CRYPTOCHROMES

- **Light causes photosynthesis, it influences development by causing phototropism and also controls the appearance of plant called photomorphogenesis .**
- **There are two major families of plant responses to light signals: the phytochrome responses, and the blue-light responses.**
- **Four kinds of photoreceptors known to affects photomorphogenesis in plants**

    1. **Phytochrome**
    2. **Cryptochrome**
    3. **UVBphotoreceptor**
    4. **Protochlorophyllide a**

Cryptochrome: Blue or near UV light absorbing pigment.

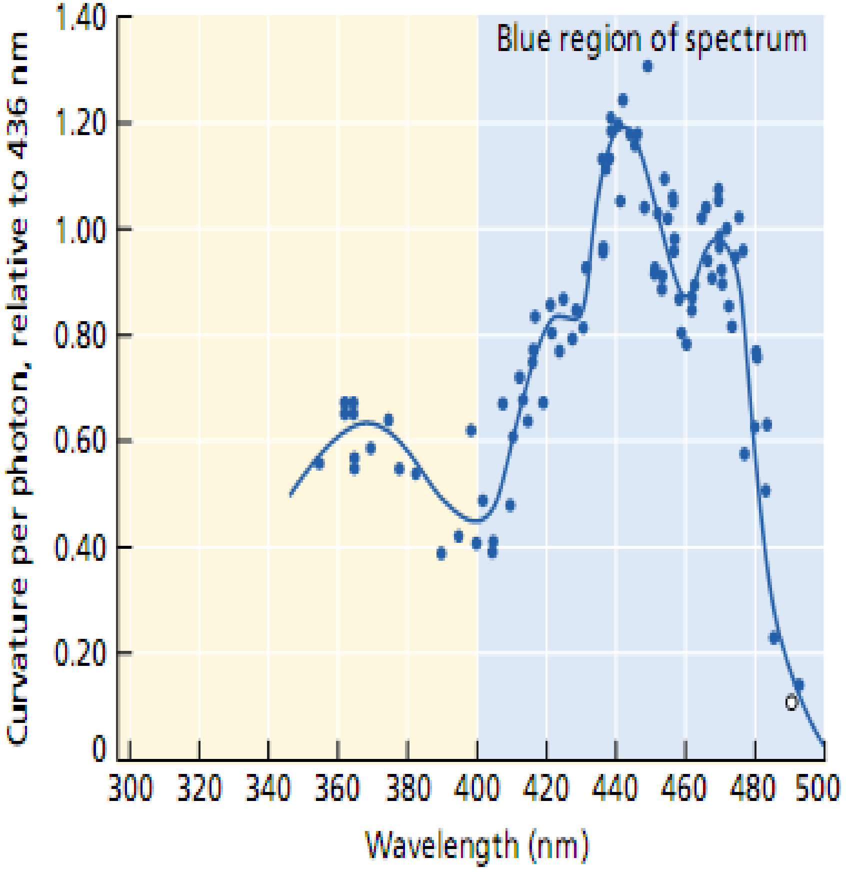
- there are numerous plant responses to blue light: phototropism, inhibition of hypocotyl elongation, stimulation of chlorophyll and carotenoid synthesis, activation of gene expression, stomatal movements, phototaxis (the movement of motile unicellular organisms towards or away from light), enhancement of respiration, and anion uptake in algae
- Blue-light responses have been reported in higher plants, algae, ferns, fungi, and prokaryotes.
- Also absorbs long wave UV (320-340nm).
- Named because of its special importance in cryptogams.
- It is a signaling protein .

How, can we distinguish specific responses to blue light?

One important identification criterion is that in specific blue-light responses, blue light cannot be replaced by a red-light treatment, and there is no red/far-red reversibility

- **Another key distinction is that blue light responses of higher plants share a characteristic action spectrum**

Action spectrum for blue light stimulated phototropism in oat coleoptiles.

