Light perception

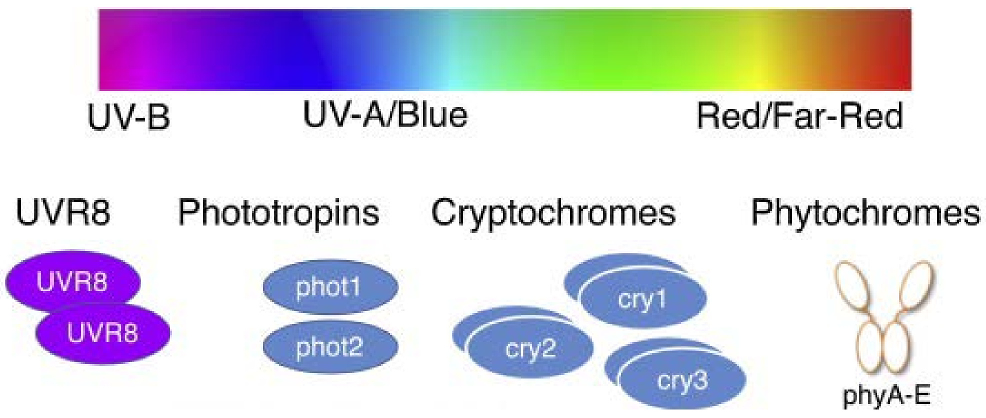
What does a plant see?

Light: y/n	Direction	Intensity	Color	Duration
Photomorphogenesis	**Phototropism**	**Photons / time x area**	**Spectral distribution**	**Day length**
Skotomorphogenesis				
			daytime	season
below / above ground				flowering time
shade (avoidance)				
day / night				

Four photoreceptor systems

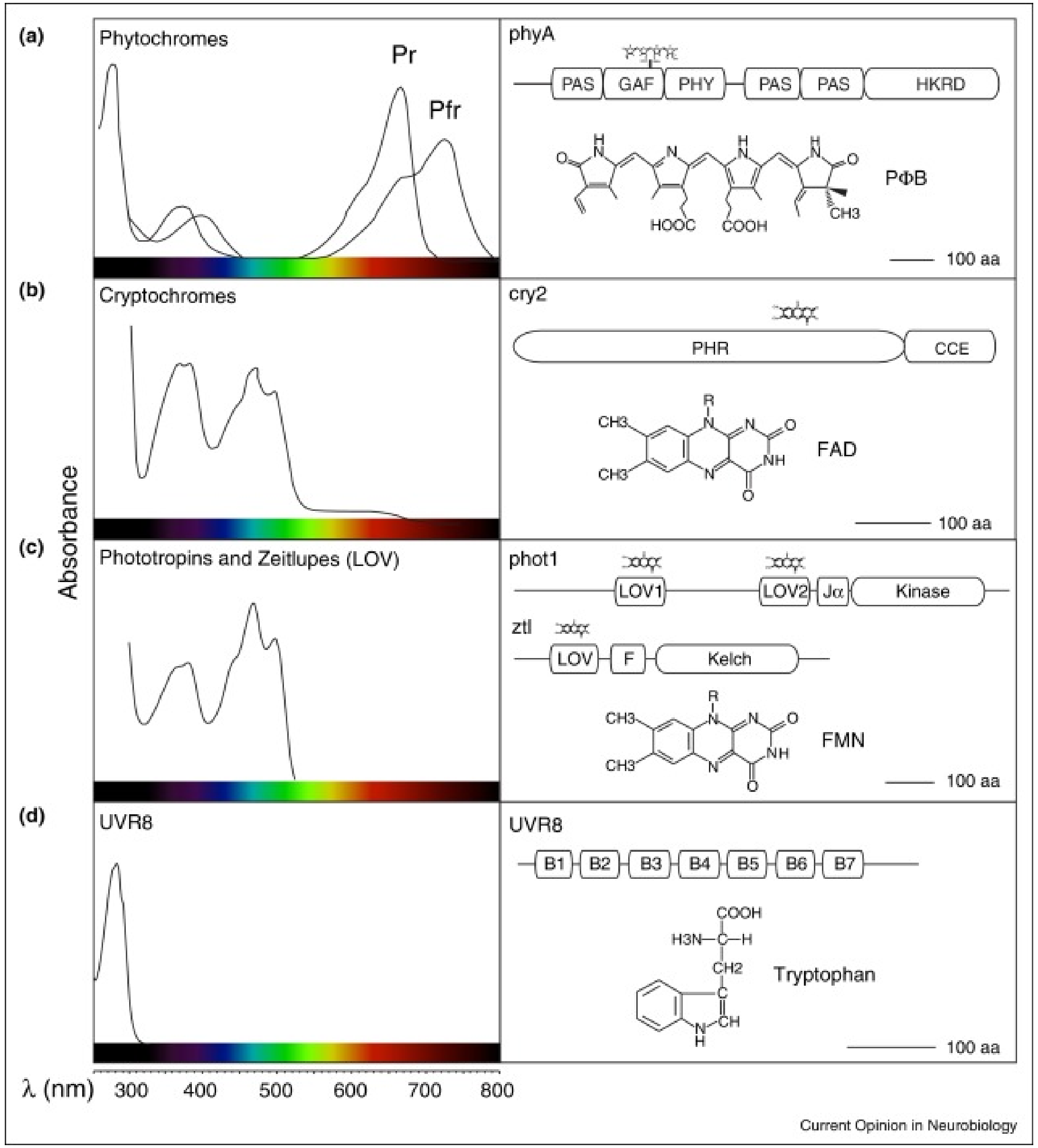
- light perception: ~ 260 bis ~730 nm
- perception by eye (max:~ = 550 nm)
- cytosolic photoperception *vs.* photosynthetic pigments in plastids

- <u>phytochromes</u>
  - absorption maxima: red/far-red
  - photoreversible
- 2 types of blue light/UV-A-photoreceptors
  - <u>phototropins</u> and <u>cryptochromes</u>
- <u>UV-B photoreceptor</u> (UVR8)
  - UV-B protection

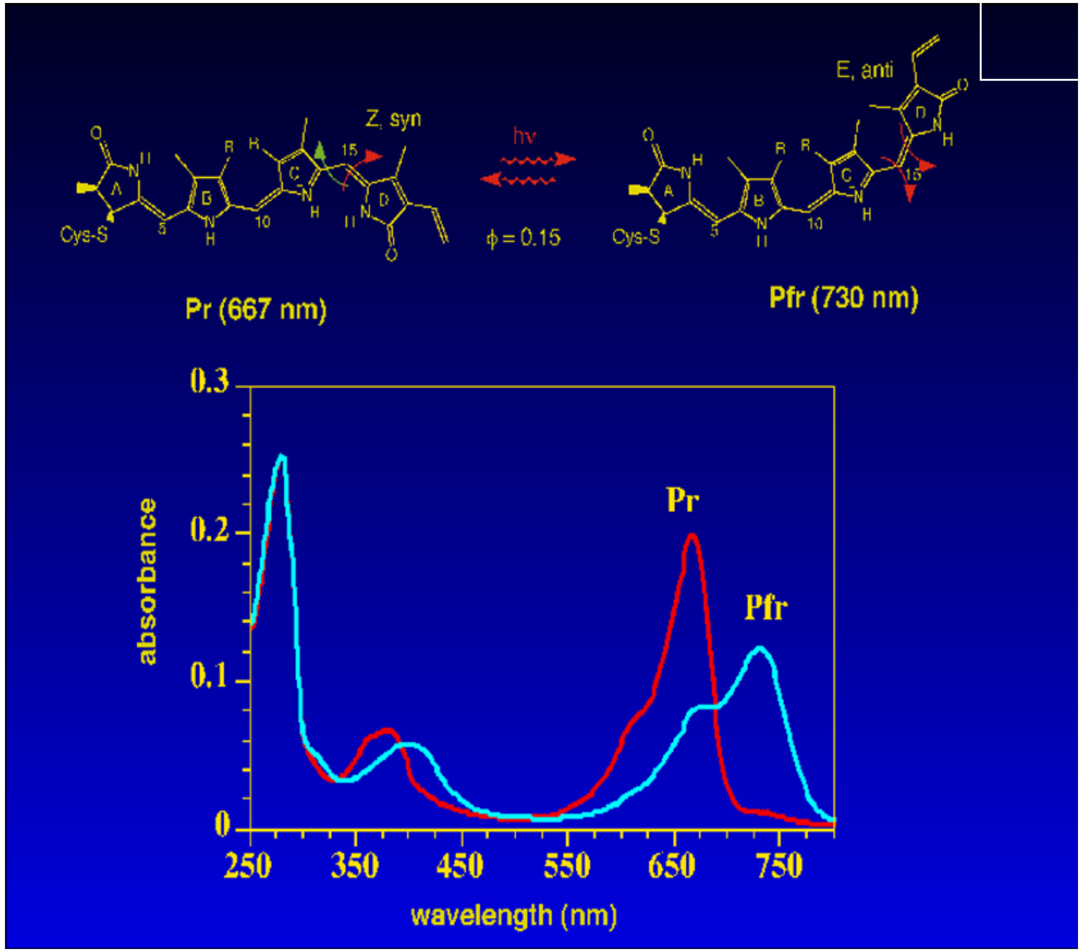


	photoreceptor	genes	evolutionary precursor	photo-responses
	UV-B receptor	–	–	hypocotyl growth flavonoid synthesis
UV-A / blue	cryptochormes	CRY1 CRY2	photolyases	hypocotyl growth flavonoid synthesis flower induction
	phototropins	PHOT1 PHOT2	bacterial light, oxygen, voltage receptors	phototropism stomata opening chloroplast movement
red / far-red	phytochromes	PHYB PHYC PHYD PHYE PHYA	bacterial two-component histidine kinases	hypocotyl growth flower induction flavonoid synthesis root growth shade avoidance greening etc.

Chromophores



1. Phytochromes



photoreversible pigment

Red light changes P_r to P_{fr}
Far-red light changes P_{fr} to P_r
P_{fr} reverts to P_r in the dark

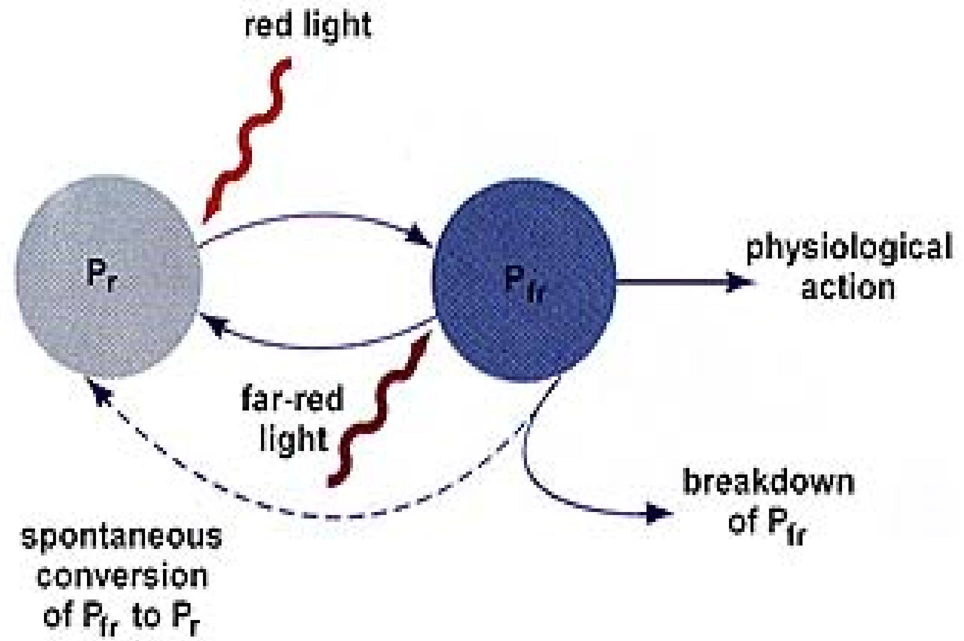
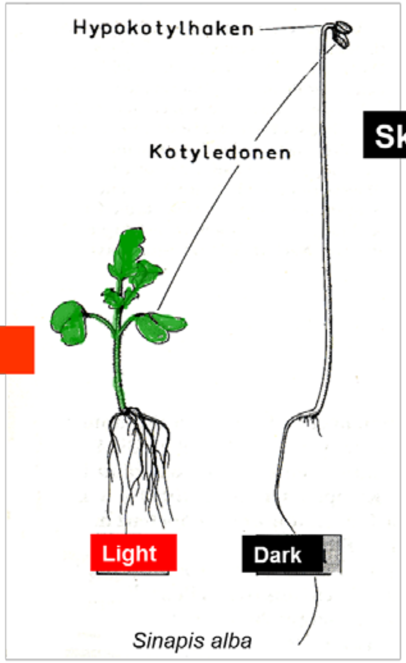


