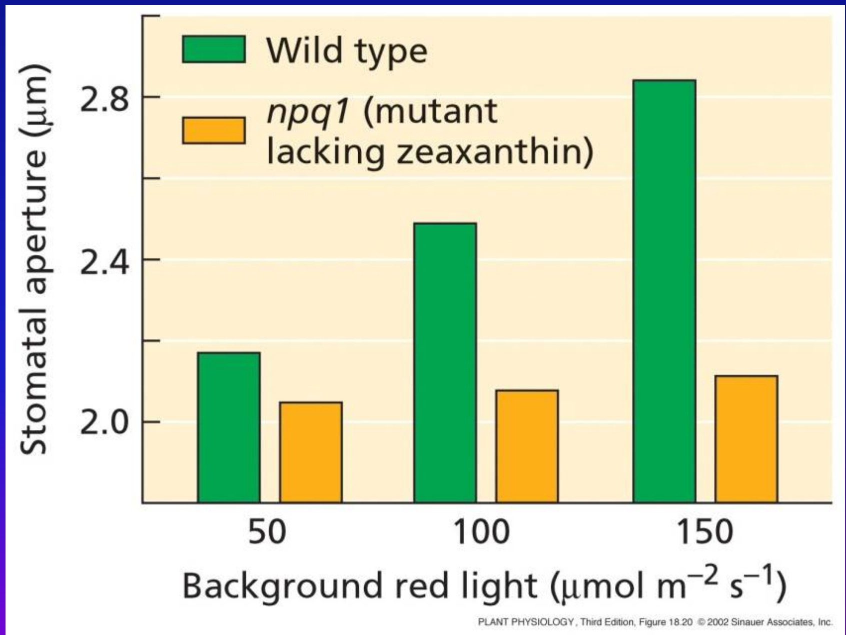


Action spectrum of stomata opening

- Content of zeaxanthin in guard cells corresponds with size of stomatal aperture

- Sensitivity of guard cells to blue light increases with zeaxanthin concentration

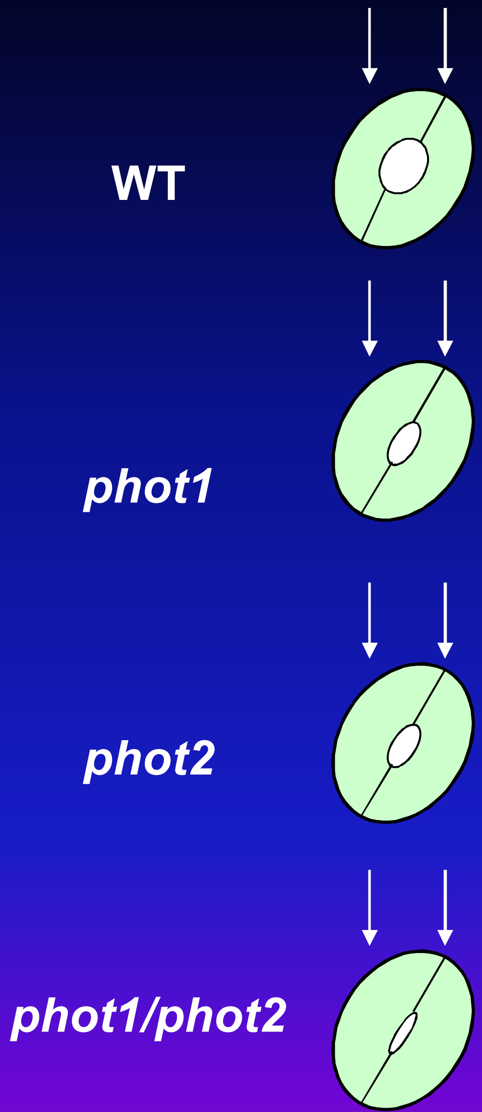
***Arabidopsis* mutant *npq1* (*nonphotochemical quenching*)**



***npq1* does not accumulate zeaxanthin in chloroplasts => lack of specific opening of stomata induced by blue light**

***npq1* shows only basal stomata opening induced by photosynthesis**

Stomata opening is controlled also by phototropins



Responses of stomata to blue light involve genes *PHOT1* and *PHOT2*.

Mechanisms of interaction between PHOTs and zeaxanthin are not known.

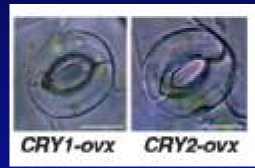
Stomata function autonomously – response of one stoma to blue light does not depend on response of the another one.