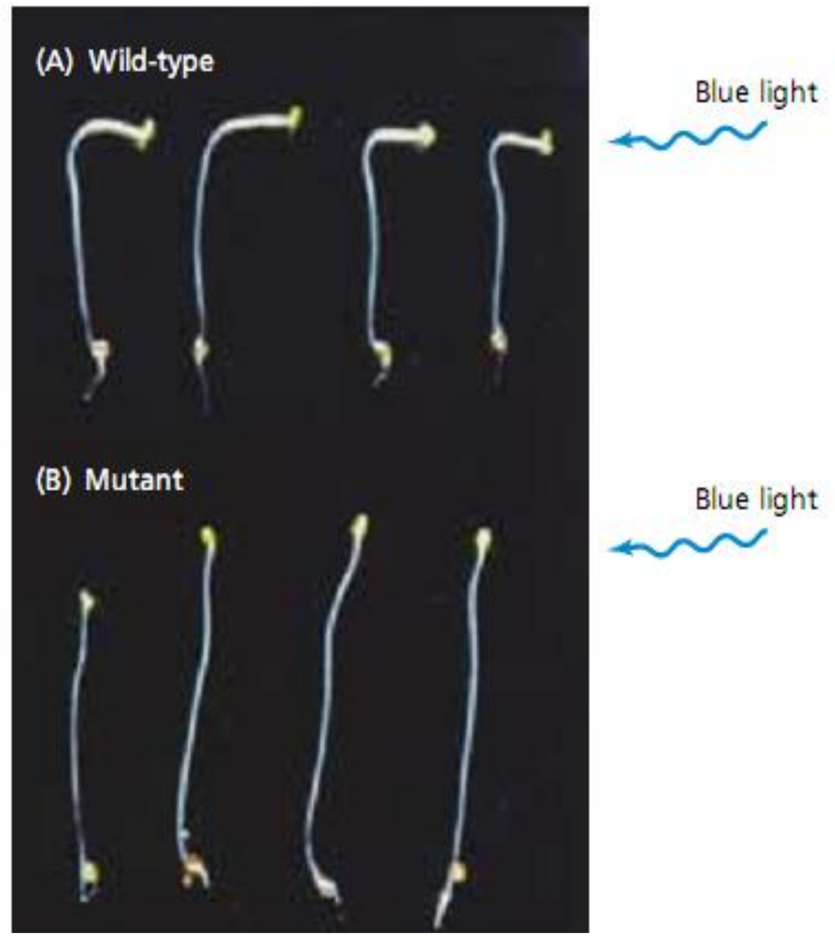


Action spectra for blue light–stimulated phototropism, stomatal movements, inhibition of hypocotyl elongation, and other key blue-light responses share a characteristic "three-finger" fine structure in the 400 to 500 nm region

1) Blue Light Regulates Gene Expression

- **the genes that code for the enzyme a) chalcone synthasec (which catalyzes the first step in flavonoid biosynthesis), b) for the small subunit of rubisco, and c) for the proteins that bind chlorophylls a and b**
- **SIG5, one of six SIG nuclear genes in Arabidopsis that play a regulatory role in the transcription of the chloroplast gene psbD, which encodes the D2 subunit of the PSII reaction center, is specifically activated by blue light. In contrast, the other five genes are activated by both blue and red light.**