Photo-/scoto-morphogenesis: classical phytochrome experiment

Photomorphogenesis



Skotomorphogenesis

Photomorphogenesis

SB21.17

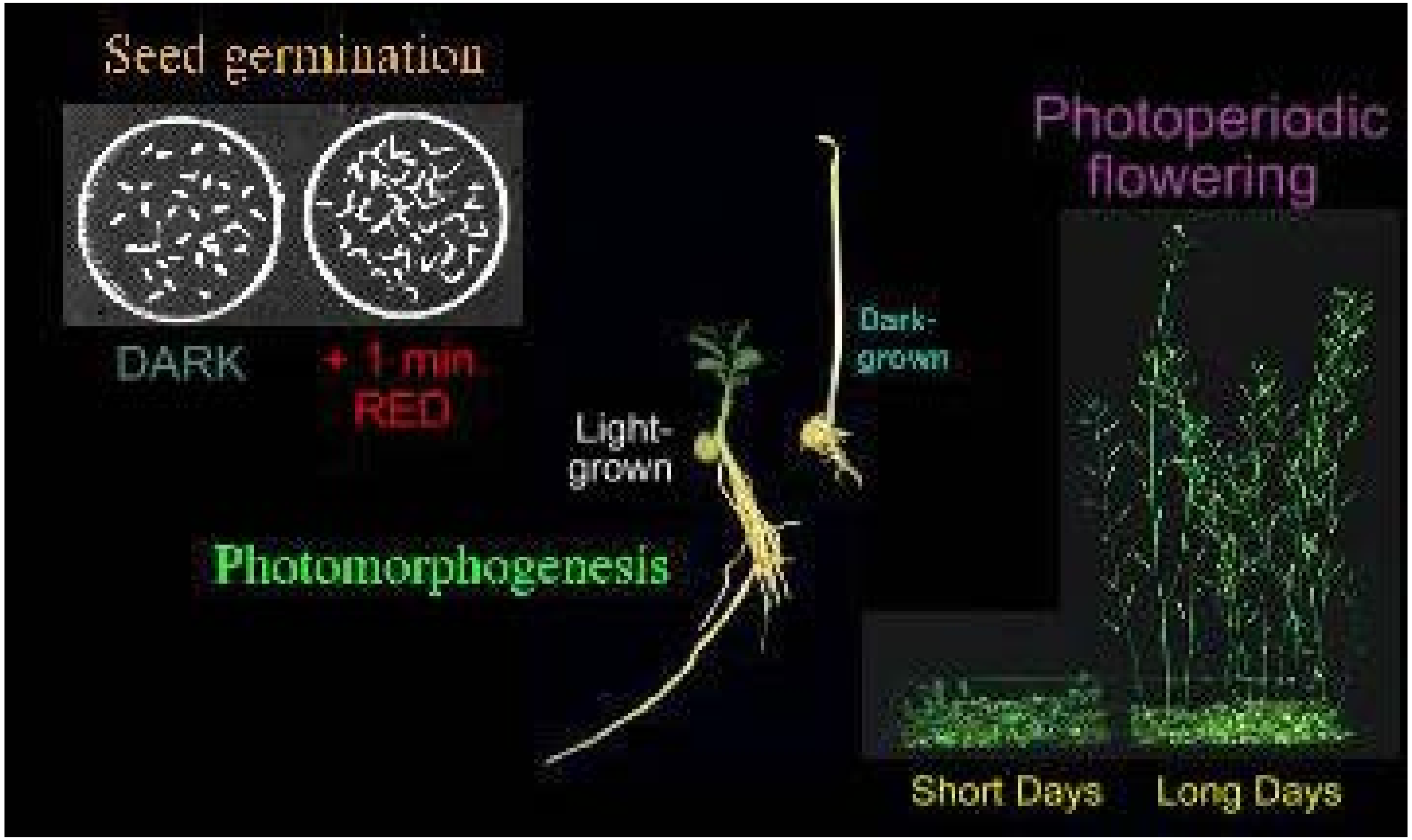
Dicotyledoneous seedlings

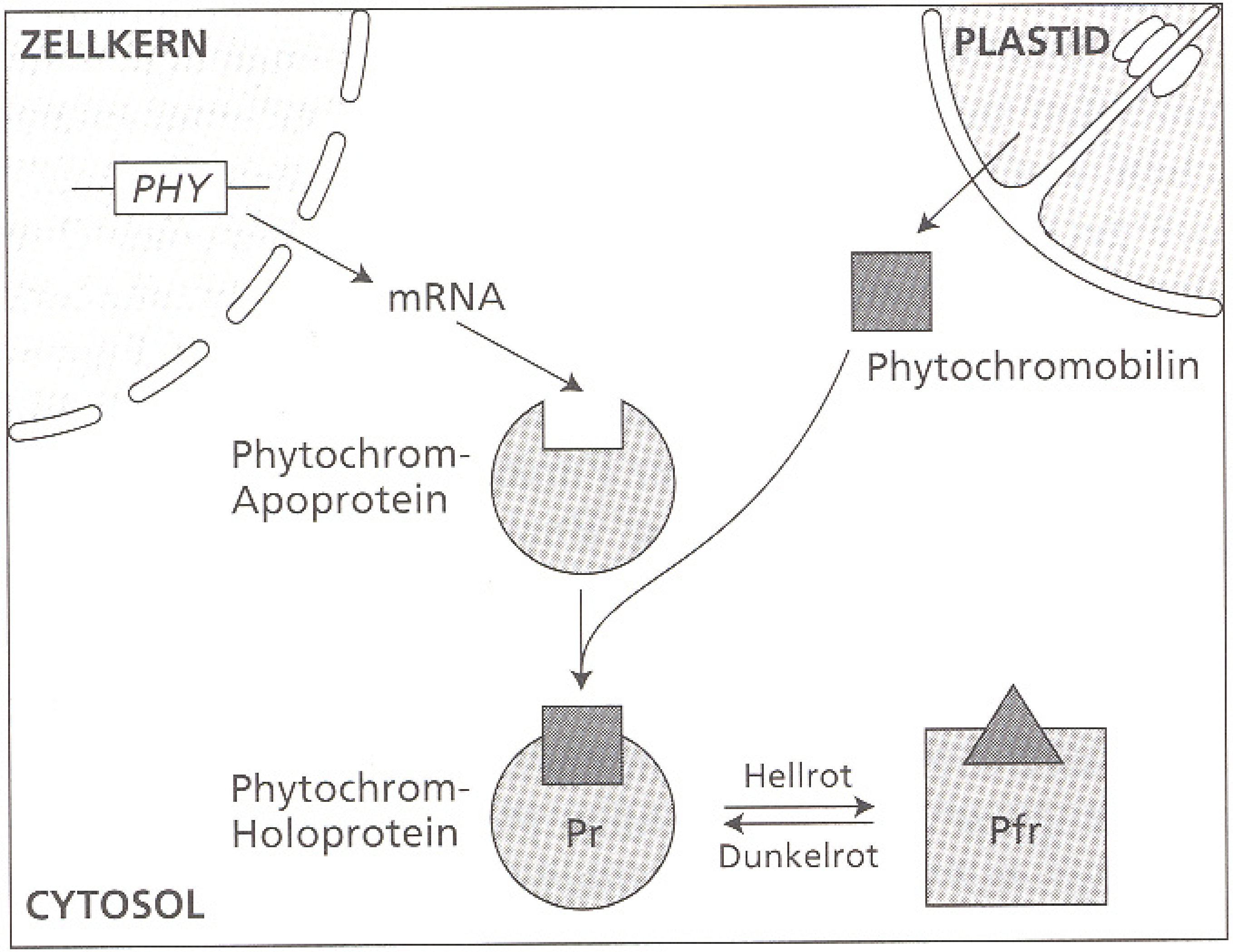
- germination is normally light-inducible

- 3 criteria for photomorphogenesis
  - hypocotyl elongation
  - hock opening
  - cotyledon developmen

-Questions:
- why such a developmental change?
- isolation and characterization of mutants
- involvement of photorecpetor?

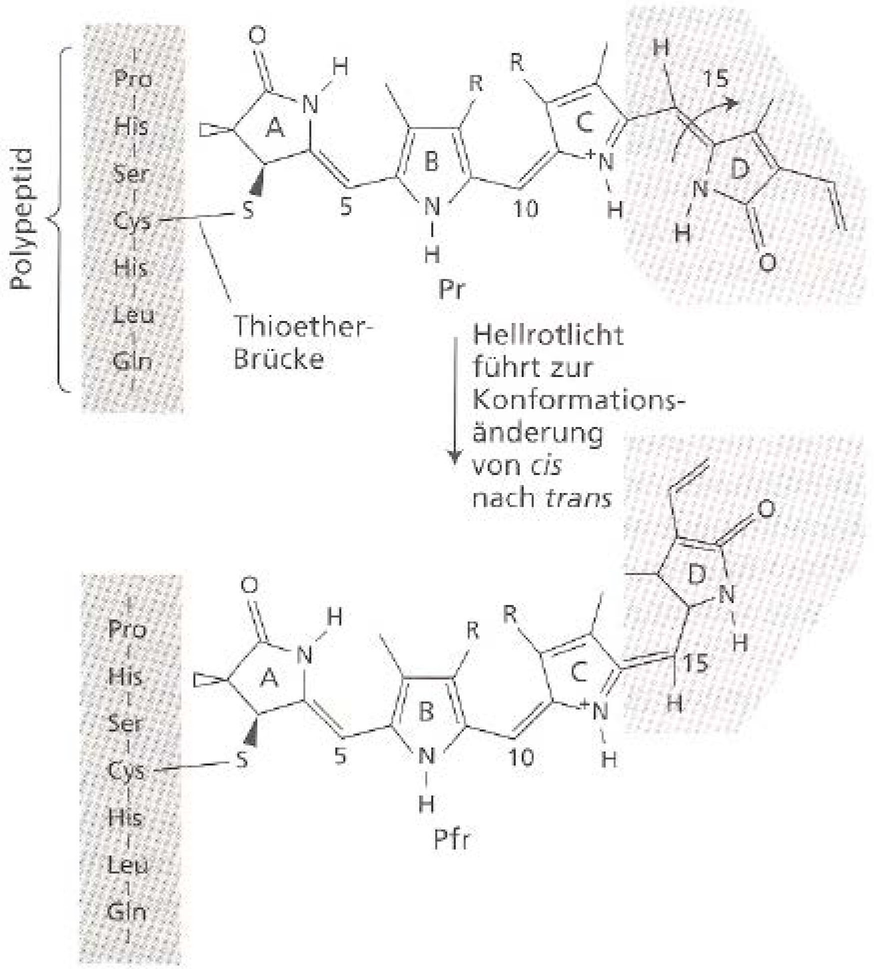
phytochrome controls





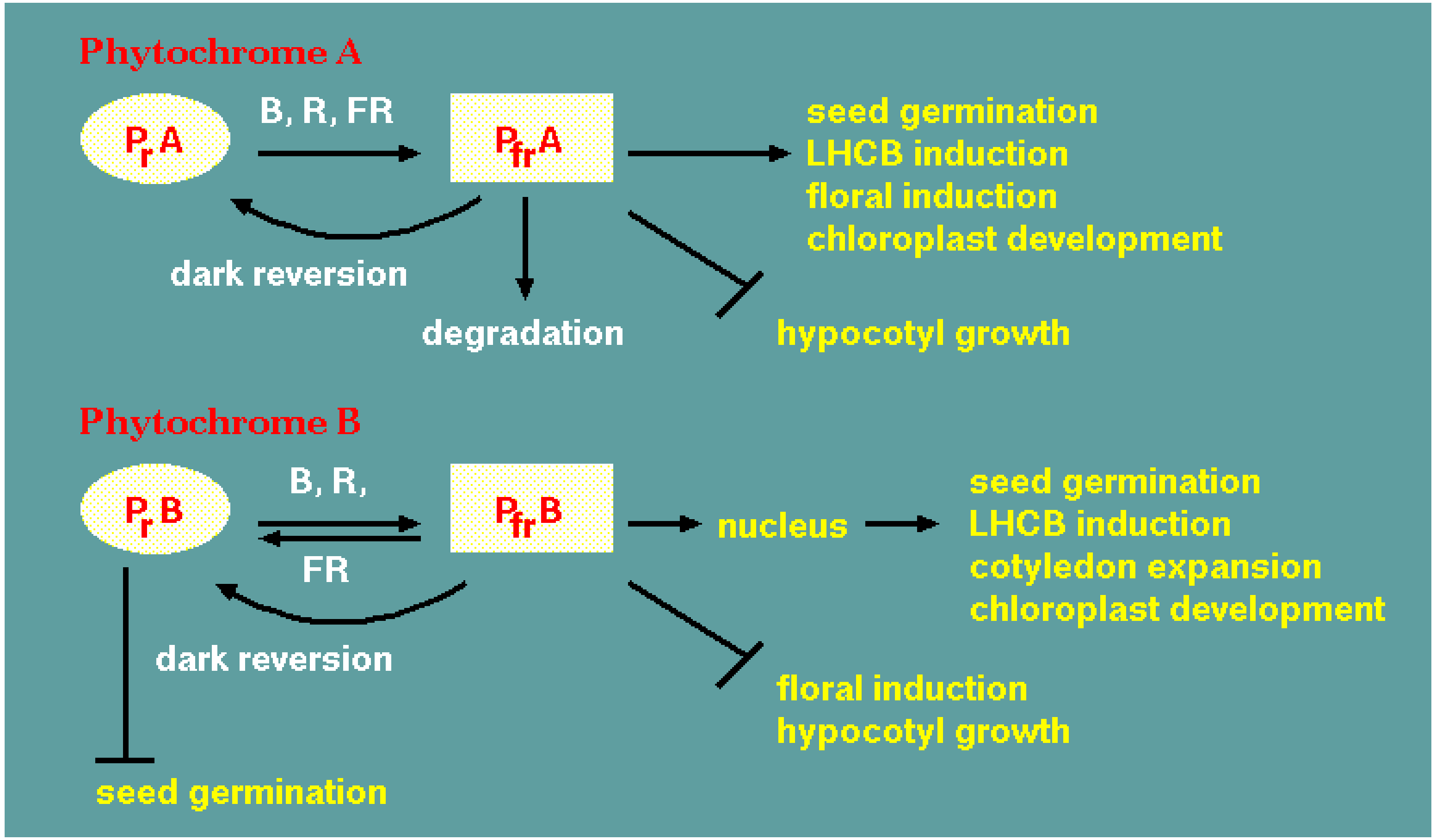
17.6 Phytochromobilin wird in den Plastiden synthetisiert und in das Cytosol ausgeschleust, wo es mit dem Phytochrom-Apoprotein zusammentritt. (Nach Kendrick et al. 1997)

phytochrome photoconversion



17.4 Struktur der Pr- und Pfr-Form des Chromophors (Phytochromobilin). Der Peptidanteil ist über eine Thioetherbrücke mit dem Chromophor verbunden. Der Chromophor erfährt als Reaktion auf Hellrot- und Dunkelrotlicht eine *cis-trans*-Isomerisierung am Kohlenstoffatom 15. (Nach Andel et al. 1997)

Light-lable PhyA and light-stable PhyB



Feedback regulation for Phytochrome A

PHYA gene ’ *PHYA* mRNA ’ PHYA protein accumulates in Pr form in the dark

Down-regulation

- LIGHT

Pfr ’ ubiquitin ’ degradation

“

Reaction

“

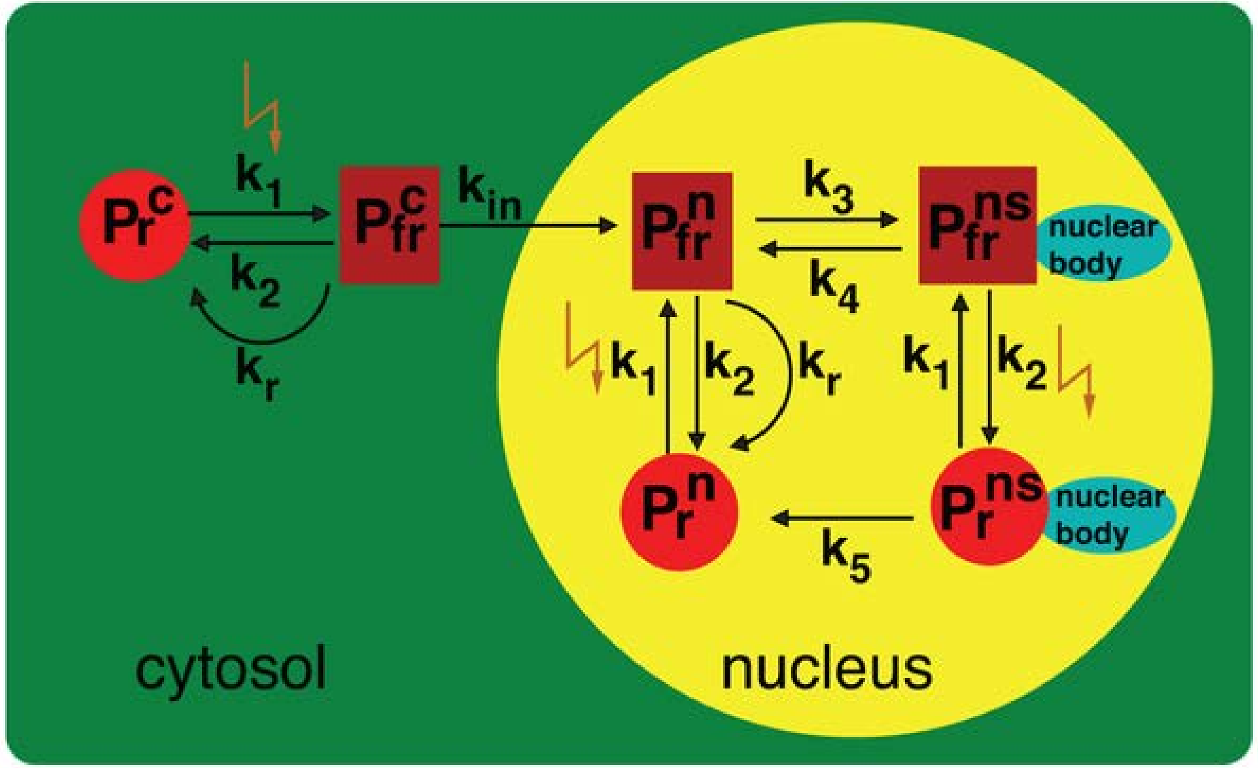
De-etiolement

The Pfr form of PHYA shuts down expression of its own gene and degrades PHYAfr via ubiquitin.