Into the process of stomata opening cryptochromes and COP1 are involved

Opening of stomata induced by blue light

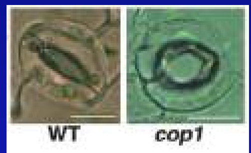
WT > cry1 = cry2 > cry1cry2

WT < CRY1-ovx = CRY2-ovx

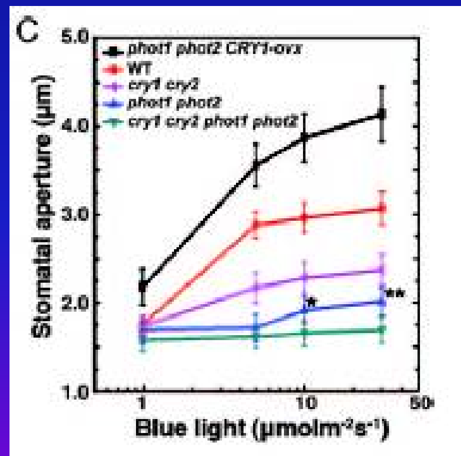


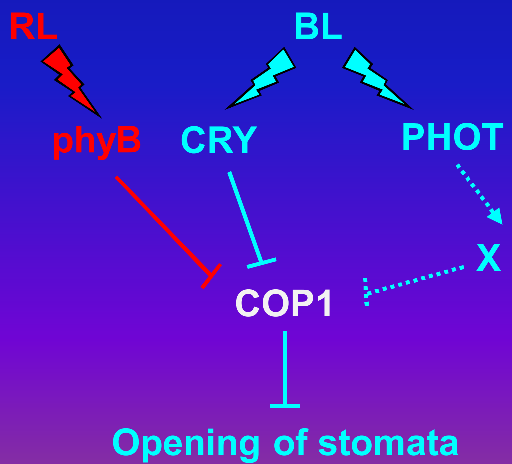
cry1cry2 > phot1phot2 > cry1cry2phot1phot2