2) Blue Light Stimulates Asymmetric Growth and Bending

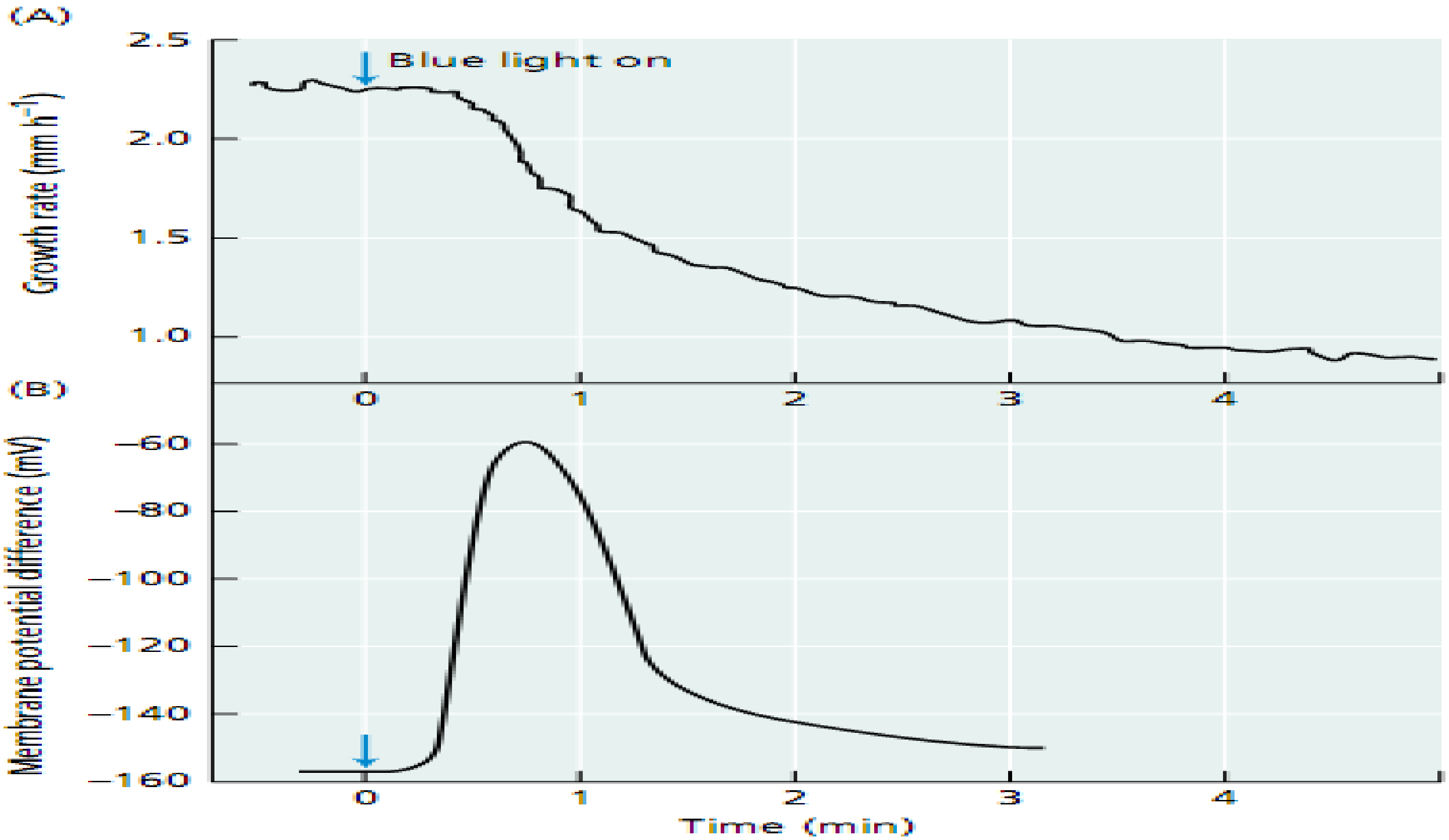


- **phototropic bending occurs only in growing organs, and that coleoptiles and shoots that have stopped elongating will not bend when exposed to unilateral light.**
- **On the other hand, dark-grown, etiolated coleoptiles continue to elongate at high rates for several days and, depending on the species, can attain several centimeters in length.**
- **When irradiated with unilateral blue light, the sporangiophore of phycomyces bends toward the light with an action spectrum similar to that of coleoptile phototropism.**
- **These studies have led to the isolation of many mutants with altered phototropic responses and the identification of several genes that are required for normal phototropism.**

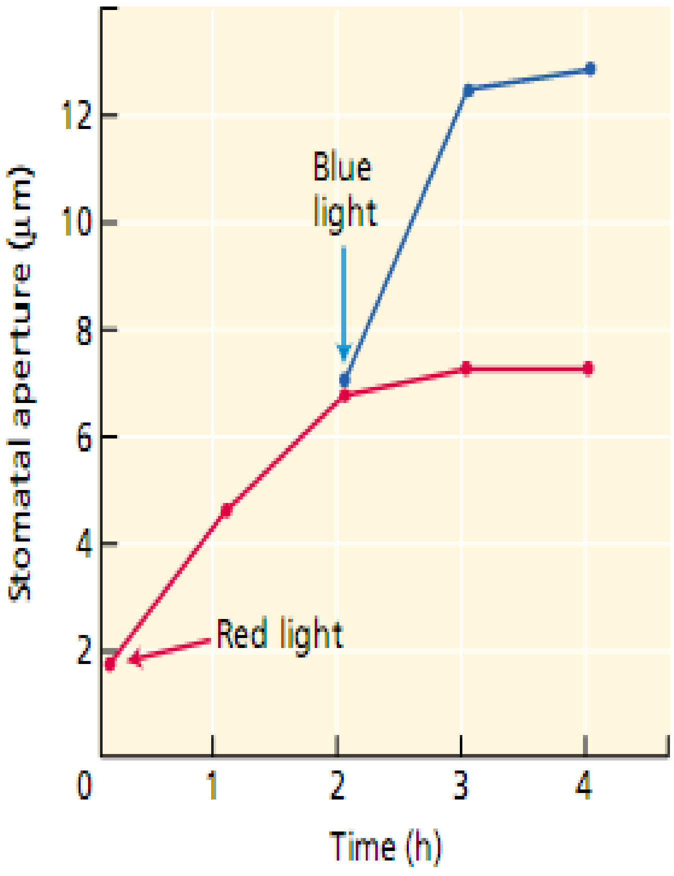
3) Blue Light Rapidly Inhibits Stem Elongation