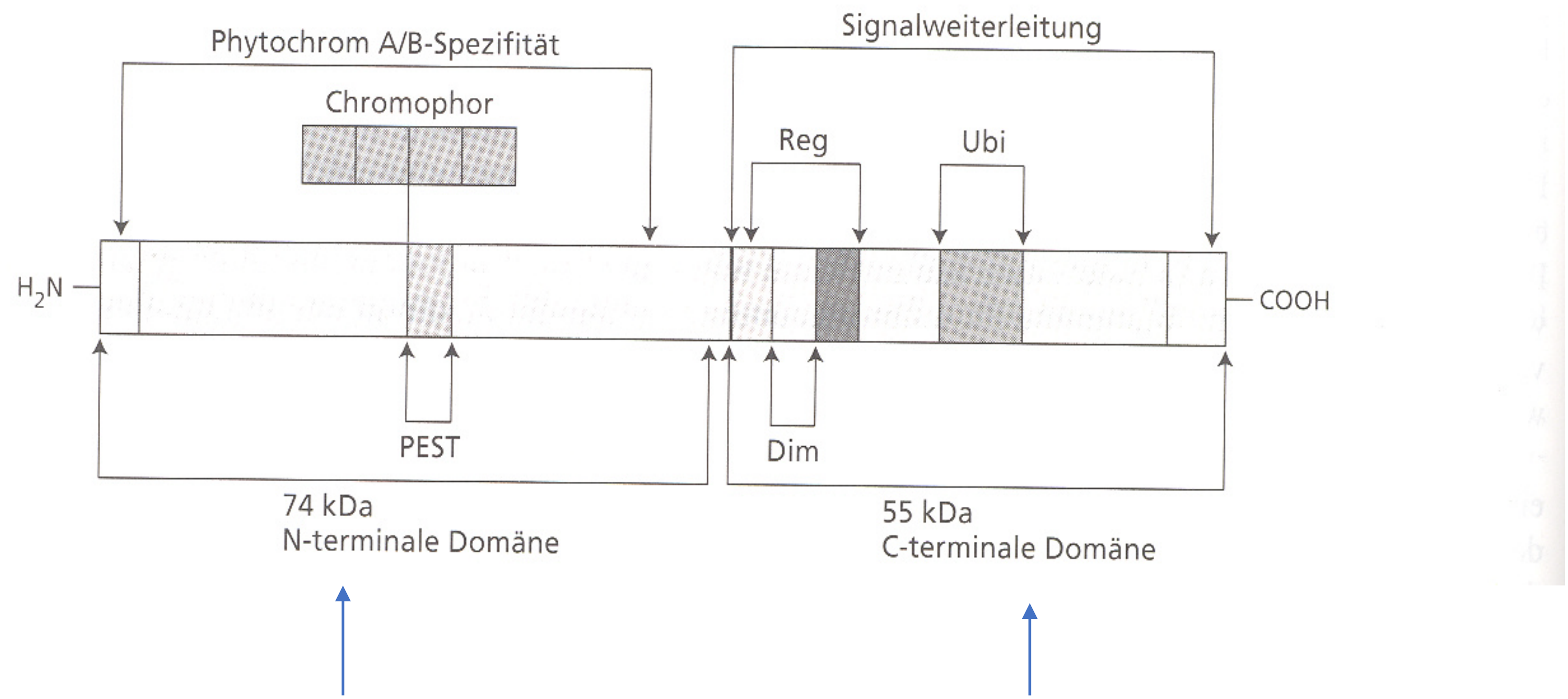
No such regulation for PHYB.
PHYB operates preferentially in green plants.

During de-etiolation, control shifts from PhyA to PhyB.

PhyB migrates to the nucleus



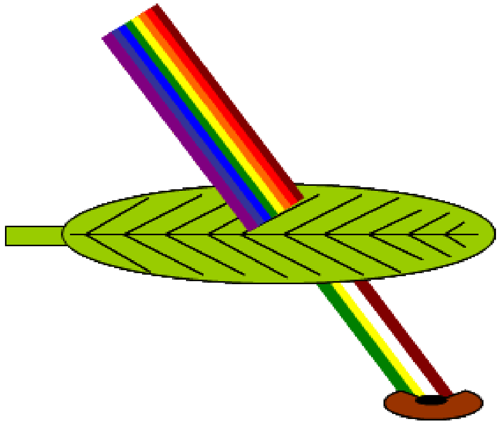
Domain structure of PhyA and PhyB



- PhyA/B specificity
- Chromophor binding

- dimerisation
- ubiquitin binding domain ONLY in PHYA
- signaling domain / interaction partner binding sites

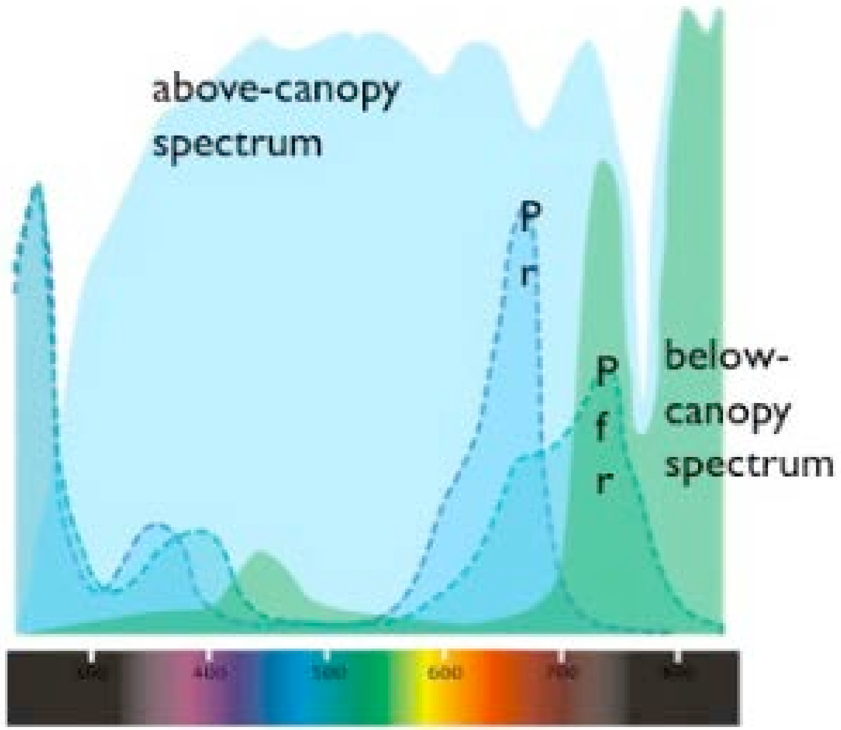
Shade avoidance response of phytochrome B



Chl absorbs most of red, but not far-red light

=>

Plants in the shade: more inactive Pr



High R:FR Low R:FR

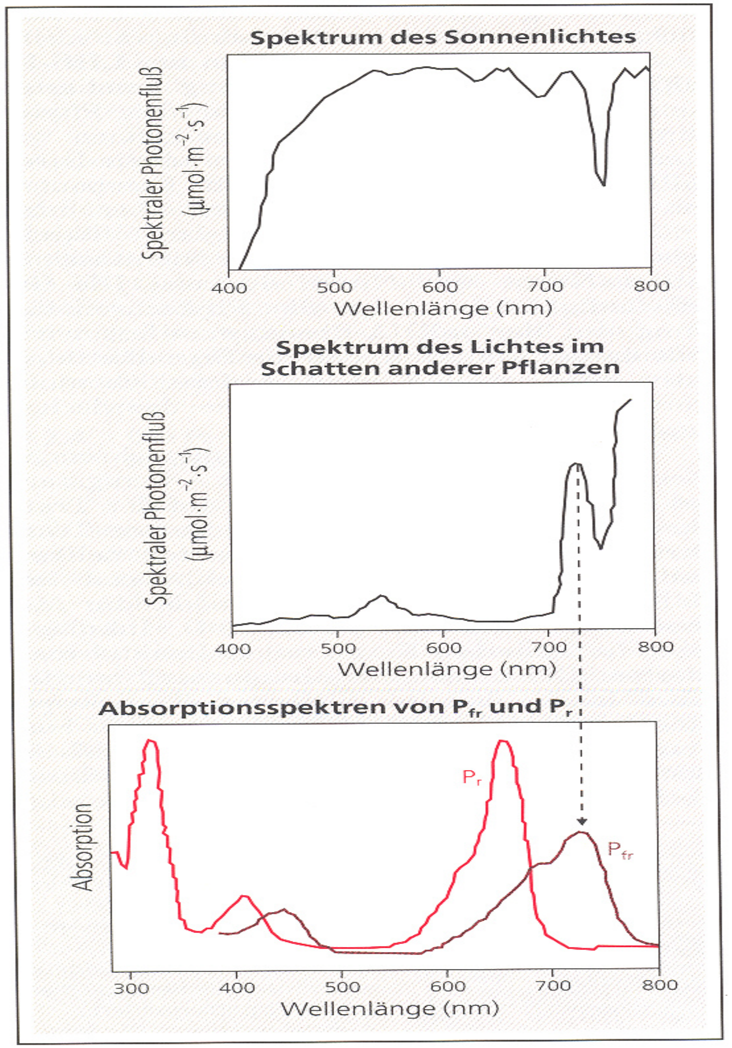


Abb. 5.3. Die Bedeutung des Phytochromsystems zur Messung der Lichtqualität für die pflanzliche Entwicklung im Schatten. Die Funktion des Phytochromsystems beschränkt sich nicht nur auf die frühe Keimlingsentwicklung, sondern es mißt auch Lichtqualitäten in der adulten Pflanze. Dieses Meßsystem ist für Pflanzen wichtig, die im Schatten anderer Pflanzen oder dicht neben konkurrierenden Pflanzen wachsen, weil es der Pflanze bereits zu einem Zeitpunkt Informationen über die Umwelt liefert, zu dem die pflanzliche Konkurrenz noch keinen direkten Schatten wirft. Die spektrale Zusammensetzung des eingestrahlten Sonnenlichtes ist im oberen Teil der Abbildung gezeigt. Unterhalb von 400 nm fällt vergleichsweise wenig Lichtenergie ein, der Abfall der Lichtenergie bei 760 nm wird auf den Wasserdampf der Atmosphäre zurückgeführt. Das Licht, das die Pflanzendecke passiert hat, besitzt eine charakteristische spektrale Zusammensetzung (mittleres Diagramm). Neben einer geringen Menge grünen Lichtes (Grünlücke) tritt vornehmlich Licht oberhalb 700 nm auf. Der Pfeil verdeutlicht, daß dieses Licht Pfr im Maximum anregt und das Gleichgewicht weitgehend in Richtung auf Pr verschiebt. Eine entsprechende Zusammensetzung hat auch von grünen Pflanzen reflektiertes Licht; es signalisiert einer Pflanze durch die Bildung von Pr in den Seitenblättern die Gefahr des Überwachsenwerdens. Dieses Phänomen könnte den „Randeffekt" in Feldern erklären, nämlich daß Pflanzen im Inneren eines Bestandes einheitlich und deutlich höher wachsen als am Rand

Shaddow

> Pfr > Pr

- **long hypokotyl**
- **field – plants at the edges are shorter, because less shaded (contain more Pfr)**

phytochrome regulates gene expression

Dark ’ light transition: > 1500 genes are turned on

Signaling mutants

- **Chromophor mutants**

  (all phytochromes are affected)

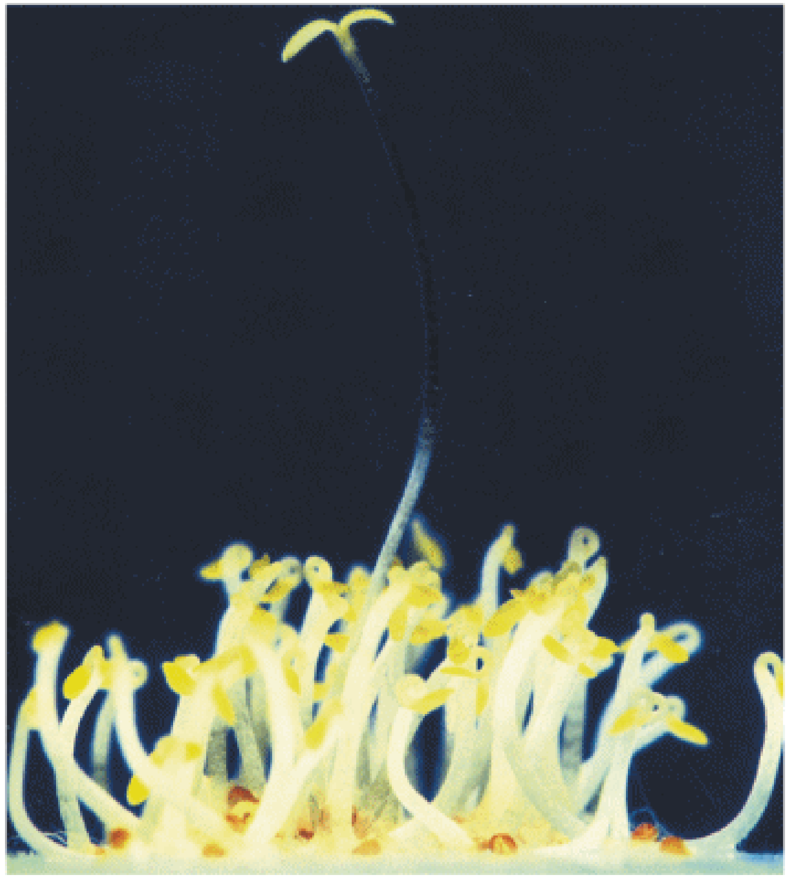
- **Apoprotein mutants**

- ***loss of function* mutants**

  (e.g. defect in signaling component)