WT < cop1



cry1cry2cop1 = phot1phot2cop1 > phot1phot2CRY1-ovx



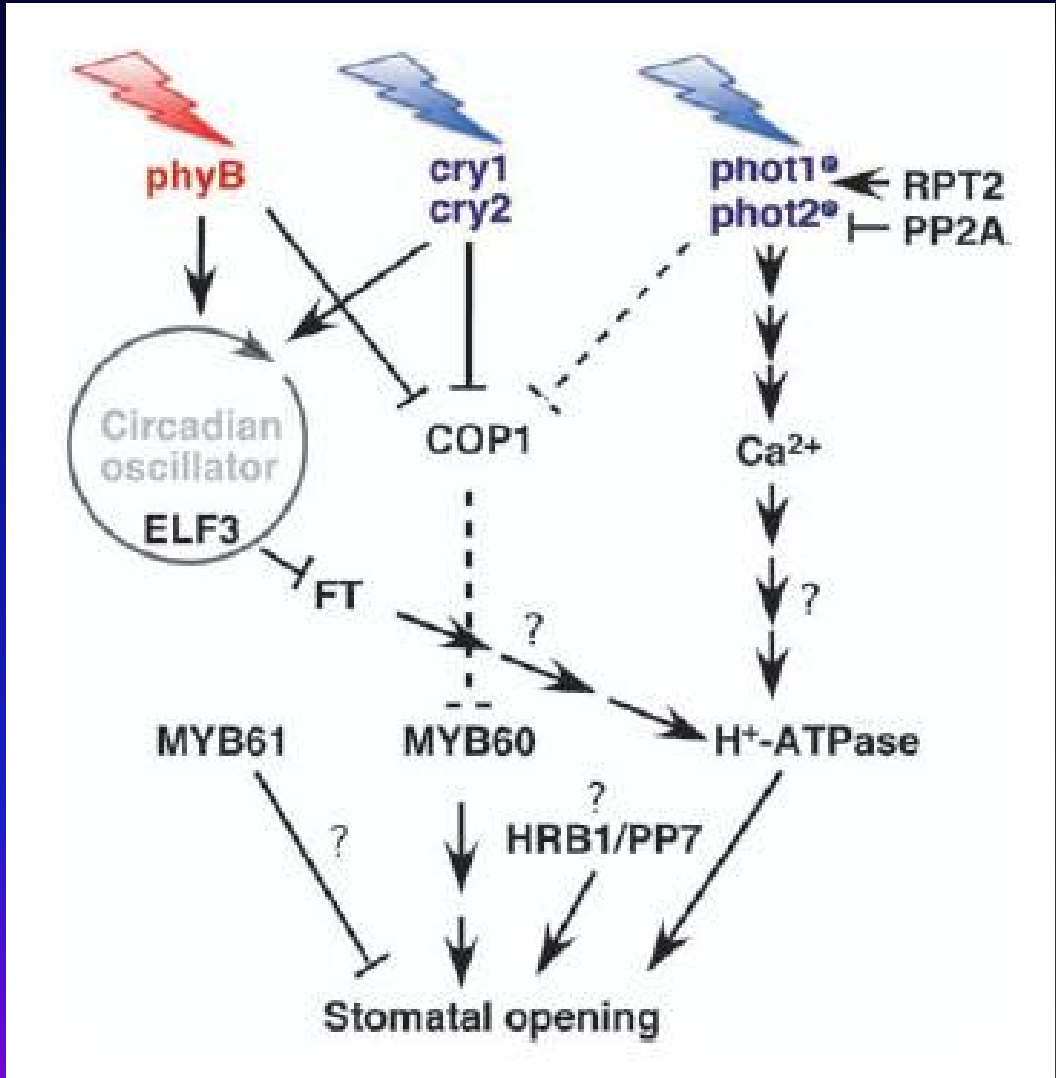


Mao J et al. (2005) PNAS 102: 446-452

UPDATE 2012

Chen C et al. (2012) Mol Plant 5: 566-572

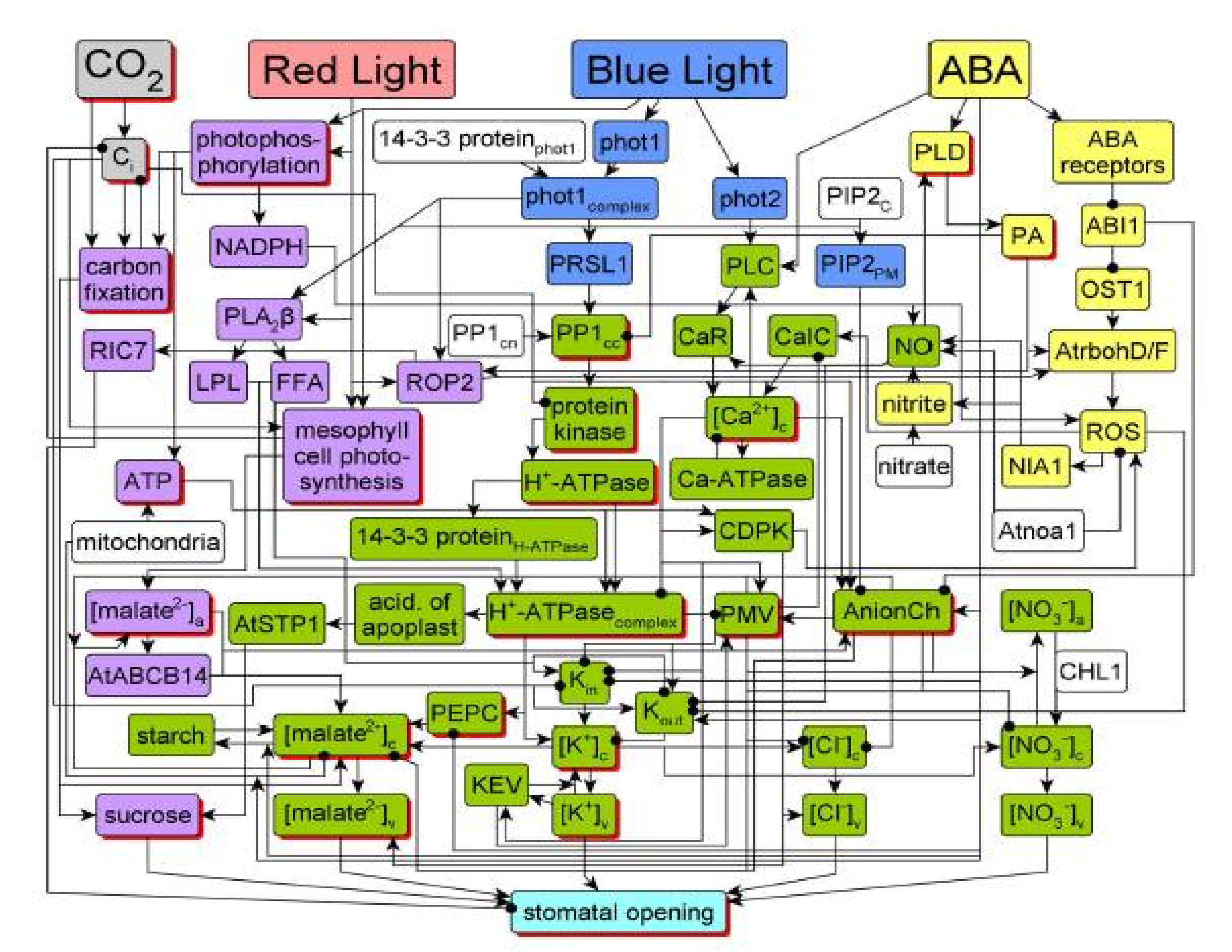
New model of involvement of photoreceptors in stomata opening.