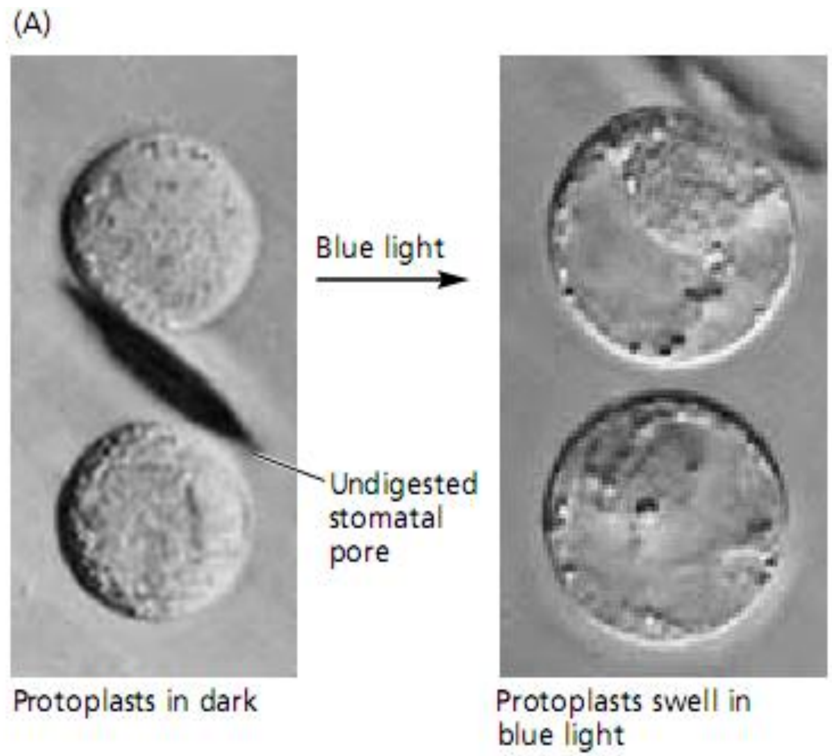
- **Another fast response elicited by blue light is a depolarization of the membrane of hypocotyl cells that precedes the inhibition of growth rate**
- **The membrane depolarization is caused by the activation of anion channels which facilitates the efflux of anions such as chloride.**
- **Use of an anion channel blocker prevents the blue light dependent membrane depolarization and decreases the inhibitory effect of blue light on hypocotyl elongation**

Blue light induced (A) changes in elongation rates of etiolated cucumber seedlings and (B) transient membrane depolarization of hypocotyl cells. As the membrane depolarization (measured with intracellular electrodes) reaches its maximum, growth rate (declines sharply.