- ***gain of function* mutants**

  (e.g. constitutive active signaling component)

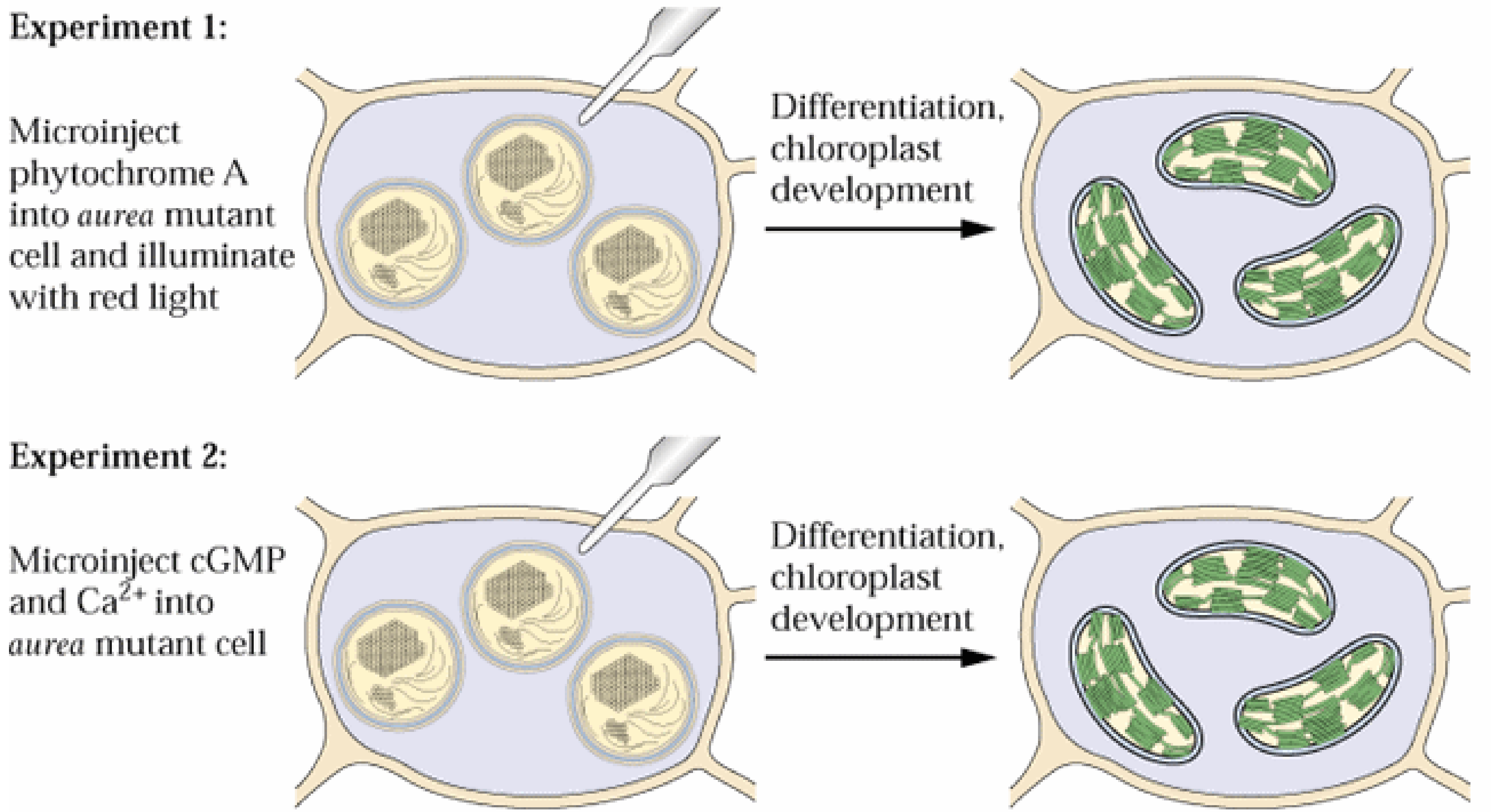


Phenotype mutant screens

dark phenotype in light

light phenotype in dark

phytochrome signaling from cytoplasma to nucleus: Ca^{2+} and cGMP



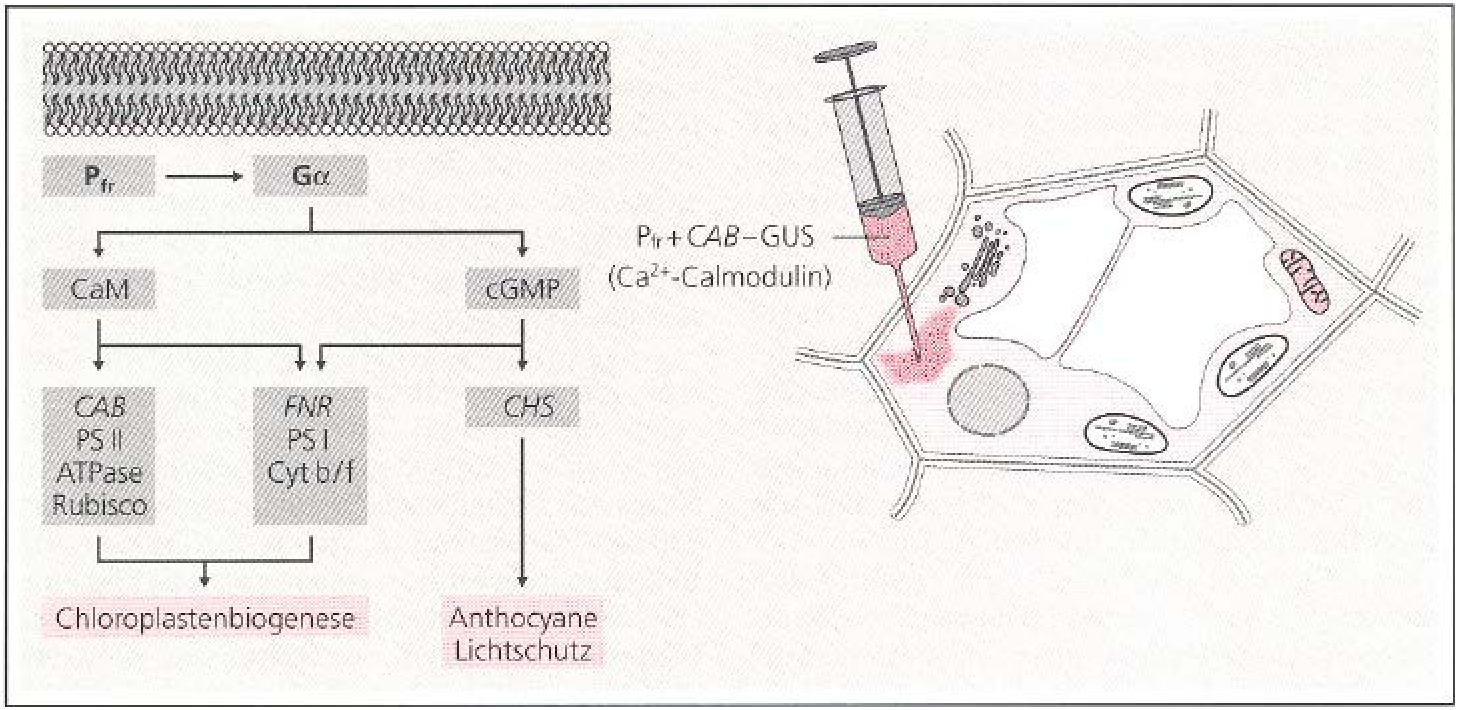
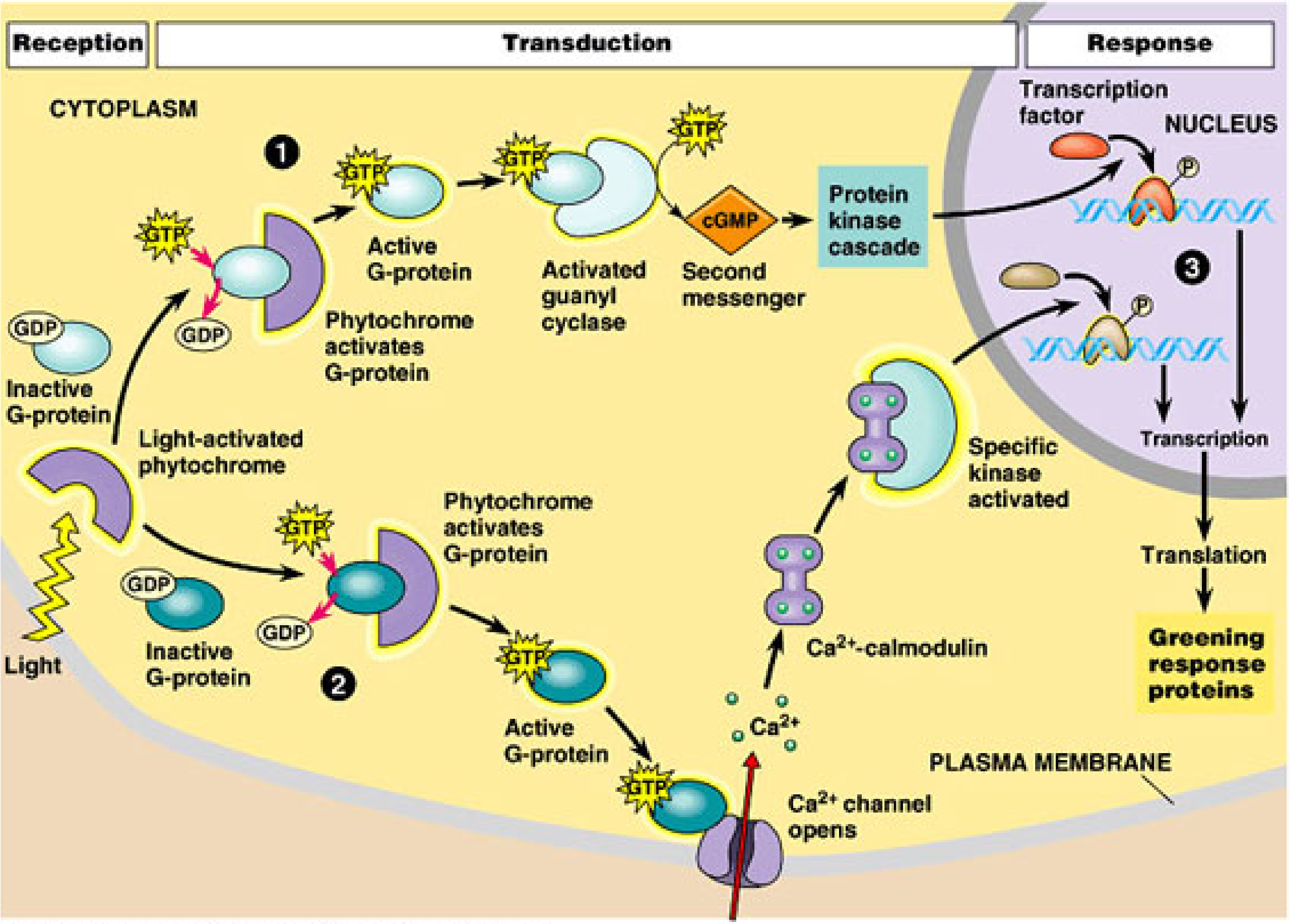
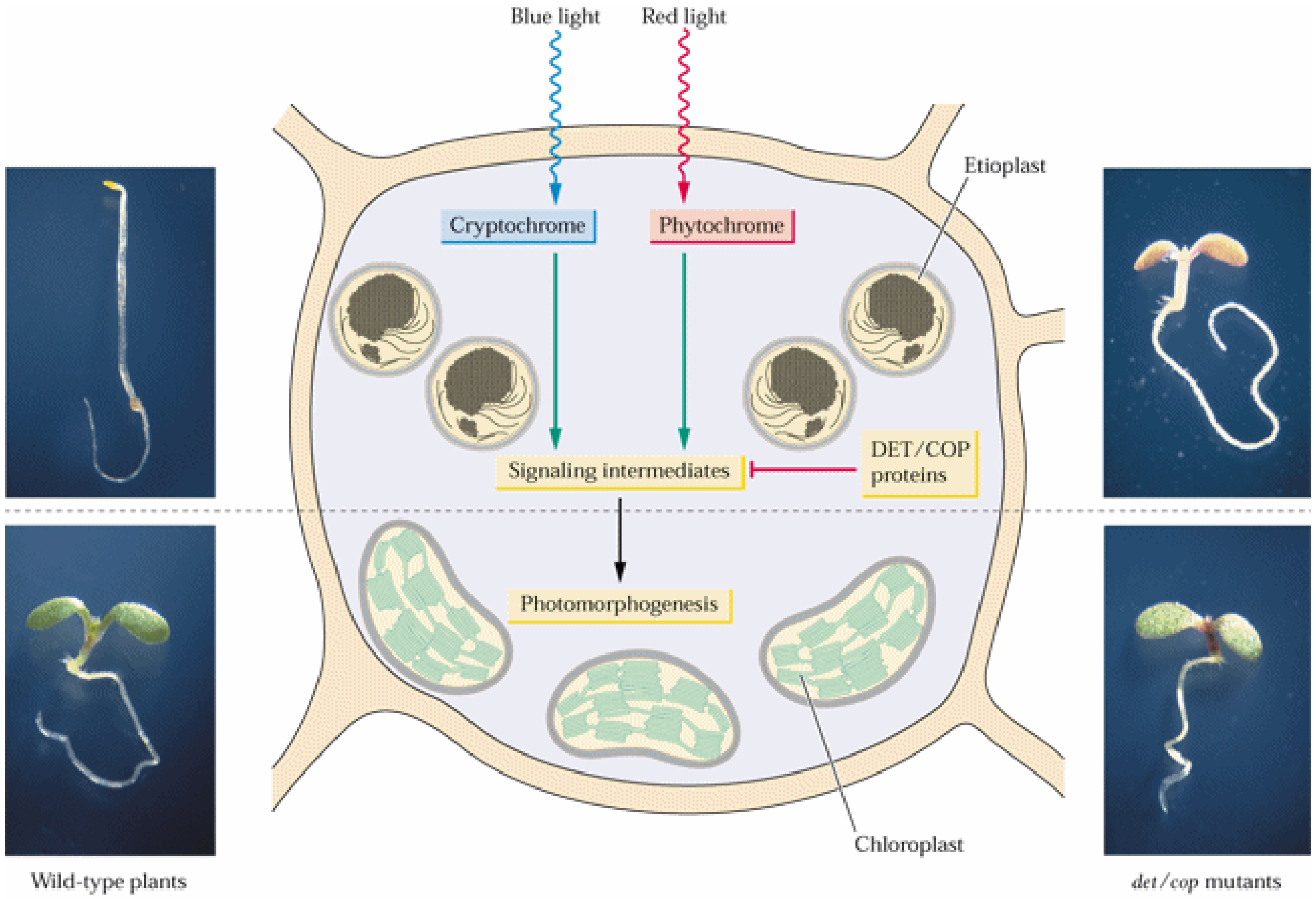