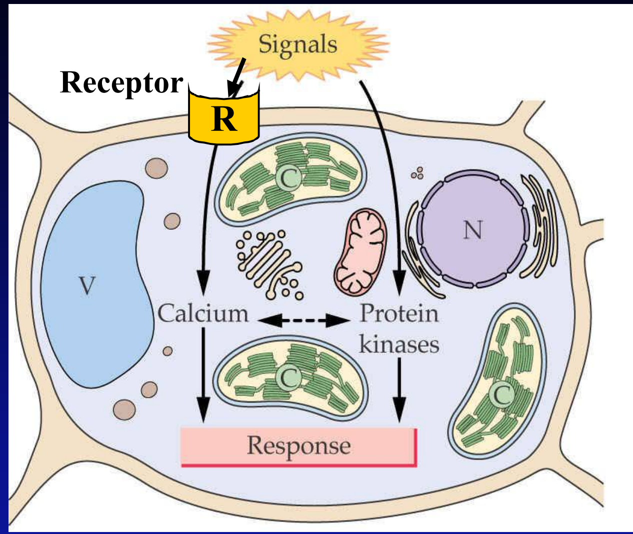
UPDATE 2014

Sun Z et al. (2014) Computational Biology 10: e1003930

Current model of light-induced opening of stomata and regulation by CO_2 and ABA



c) Signal transduction



Signaling pathways involving cryptochromes

CRY1 and CRY2 – homologous to photolyase, but the photolyase activity is missing

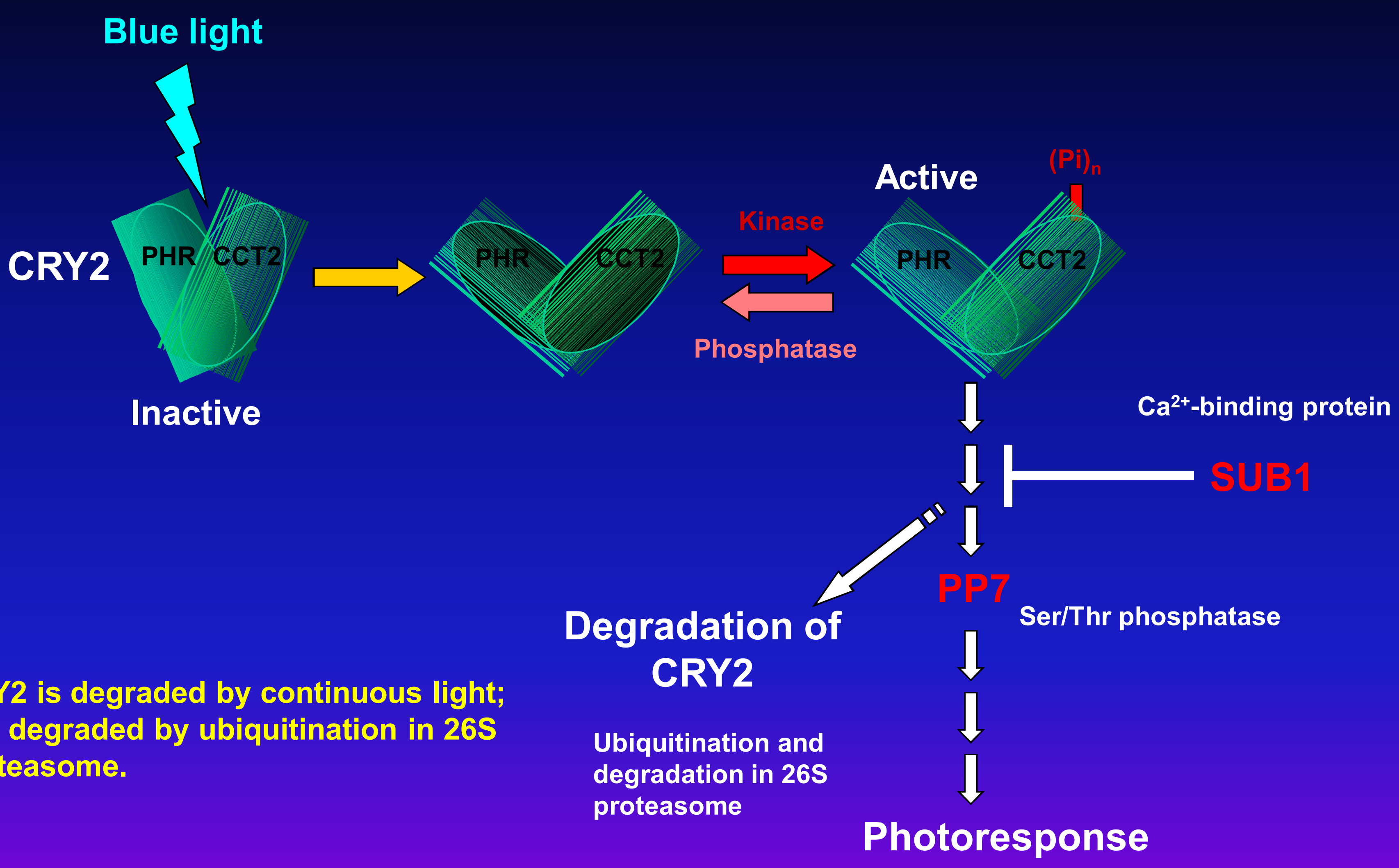
Proposed another mechanism of signal transduction