4) Blue Light Stimulates Stomatal Opening





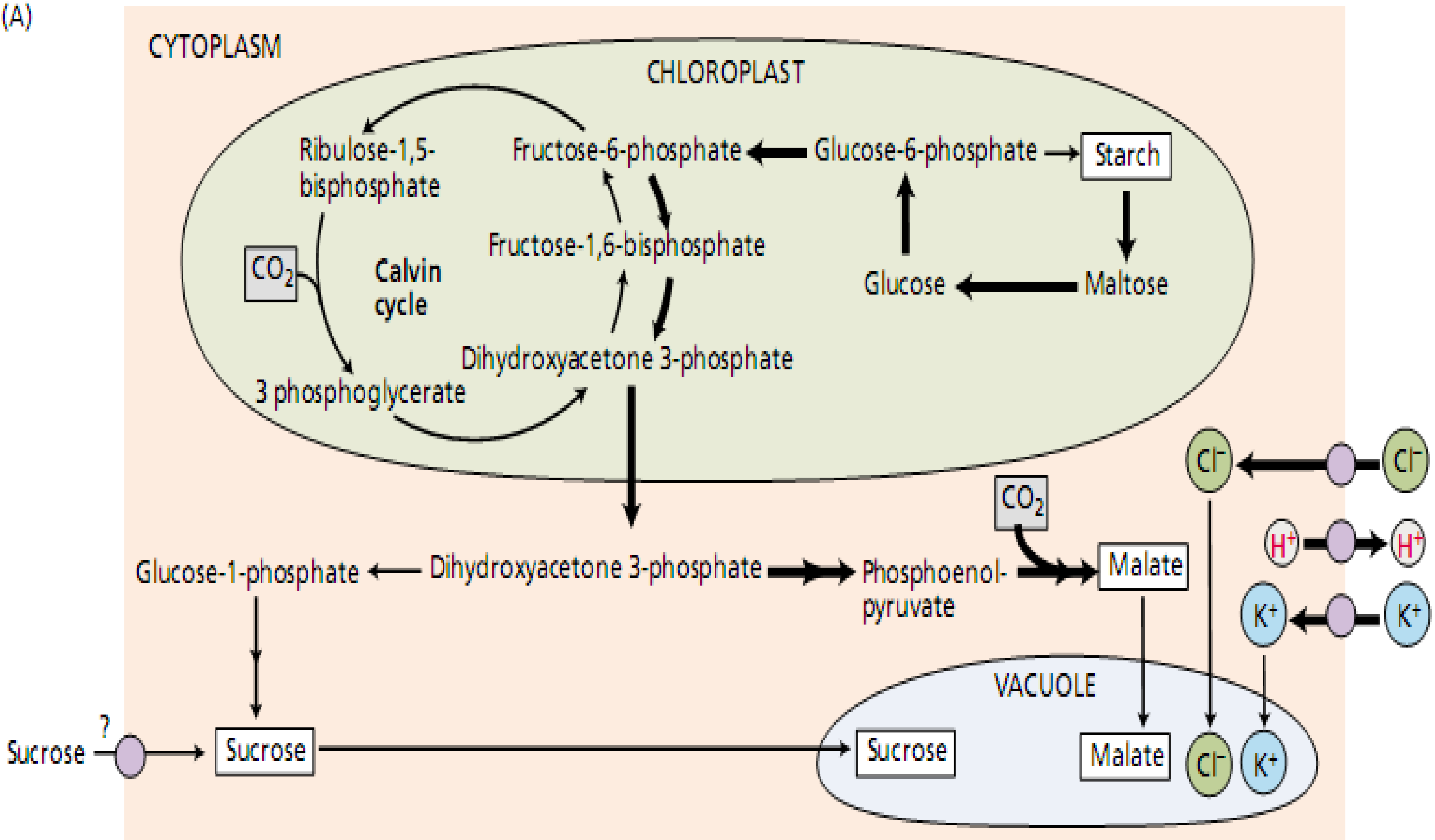
- **The light-stimulated uptake of ions and the accumulation of organic solutes decrease the cell’s osmotic potential (increase the osmotic pressure).**
- **Water flows in as a result, leading to an increase in turgor of guard cells which finally is mechanically transduced into an increase in stomatal apertures**

Blue Light Activates a Proton Pump at the Guard Cell Plasma Membrane

- **When guard cell protoplasts from broad bean (*Vicia faba*) are irradiated with blue light under background red-light illumination, the pH of the suspension medium becomes more acidic.**
- **This blue light induced acidification is blocked by inhibitors that dissipate pH gradients, such as CCCP, and by inhibitors of the ATPase, such as vanadate**
- **this blue-light stimulation of proton pumping lowers the pH of the apoplastic space surrounding the guard cells.**

Blue Light Regulates Osmotic Relations of Guard Cells

(A)



- **The electrical component of the proton gradient provides a driving force for the passive uptake of potassium ions via voltage-regulated potassium channels**
- **Chloride is thought to be taken up through anion channels. Thus, blue light dependent stimulation of proton pumping plays a key role in guard cell osmoregulation during light-dependent stomatal movements**
- **Potassium concentration in guard cells increases several fold when stomata open, from 100 mM in the closed state to 400 to 800 mM in the open state, depending on the plant species and the experimental conditions.**
- **These large concentration changes in the positively charged potassium ions are electrically balanced by the anions Cl- and malate**

Four distinct metabolic pathways that can supply osmotically active solutes to guard cells have been characterized

1. The uptake of K^+ and Cl^- coupled to the biosynthesis of malate^{2-}
2. The production of sucrose from starch hydrolysis
3. The production of sucrose by photosynthetic carbon fixation in the guard cell chloroplast
4. The uptake of apoplastic sucrose generated by mesophyll photosynthesis