Abb. 5.8 Ein Modell der Signaltransduktionskette des Phytochroms. Als Genmarker für die Anthocyansynthese wurde der Chalkonsynthasepromotor *(CHS)* (s. 4.1.5 u. Abb. 4.**7**) eingesetzt, die Promotoren eines LHC II- *(CAB)* und NADP$^+$-Ferredoxinoxidoreductase-Gens *(FNR)* dienten als repräsentative Photosystem-II- bzw. Photosystem-I-assoziierte Gene. Untereinheiten der beiden Photosysteme selbst (PS I, PS II), des Cytochrom-b_6f-Komplexes (Cyt b/f), der ATP-Synthase (ATPase) und der Ribulose-1,5-bisphosphat-Carboxylase/Oxygenase (Rubisco) wurden immuncytochemisch nachgewiesen. Ausgehend von Pfr, das nach diesen Vorstellungen zumindest temporär an der Plasmamembran lokalisiert sein müßte, wird die α-Untereinheit eines heterotrimeren G-Proteins (G_α) aktiviert. Das führt einerseits zu einer Erhöhung der intrazellulären Ca^{2+}-Konzentration, andererseits zu einer Stimulierung der Guanylatcyclaseaktivität. Calmodulin (CaM) induziert die *CAB*-Transkription, aktiviert aber auch andere Photosystem-II-Gene sowie die Gene für die kleine Untereinheit der Rubisco (*rbcS*) und für Untereinheiten der ATP-Synthase. Über cGMP allein wird der Sekundärstoffwechsel aktiviert, der zur Synthese der Anthocyane führt. Das Anschalten des *fnr*-Promotors sowie der Gene für Untereinheiten des Photosystems I und des Cytochrom-b_6f-Komplexes ist cGMP- und Ca^{2+}-Calmodulin-abhängig. Die Biogenese funktionsfähiger Chloroplasten erfordert damit die gleichzeitige Aktivierung beider Regulationswege. Inzwischen mehren sich die Hinweise, daß der cGMP- und der Ca^{2+}-Calmodulin-Weg sich gegenseitig negativ beeinflussen (nach Neuhaus u. Mitarb. und Bowler u. Mitarb.)

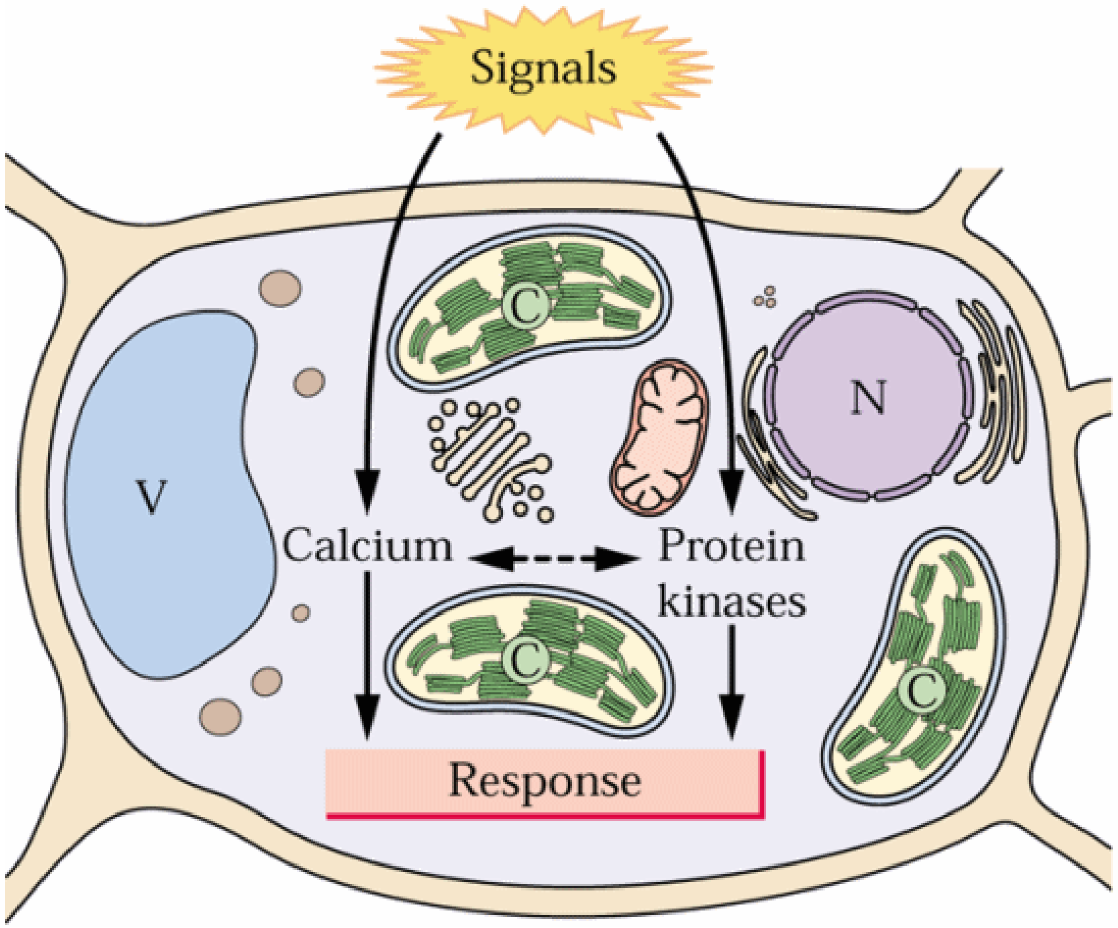


Reception
Transduction
Response
CYTOPLASM
1
GTP
GDP
Inactive G-protein
Phytochrome activates G-protein
Active G-protein
Activated guanyl cyclase
cGMP
Second messenger
Protein kinase cascade
Transcription factor
NUCLEUS
P
3
Light-activated phytochrome
Light
2
Phytochrome activates G-protein
Inactive G-protein
Active G-protein
Ca2+
Ca2+-calmodulin
Specific kinase activated
Ca2+ channel opens
Transcription
Translation
Greening response proteins
PLASMA MEMBRANE

Many phytochrome signaling components are also involved in cryptochrome signaling



Light signaling fits into general signaling models from eukaryotic cells



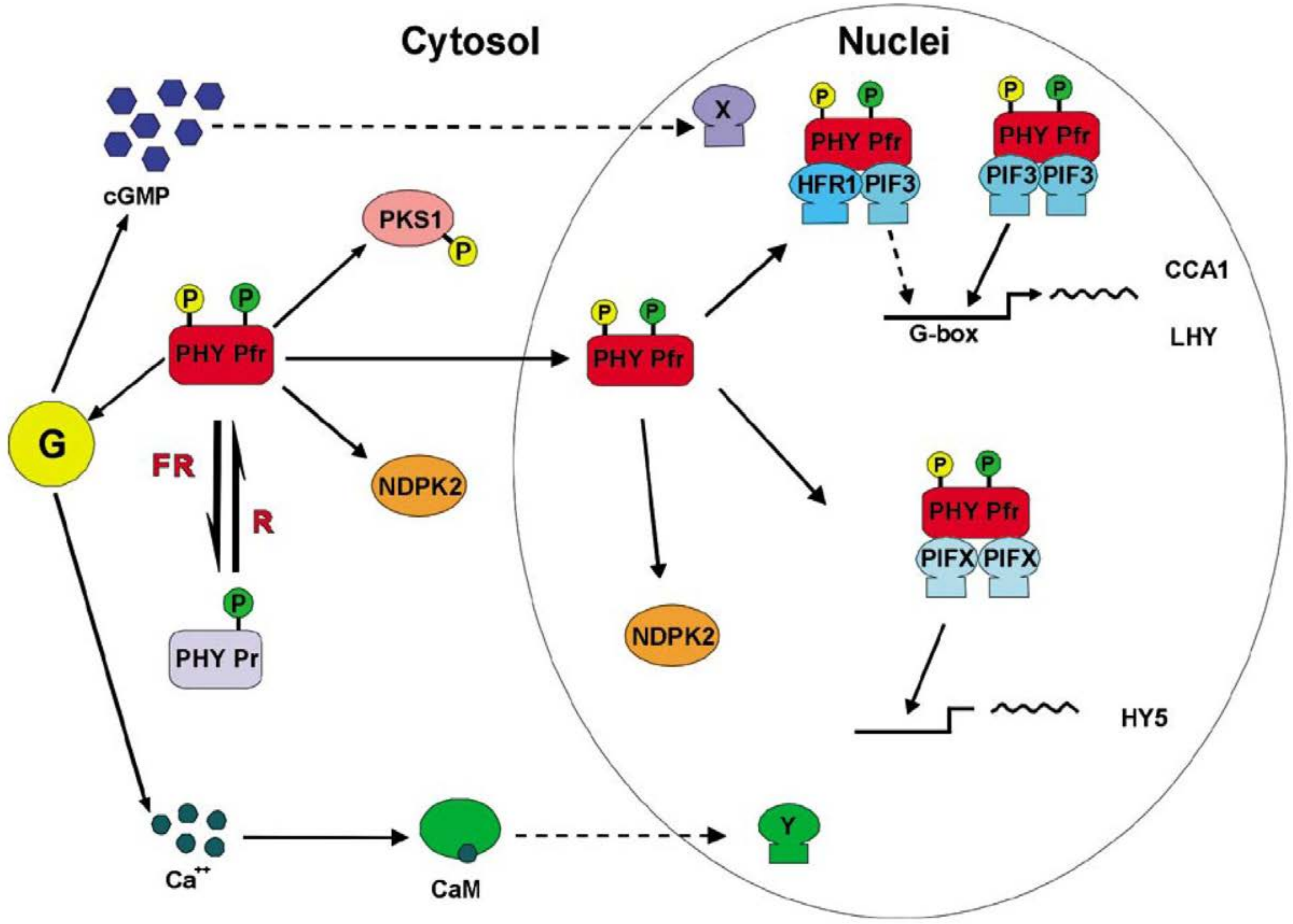
PhyB migrates to the nucleus

Pfr-form migrates, Pr-form stays in cytoplasm

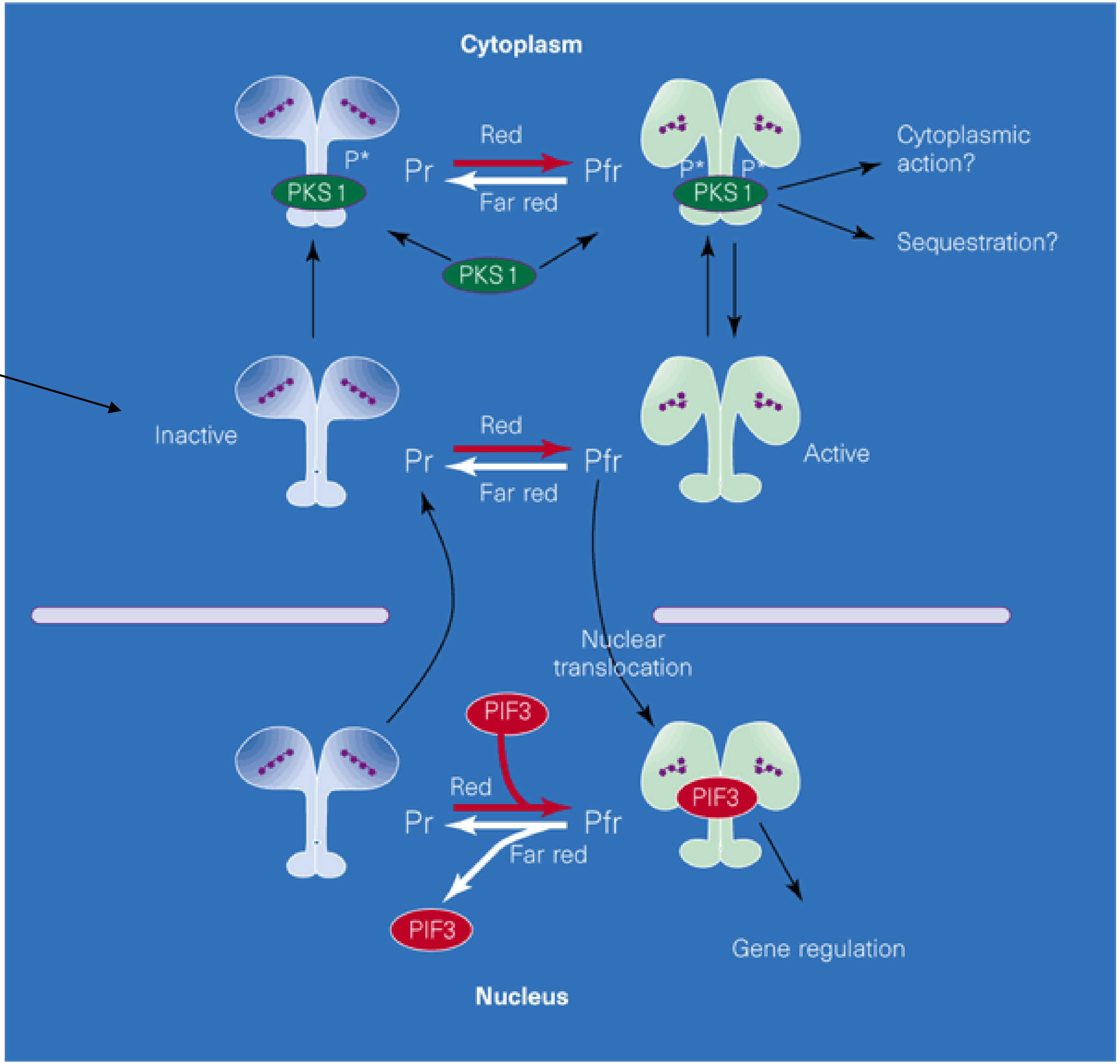
Active retardation of Pr-form

General principles of distribution of proteins between cytoplasm and nucleus

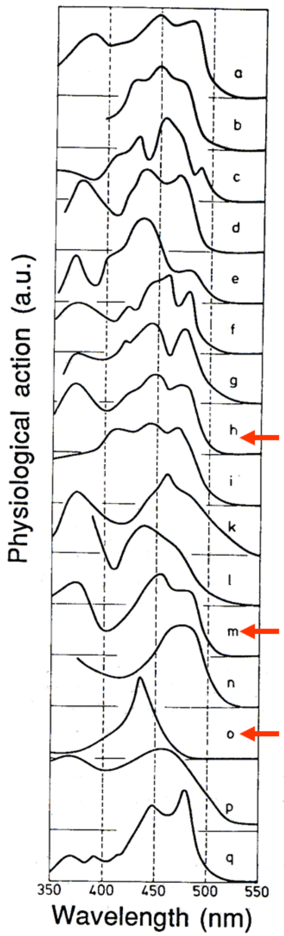
- **transcription factors**
- **„steroid hormone receptors“**
- **cryptochromes**