Phosphorylation – dephosphorylation

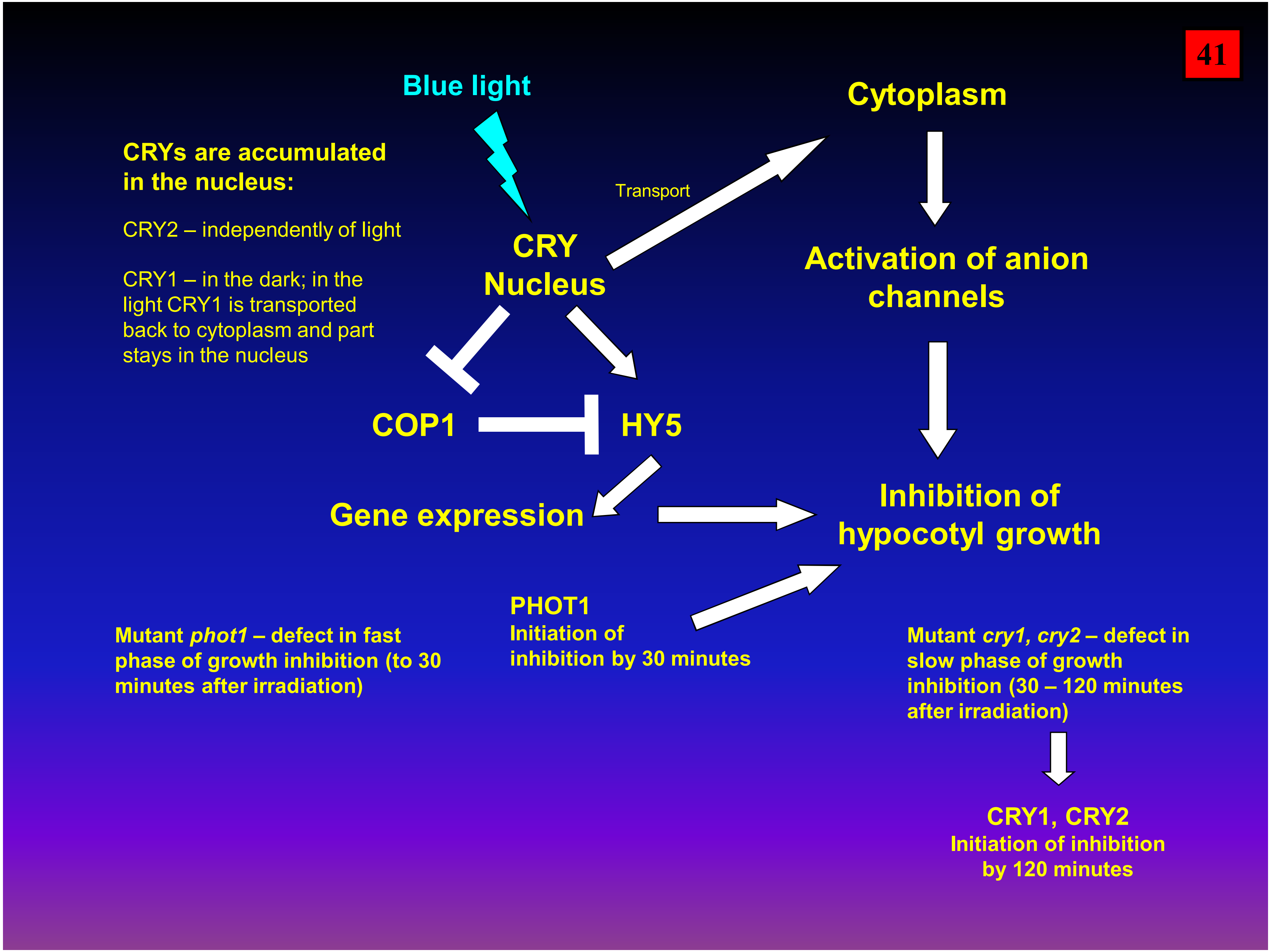
Phosphorylation = adding phosphate group to amino acid residua of a protein