Depending on environmental conditions, one or several pathways may be activated. For instance, red light–stimulated stomatal opening in detached epidermis depends solely on sucrose generated by guard cell photosynthesis,

- **The hy4 mutant of Arabidopsis lacks the blue light stimulated inhibition of hypocotyl elongation**
- **Isolation of the HY4 gene showed that it encodes a 75 kDa protein with significant sequence homology to microbial DNA Photolyase, a blue light activated enzyme that repairs pyrimidine dimers in DNA formed as a result of exposure to UV radiation**
- **The hy4 protein, later renamed cryptochrome 1 (cry1), was proposed to be a blue-light photoreceptor mediating the inhibition of stem elongation.**

- **No information is available on the chromophore(s) bound to cry1 in vivo, or on the nature of the photochemical reactions involving cry1, that would start the postulated sensory transduction cascade mediating the several blue-light responses mediated by cry1.**
- **A second gene product homologous to CRY1, named CRY2, has been isolated from Arabidopsis**
- **Both CRY1 and CRY2 appear ubiquitous throughout the plant kingdom.**
- **A major difference between them is that CRY2 is rapidly degraded in the light, whereas CRY1 is stable in light-grown seedlings**.

CRY1 & CRY 2 have differential levels of influence

Transgenic plants overexpressing the gene that encodes CRY2 show a small enhancement of the inhibition of hypocotyl elongation, indicating that unlike CRY1, CRY2 does not play a primary role in inhibiting stem elongation.

the transgenic plants overexpressing the gene that encodes CRY2 show a large increase in blue light stimulated cotyledon expansion,

In addition, CRY1 has been shown to be involved in the setting of the circadian clock in Arabidopsis