Cytosol
Nuclei
cGMP
X
PHY Pfr
HFR1
PIF3
PIF3
PIF3
PKS1
P
CCA1
G-box
LHY
G
FR
R
NDPK2
PHY Pr
PIFX
PIFX
HY5
Ca++
CaM
Y

Pr is actively inhibited to migrate to the nucleus (de-etiolion: active process)



Phototropins & Cryptochromes

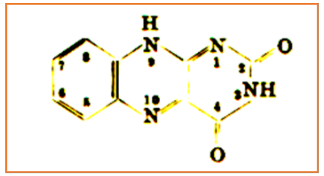


Different action spectra

Phototropismus Avena

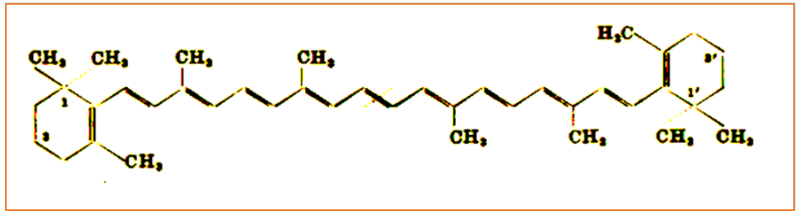
Chloroplast movement

DNA-photoreactivation



Flavine ?

Pterine ?

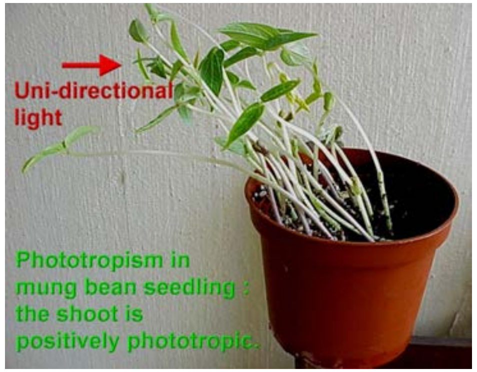


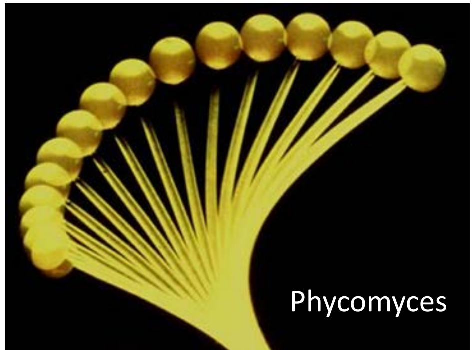
Carotene ?

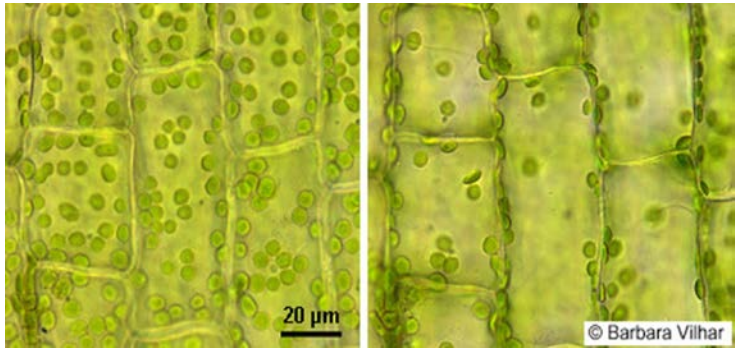
2. Phototropins

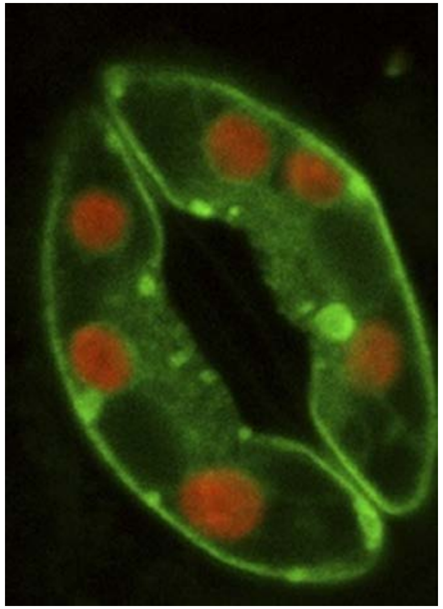
- **PHOT1** (low light intensity) vs. **PHOT2** (higher light intensity)

- **3 main functions**
    - Phototropism
    - Chloroplast movement
    - Stomata opening