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

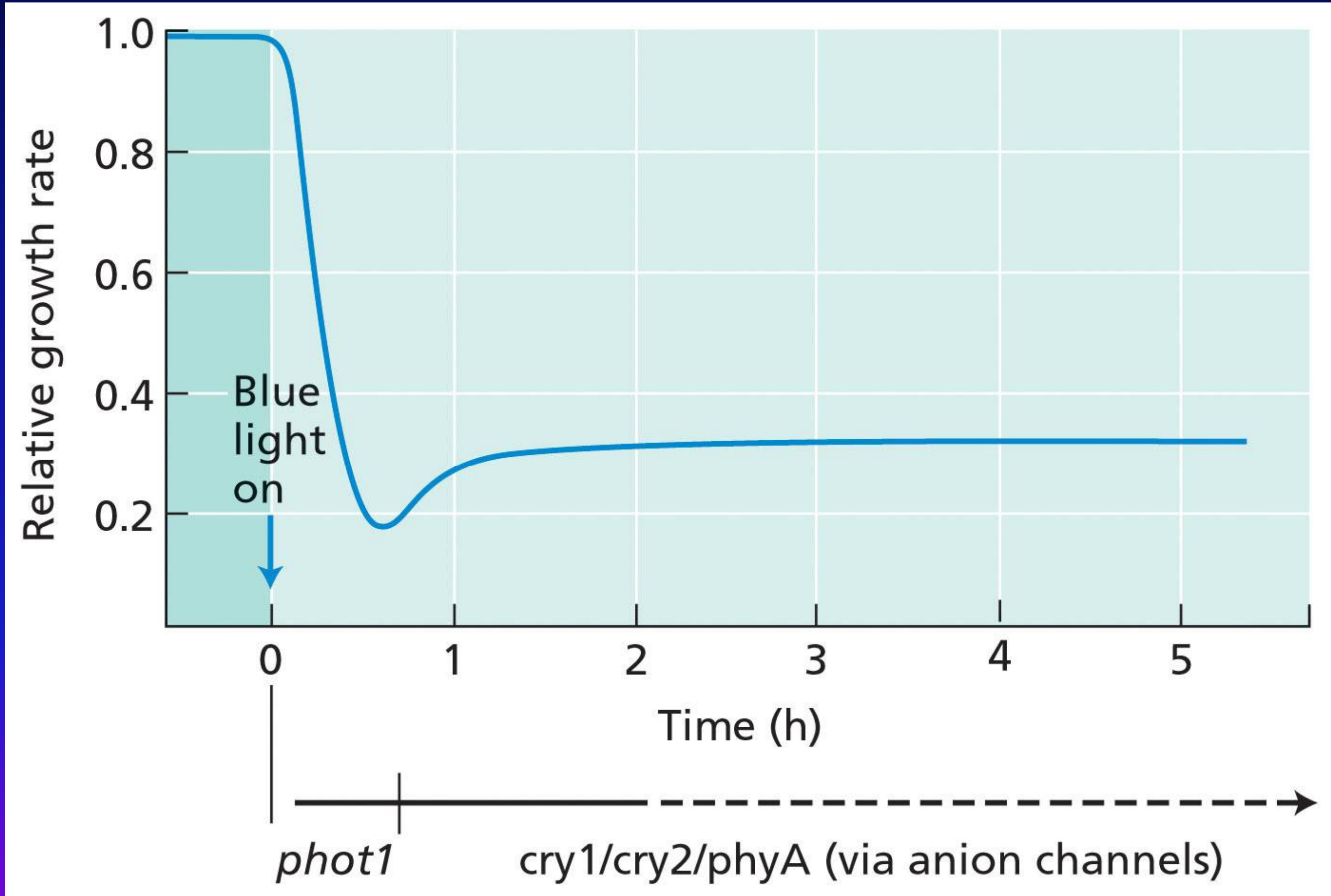
Signaling pathway of cryptochromes CRY



CRY2 is degraded by continuous light;
It is degraded by ubiquitination in 26S proteasome.


Blue light
Cytoplasm
CRYs are accumulated in the nucleus:
CRY2 – independently of light
CRY1 – in the dark; in the light CRY1 is transported back to cytoplasm and part stays in the nucleus
Transport
CRY
Nucleus
Activation of anion channels
COP1
HY5
Gene expression
Inhibition of hypocotyl growth
PHOT1
Initiation of inhibition by 30 minutes
Mutant *phot1* – defect in fast phase of growth inhibition (to 30 minutes after irradiation)
Mutant *cry1, cry2* – defect in slow phase of growth inhibition (30 – 120 minutes after irradiation)
CRY1, CRY2
Initiation of inhibition by 120 minutes

Involvement of *PHOT1* in the inhibition of hypocotyl growth induced by blue light



Signaling pathway of phototropins PHOT

Blue light

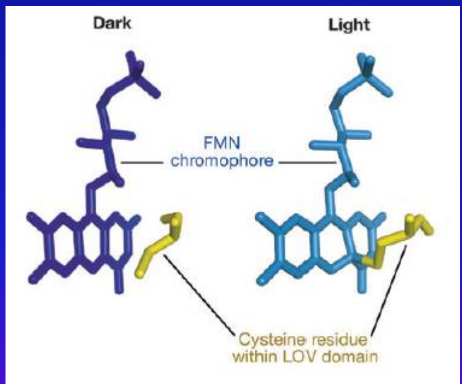
FMN + PHOT1 PHOT2 —Autophosphorylation→ PHOT1 PHOT2 → NPH3 → → ARF7 (NPH4)

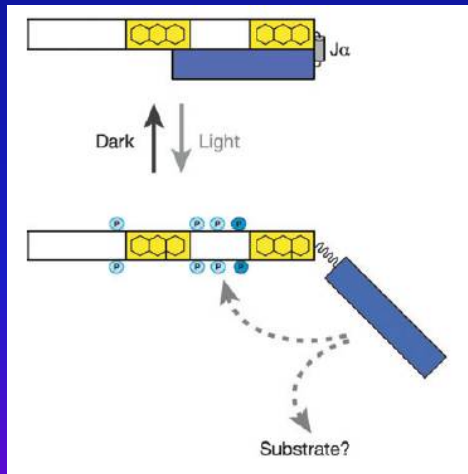
Flavin mononucleotide

Protein kinase

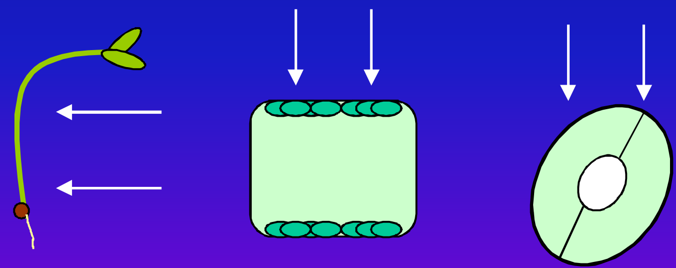
Transducers of signal from cytoplasm to the nucleus