The Carotenoid Zeaxanthin Mediates Blue-Light Photoreception in Guard Cells

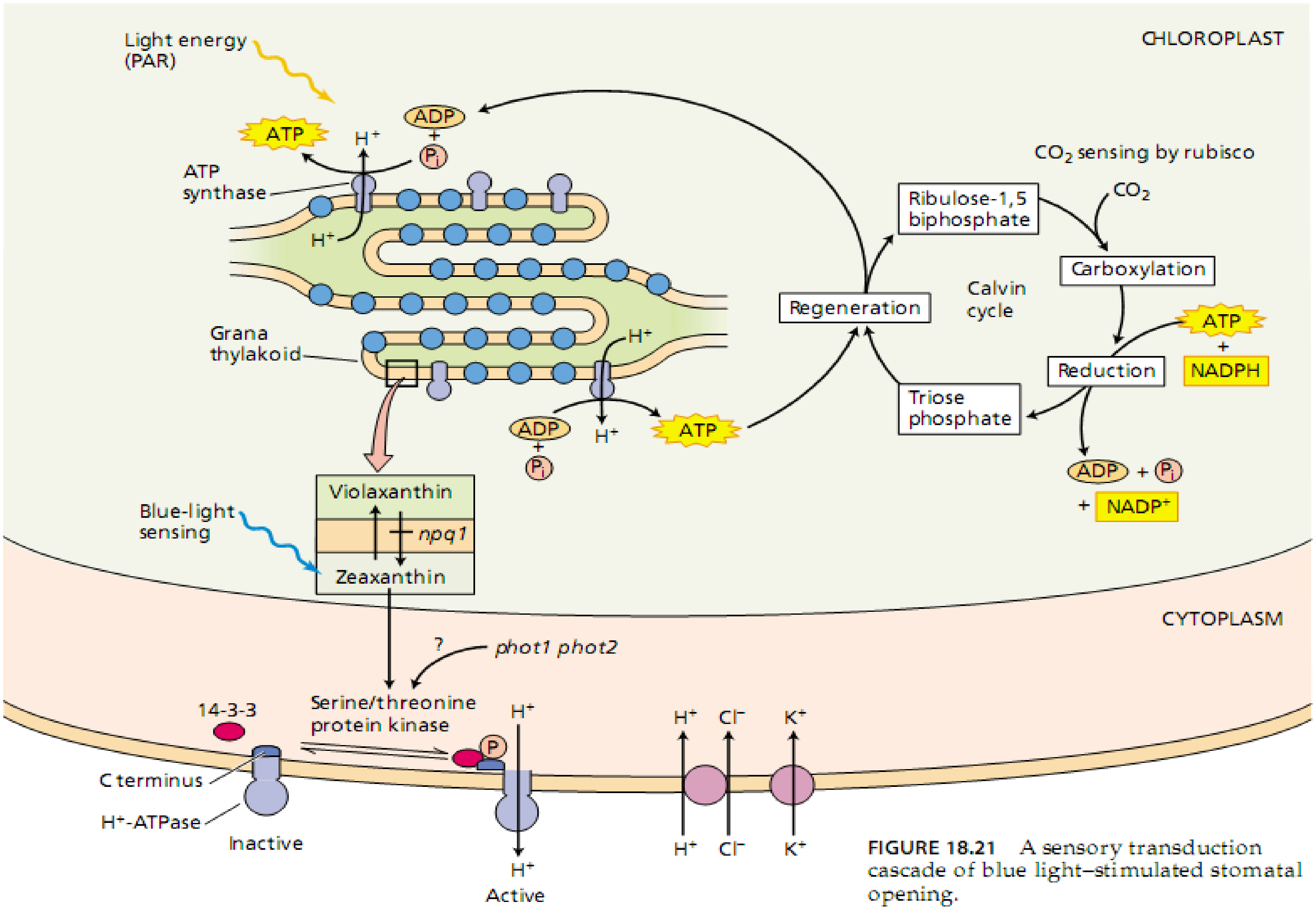


FIGURE 18.21 A sensory transduction cascade of blue light–stimulated stomatal opening.

Evidence

- Blue light–stimulated stomatal opening is completely inhibited by 3 m*M* dithiothreitol (DTT), and the inhibition is concentration dependent. Zeaxanthin formation is blocked by DTT, a reducing agent that reduces S—S bonds to –SH groups and effectively inhibits the enzyme that converts violaxanthin into zeaxanthin. The specificity of the inhibition of blue light–stimulated stomatal opening by DTT, and its concentration dependence, indicate that guard cell zeaxanthin is required for the stomatal response to blue light.

5) PRODUCTION OF ANTHOCYANIN

- In most plants anthocyanin production is frequently promoted by blue light.
- Action spectra of anthocyanin production in several species shows that general maximum responses occur in the red, far red and blue regions.

6) CONTROLS FLOWERING

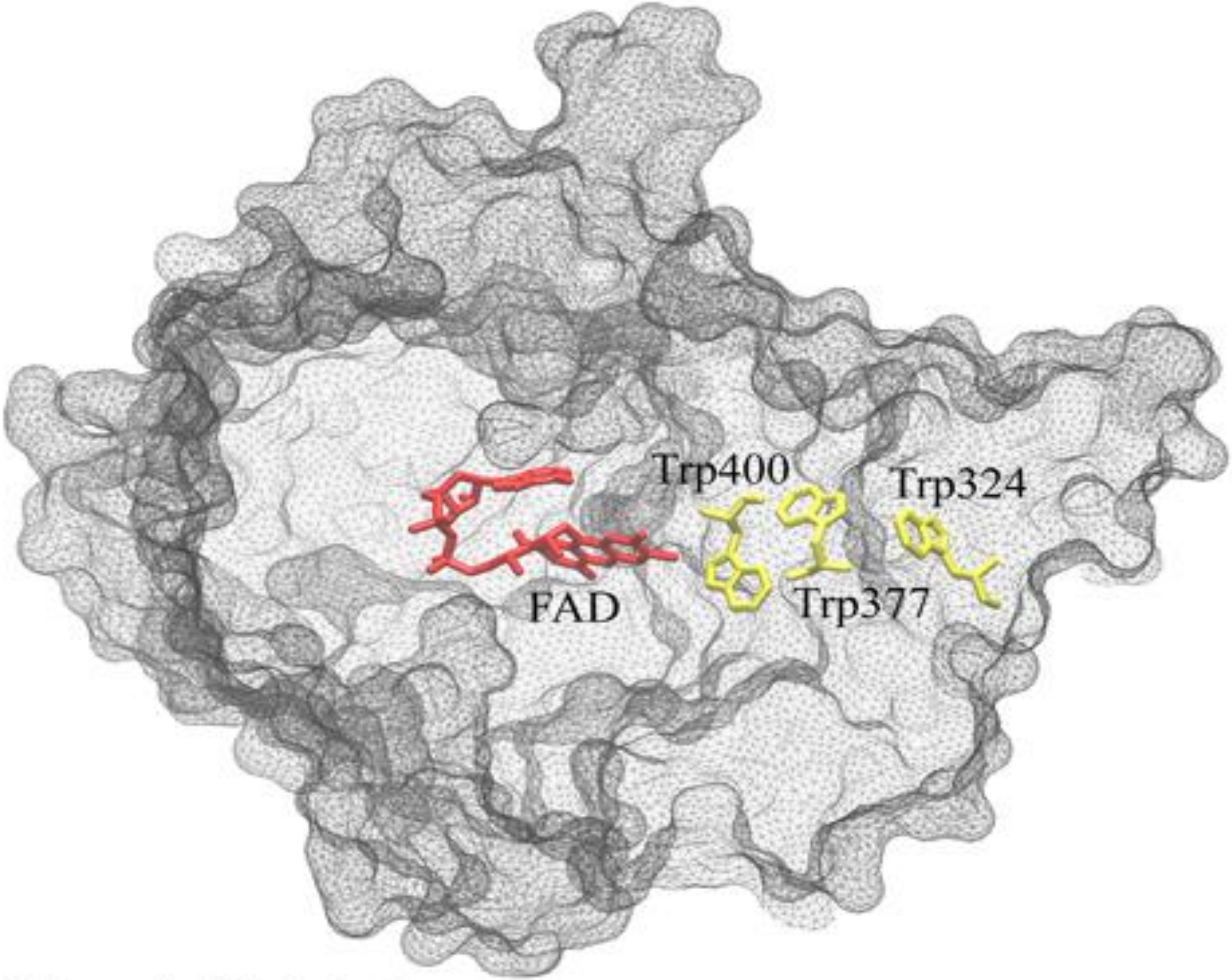
- **The CRY1 and CRY2 double mutant showed delayed flowering in monochromatic blue light.**
- **Whereas neither monogenic CRY1 nor CRY2 mutant exhibited late flowering in blue light**
- **This result suggests that, in addition to the phyB-dependent function, CRY2 also acts redundantly with CRY1 to promote floral initiation in a phyB-independent manner.**