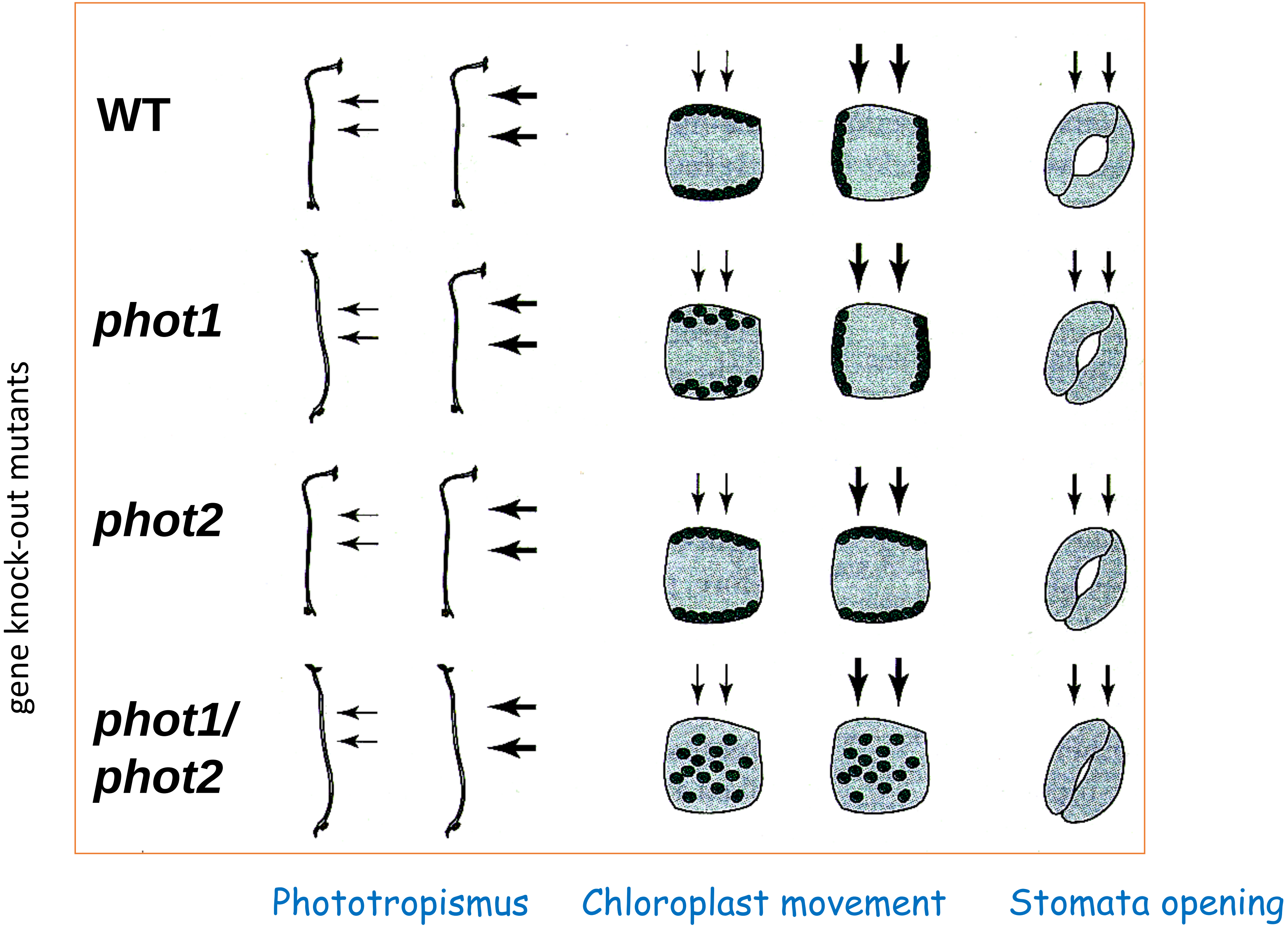




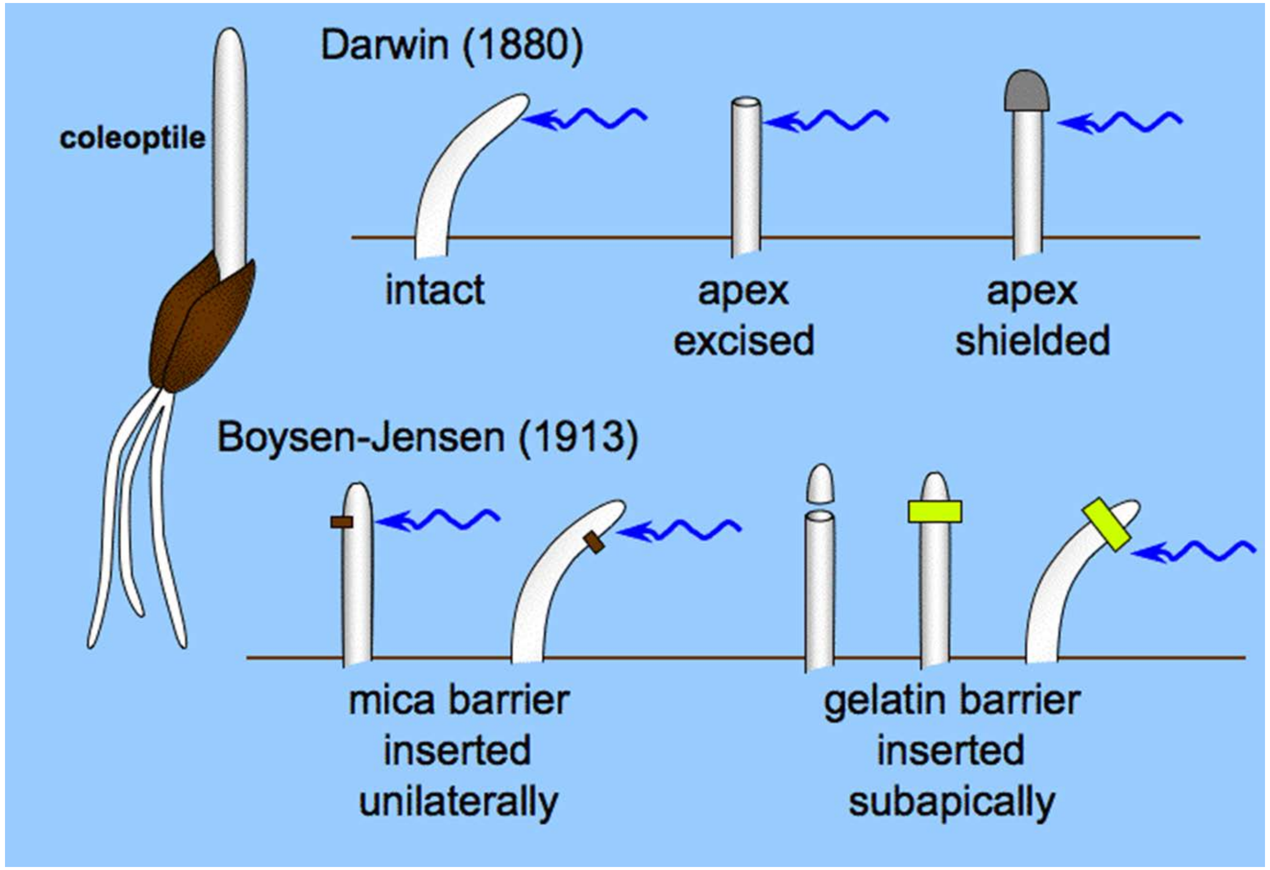


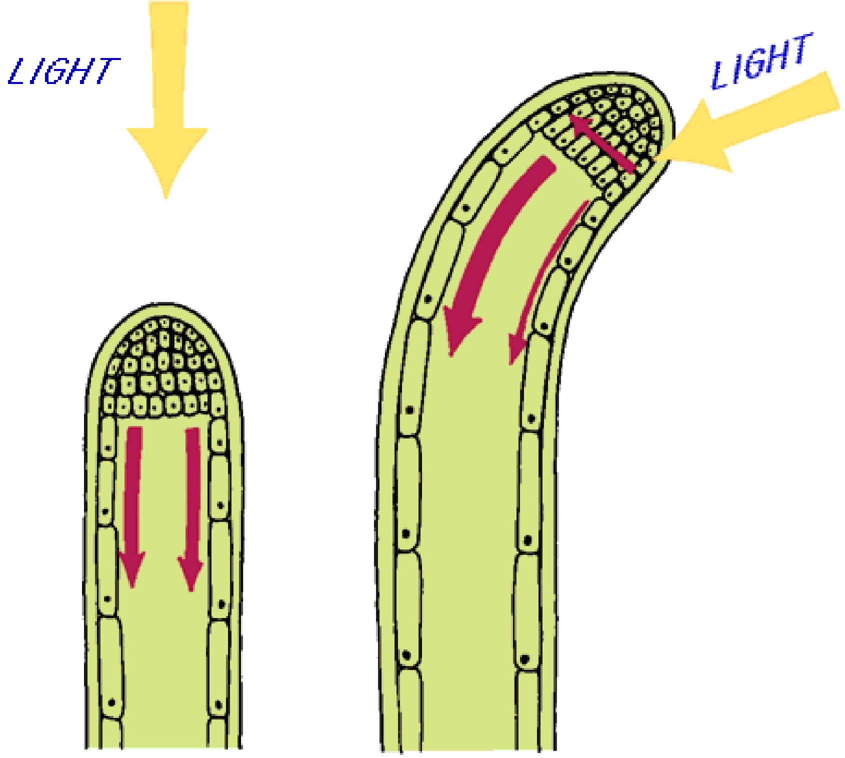


PHOT1/2 reactions

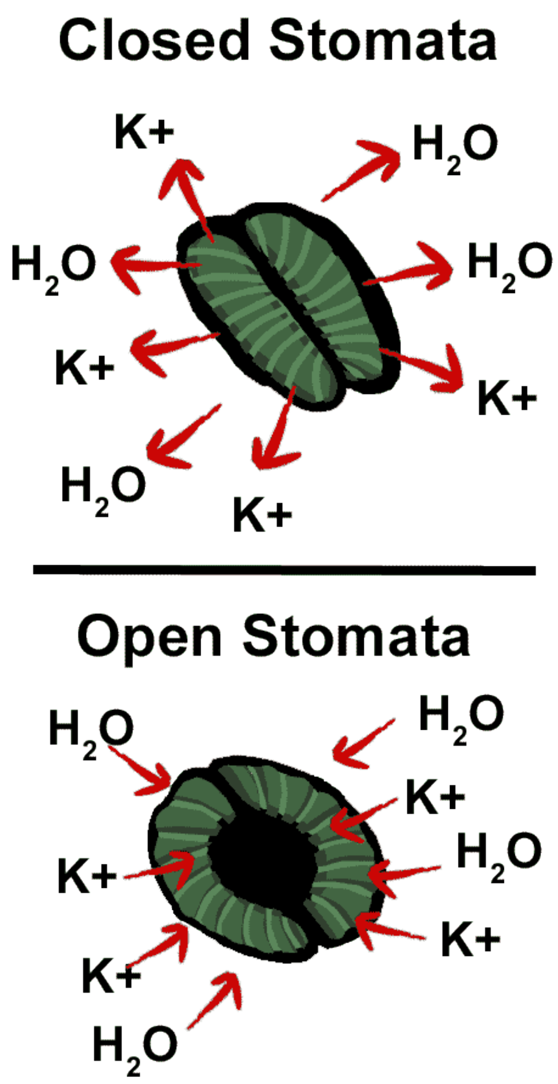


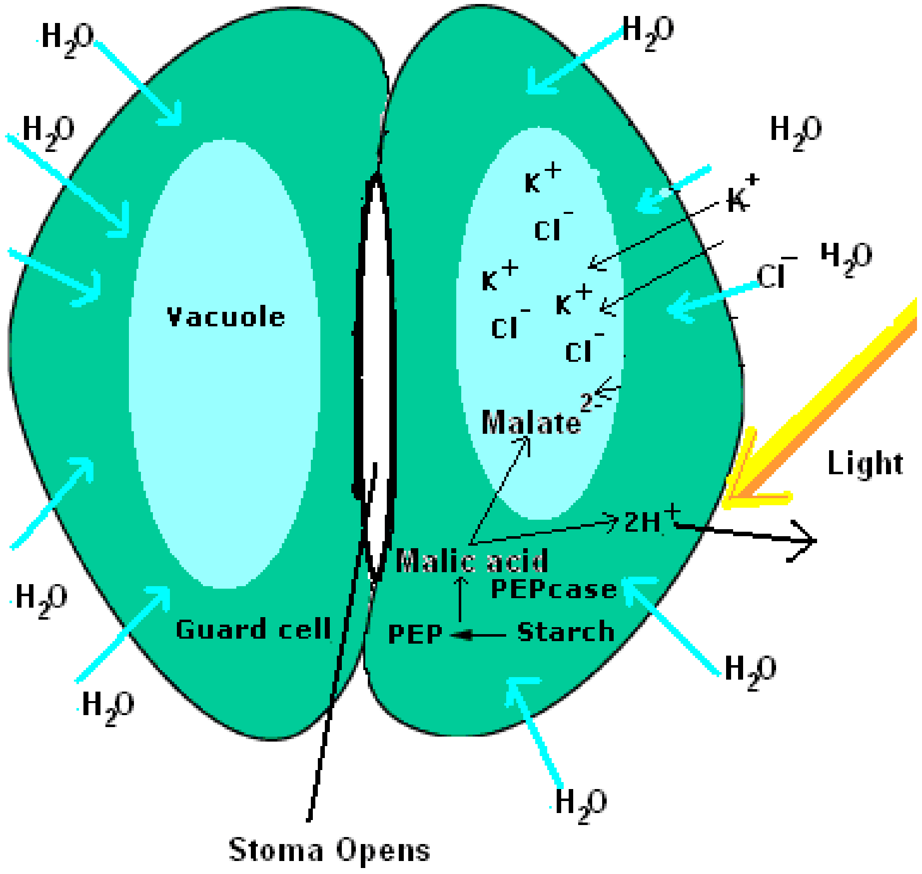
Phototropism and auxin



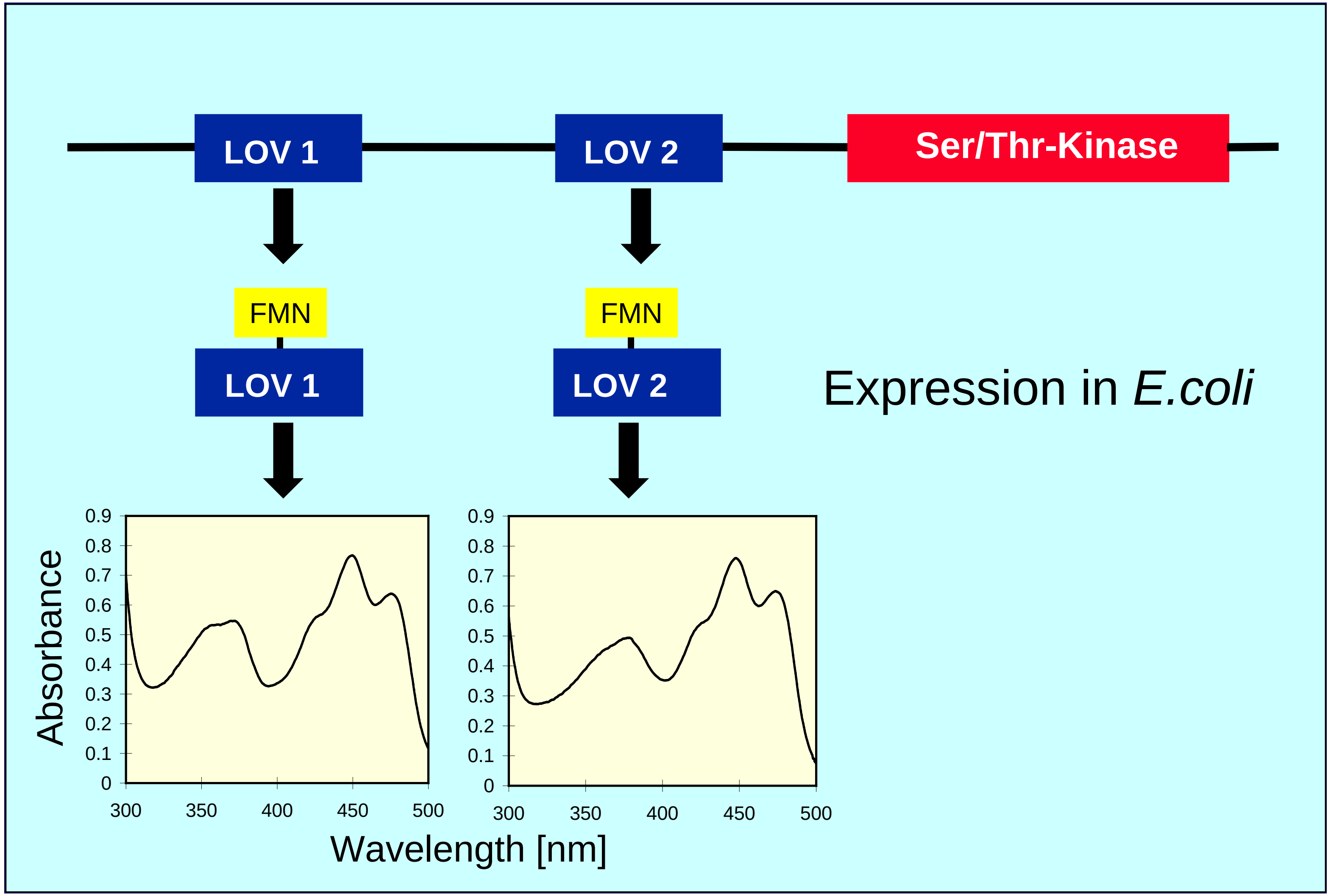


Phototropin and stomata closure





Phototropin – structure and absorption spectra



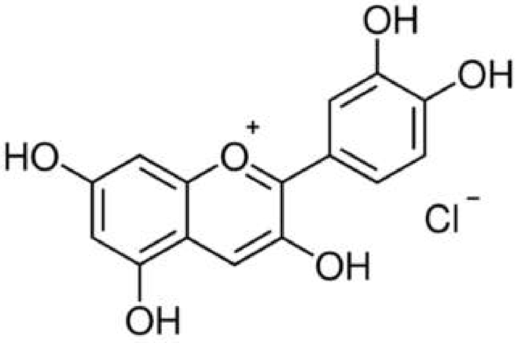
FMN = Flavin

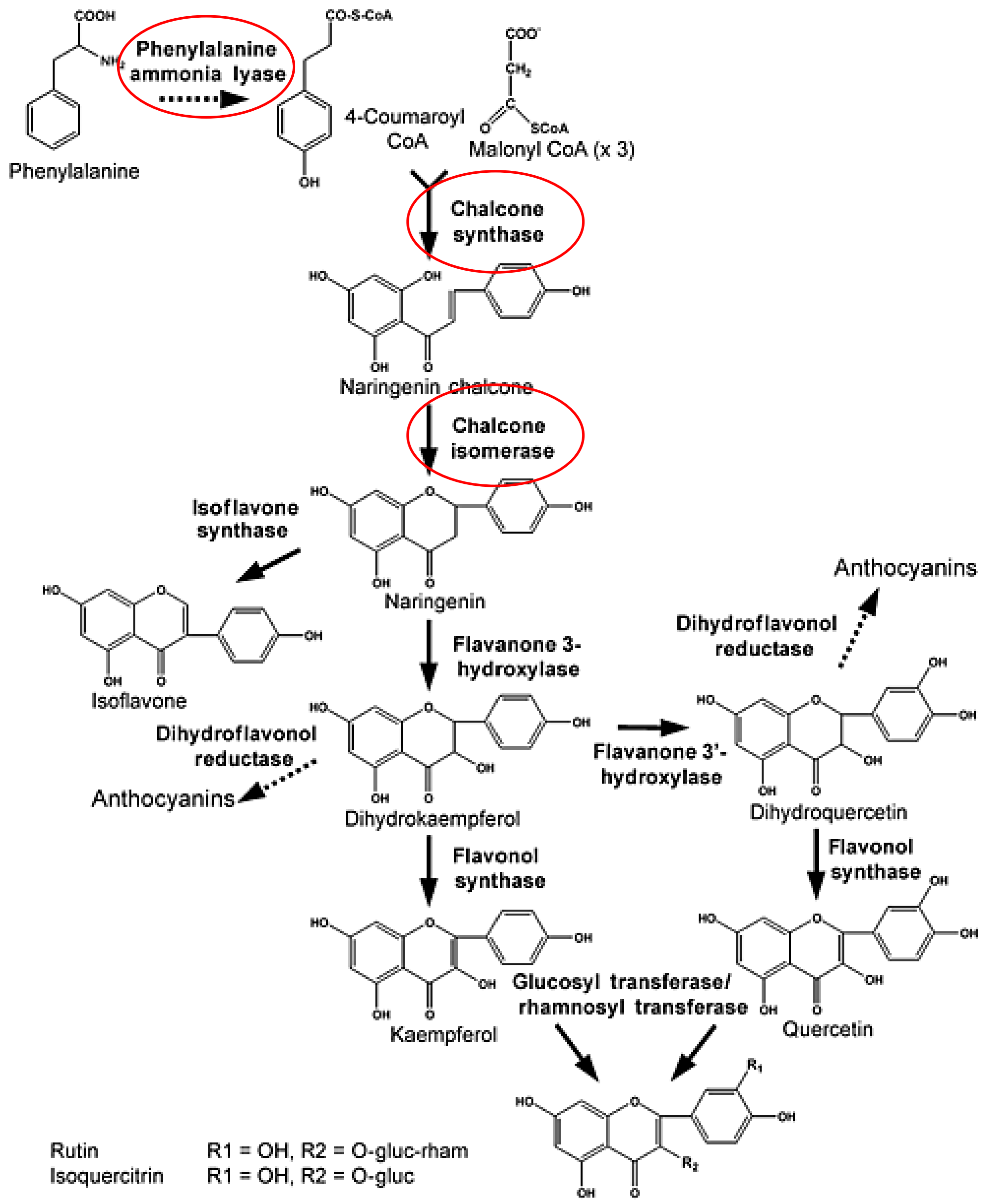
LOV = light/oxygen/voltage domain

3. Cryptochromes

- **CRY1** (low light intensity) vs. **CRY2** (higher light intensity)

- **main functions**
    - blue light perception and control of gene expression
    - Signaling merges with phytochrome action
    - Circadian rhythm