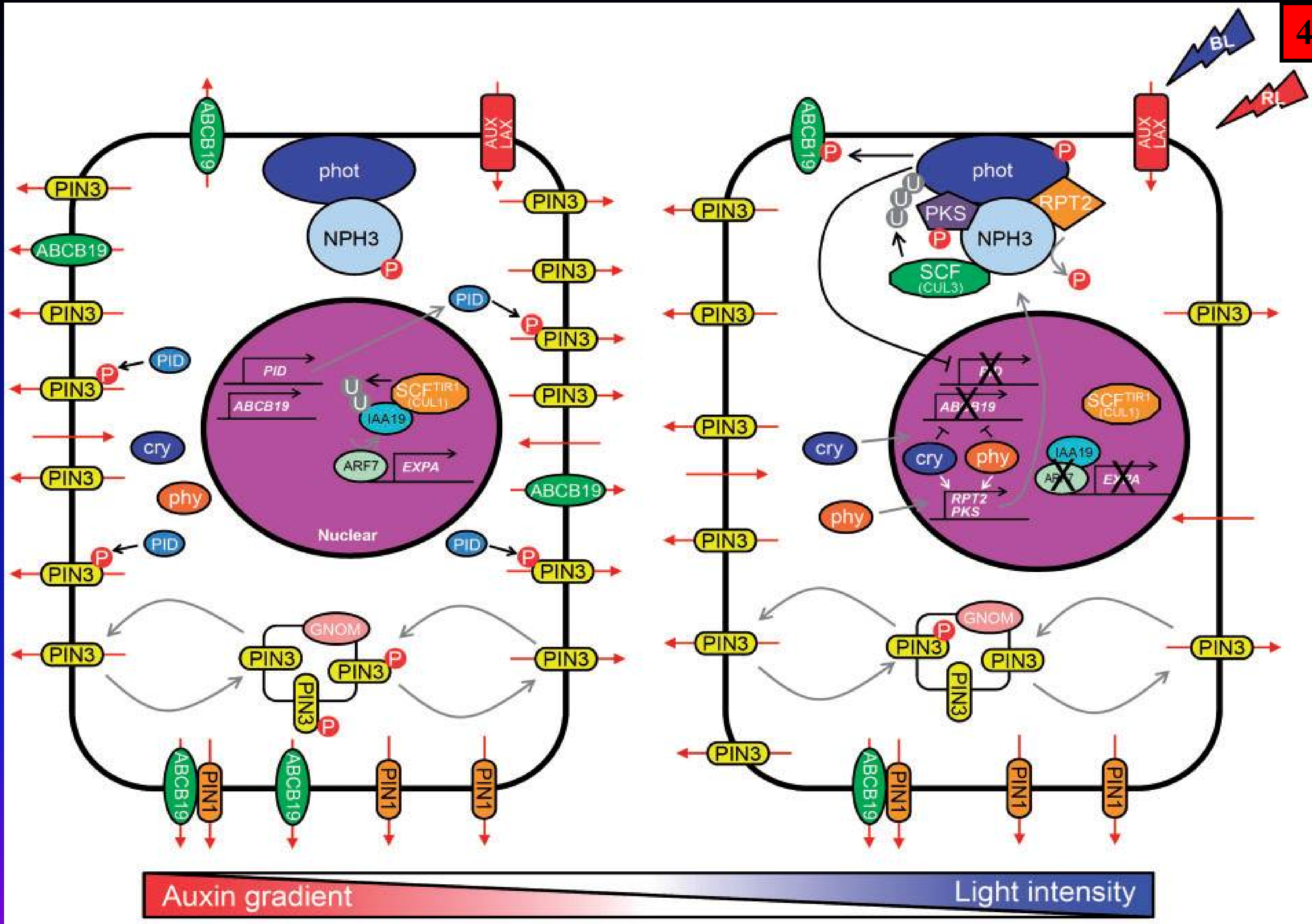
Transcription factor (auxin-responsive)





Phototropism
Chloroplast movement
Stomata opening





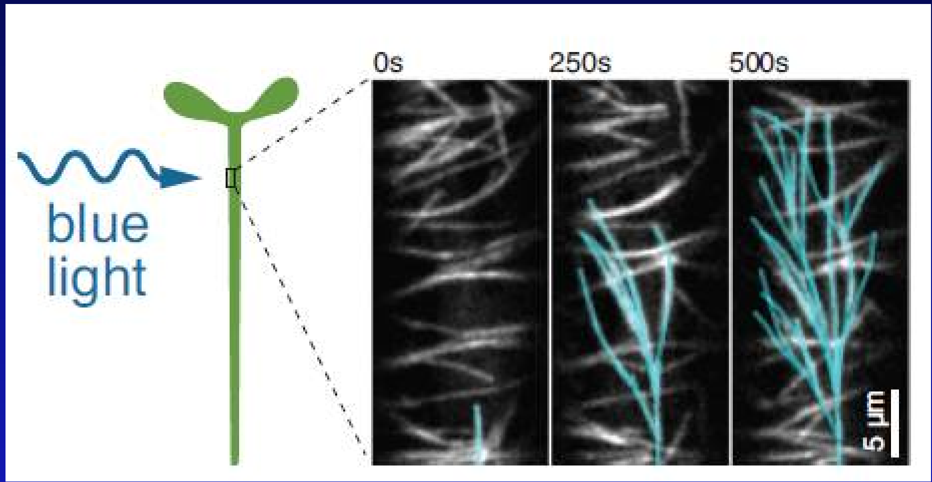
UPDATE 2012

Sakai T, Haga K (2012) Plant & Cell Physiology 53: 1517-1534

UPDATE 2013

Lindeboom JJ et al. (2013) Science 342: 1245533

Mechanism of bending caused by re-orientation of new-formed microtubules of epidermal and cortical cells.