Structure and activation

- Cryptochrome are flavo proteins that derived from DNA photolyase.

- 3 tryptophans,Trp 324,Trp 377,Trp 400 involved in the activation of cryptochrome.

- Trp 324 is located near the periphery of the protein body and Trp 400 is proximal to the protein cofacter with Trp 377 located in between.



Trp400
Trp324
FAD
Trp377

- Before activation ,the flavin cofactor become in its fully oxidised state.

- FAD absorbs blue light photons and excited to FAD.*

- FAD is then protonated producing FADH.

- In this state electron transfer is initiated .

- An electron first jumps from Trp 400 in to the hole left by excited electron in FADH forming FADH+Trp 400*.

- An electron then jumps from Trp 377 to Trp 400 forming FADH +Trp377*.

- From Trp 324 an electron then jumps to Trp 377 forming FADH+ Trp 324*.

- Finally Trp 324 becomes deprotonated to Trp 324dep. forming FADH+Trp 324dep. fixing the electron on FADH.
- The protein cryptochrome is thought to be in active state when the flavin is in FADH form.
- However cryptochrome could revert to its non active form if ever the unpaired electron on FADH back transfer to one of the 3 tryptophan.

SIGNAL TRANSDUCTION

- Sensory transduction cascades for the blue-light responses encompass the sequence of events linking the initial absorption of blue light by a chromophore and the final expression of a blue-light response, such as stomatal opening or phototropism.
- cry2, and to a lesser extent cry1, accumulates in the nucleus. This suggests that both proteins might be involved in the regulation of gene expression.
- But some of the cryptochrome action in response to blue light seems to occur in the cytoplasm because one of the earliest detected defects in cry1mutant seedlings is impaired activation of anion channels at the plasma membrane.
- In addition, cry1 and cry2 have been shown to interact with phytochrome A in vivo, and to be phosphorylated by phytochrome A in vitro

The reversal of blue light stimulated opening by green light.

- **A reversal of blue light stimulated stomatal opening by green light has been recently discovered.**
- **Stomata in epidermal strips open in response to a 30 s blue-light pulse but the opening is not observed if the blue light pulse is followed by a green-light pulse. The opening is restored if the green pulse is followed by a second blue-light pulse, in a response analogous to the red/far-red reversibility of phytochrome responses. (Frechilla et al. 2000.)**

To Conclude…………

These blue light signals are transduced in to electrical metabolic and genetic processes that allow plants to alter growth development and function in order to acclimate the changing environmental conditions.