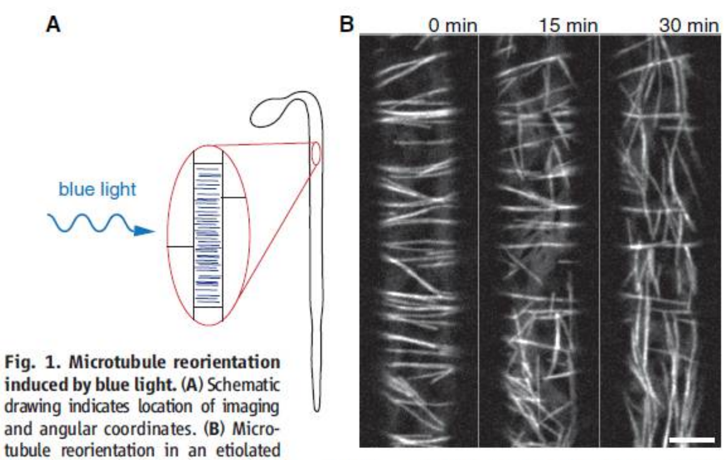
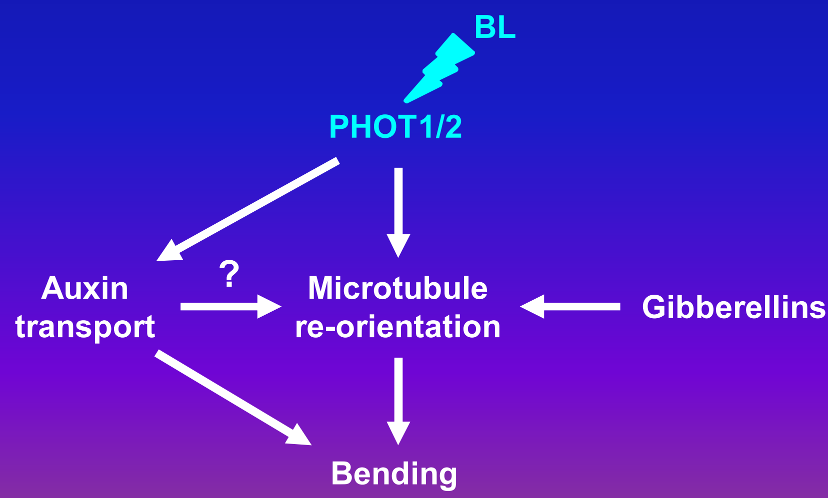
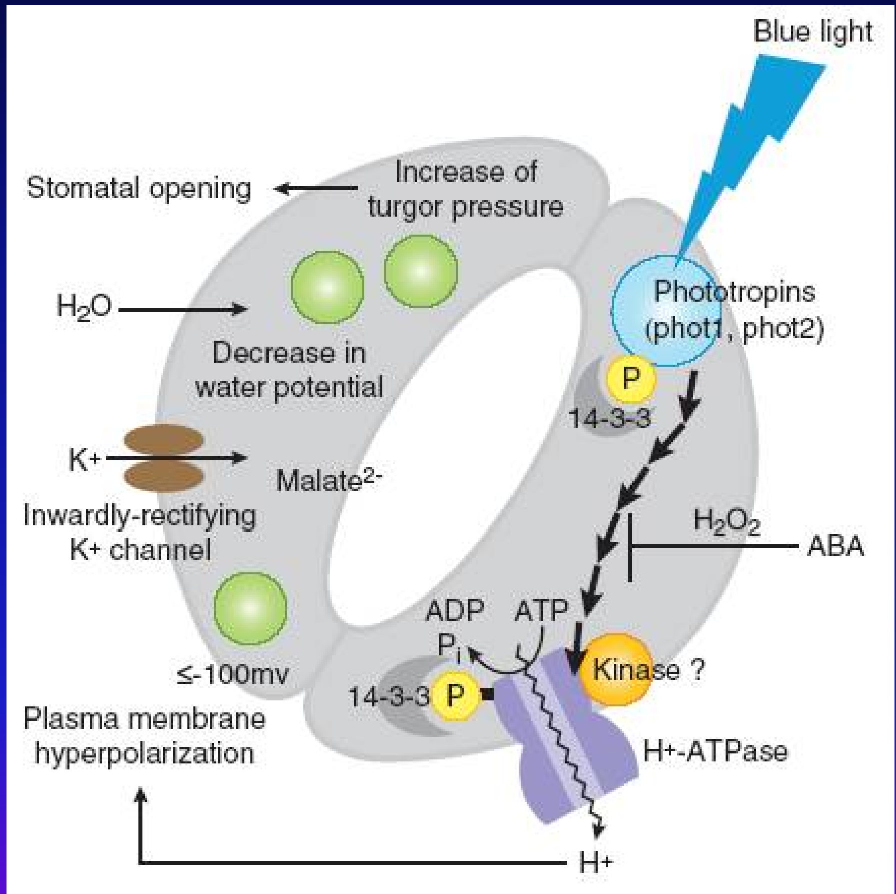


Fig. 1. Microtubule reorientation induced by blue light. (A) Schematic drawing indicates location of imaging and angular coordinates. **(B)** Microtubule reorientation in an etiolated hypocotyl cell expressing mCherry-TUA5 and GCP2-3×GFP (only mCherry-TUA5 signal is shown). Scale bar, 5 µm. See also movie S1.

Using photoreceptors of PHOT1 and PHOT2, blue light stimulates rise of new oriented microtubules. Formation of new microtubules is directed by protein katanin, which severs existing microtubules. Growth of ends of new assembled microtubules results to formation of re-oriented microtubules in epidermal and cortical cells. This re-orientation results to change in cellulose deposition in newly formed cell wall and to bending.



Opening of stomata through phototropins PHOT1 and PHOT2



Blue light

PHOT1, PHOT2

Anion channels

H^+-ATPase

Hyperpolarization

K^+- channels

Stomata opening

H^+-ATPase: C- terminal end has autoinhibitory domain – regulates the activity of ATP-ase by blocking the catalytic site.

Activation of ATPase: phosporylation of Ser/Thr C-terminal domain of ATPase => autoinhibitory domain is removed from catalytic site.


Foto: Martin Rak - Lesní chrám