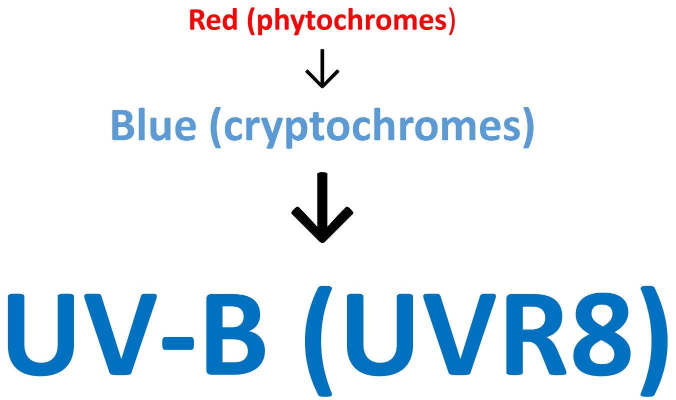




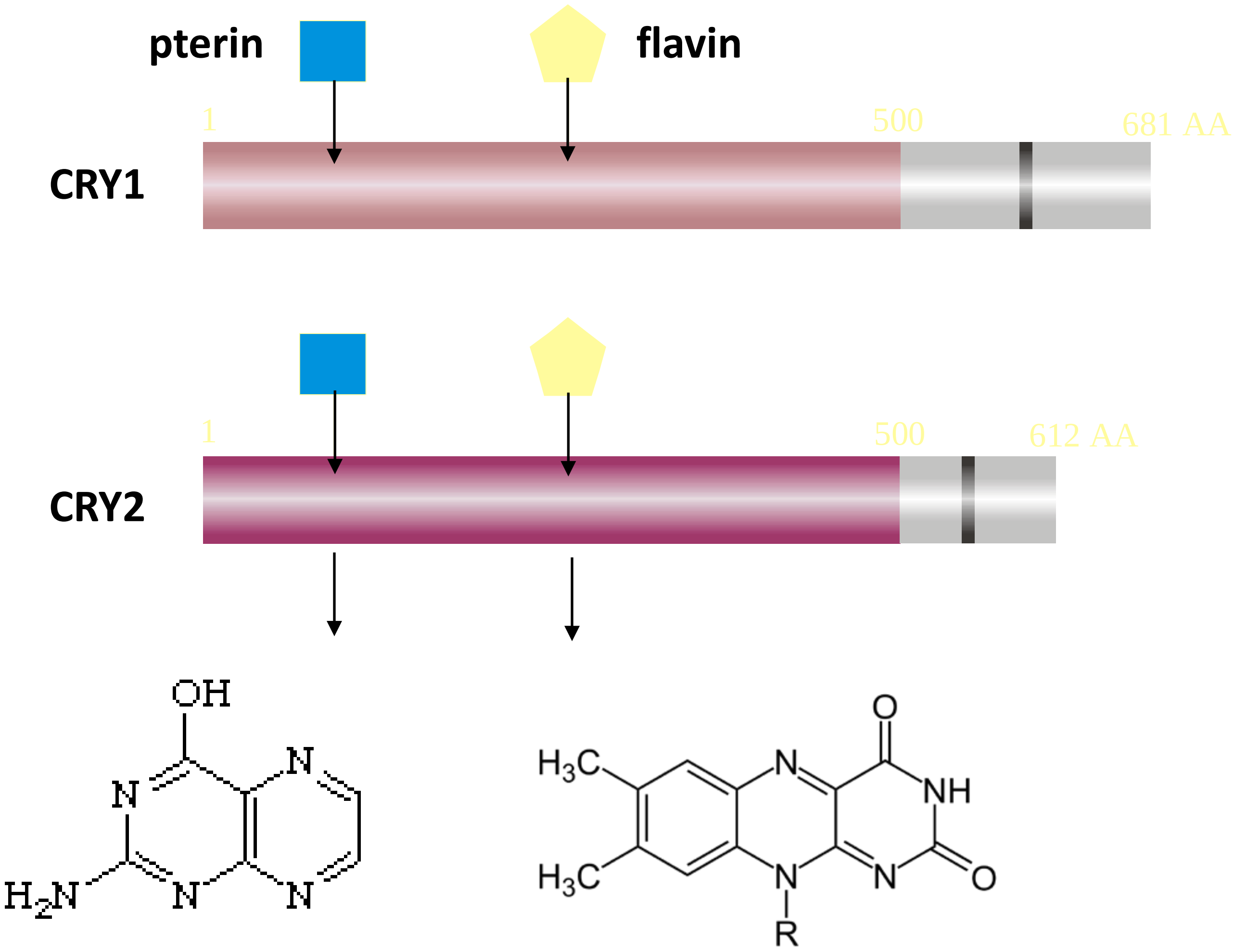
- **Flavonoid biosynthesis pathway**

- Besides red anthocyanin, flavonoids absorb UV and have UV-B protective functions.

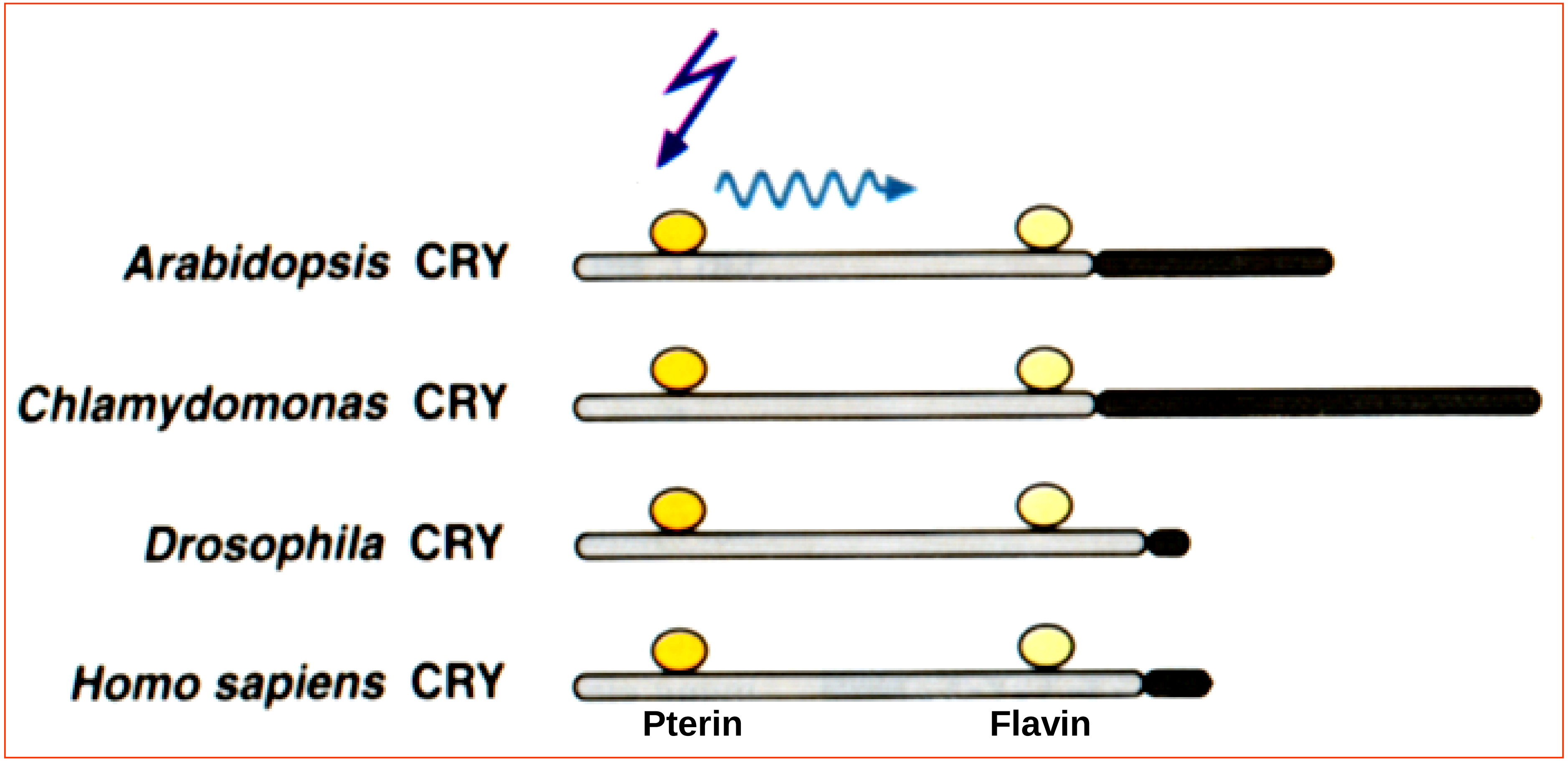
- Marker genes (*PAL*, *CS*, *CI*, *etc*.) are light-regulated.



Domain structure
CYP1 and CYP contain pterin and flavin

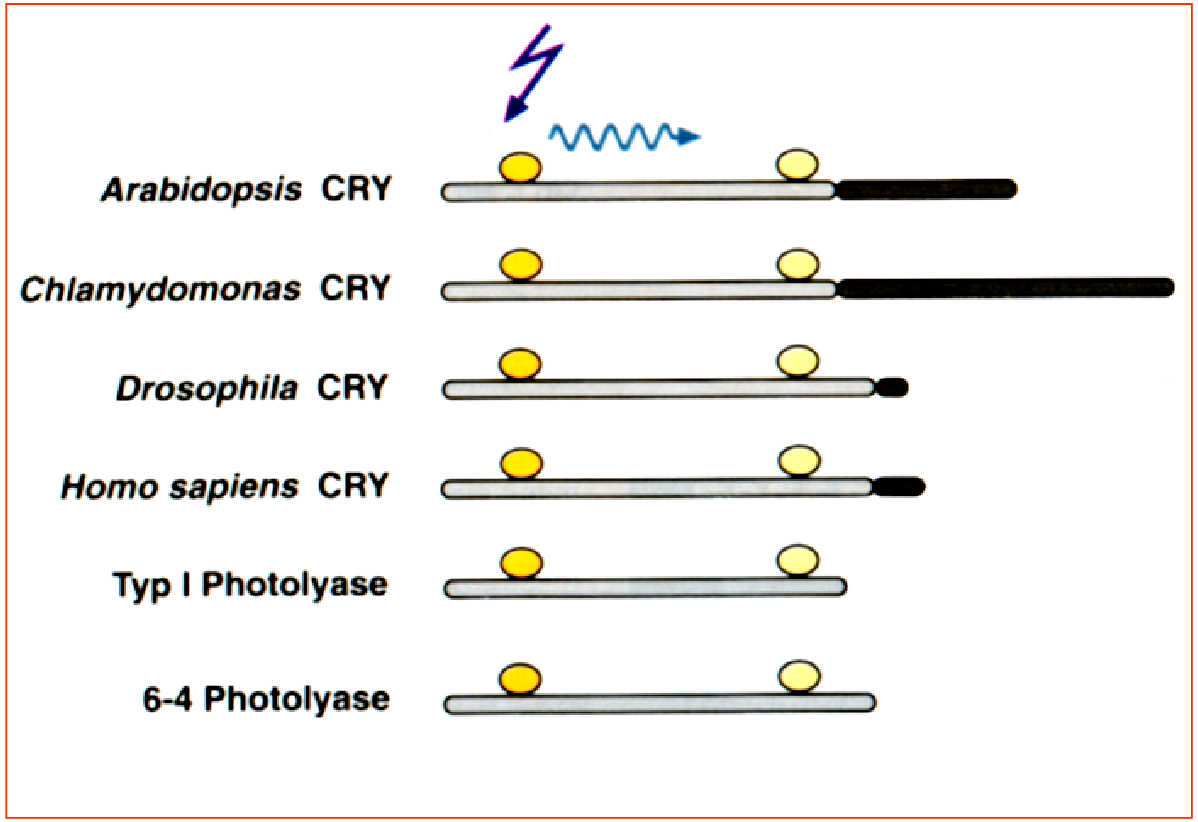


Cryptochromes are present in many organisms and play a crucial role in circadian rhythm



Cyptochromes originate from photolyases

- in prokaryotic and eukaryotic organisms
- Structure is similar to cytochromes
- Flavin (FAD) and Pterin as chromophores
- Photolyases are enzymes for DNA repair after blue-light activation



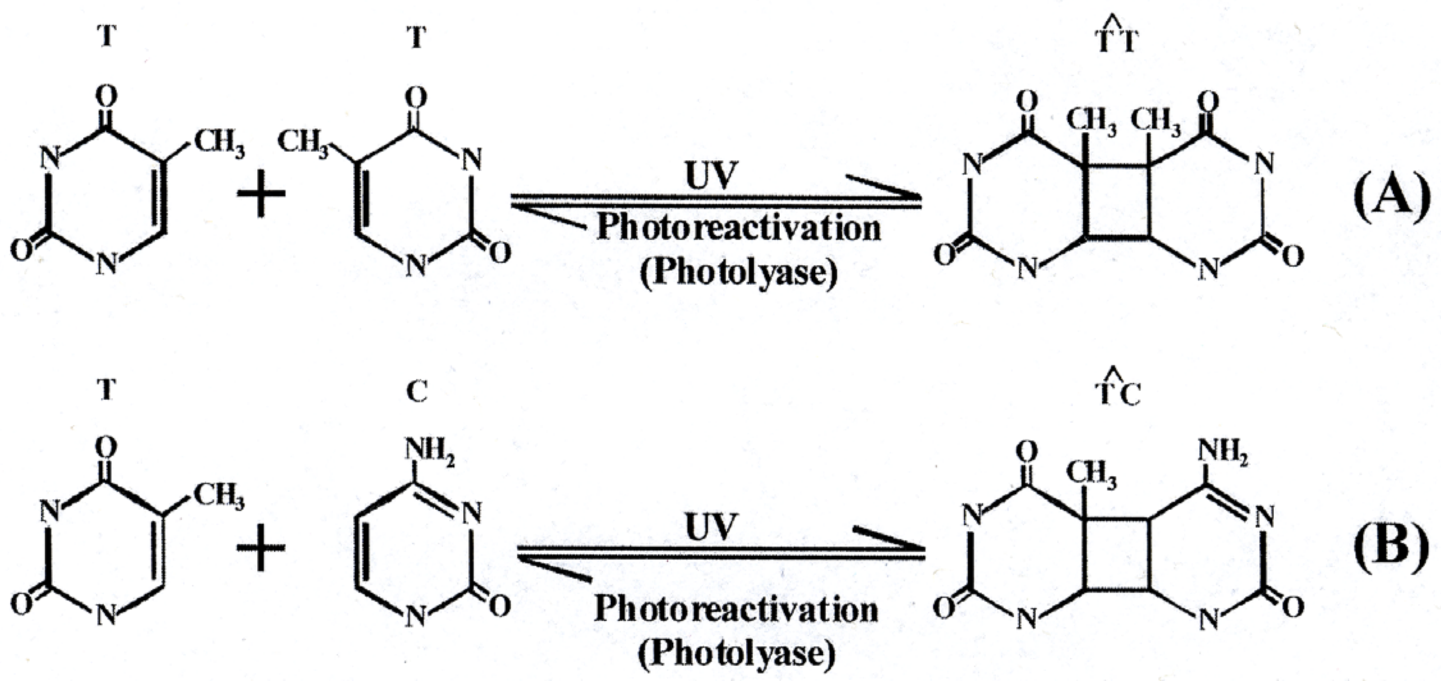
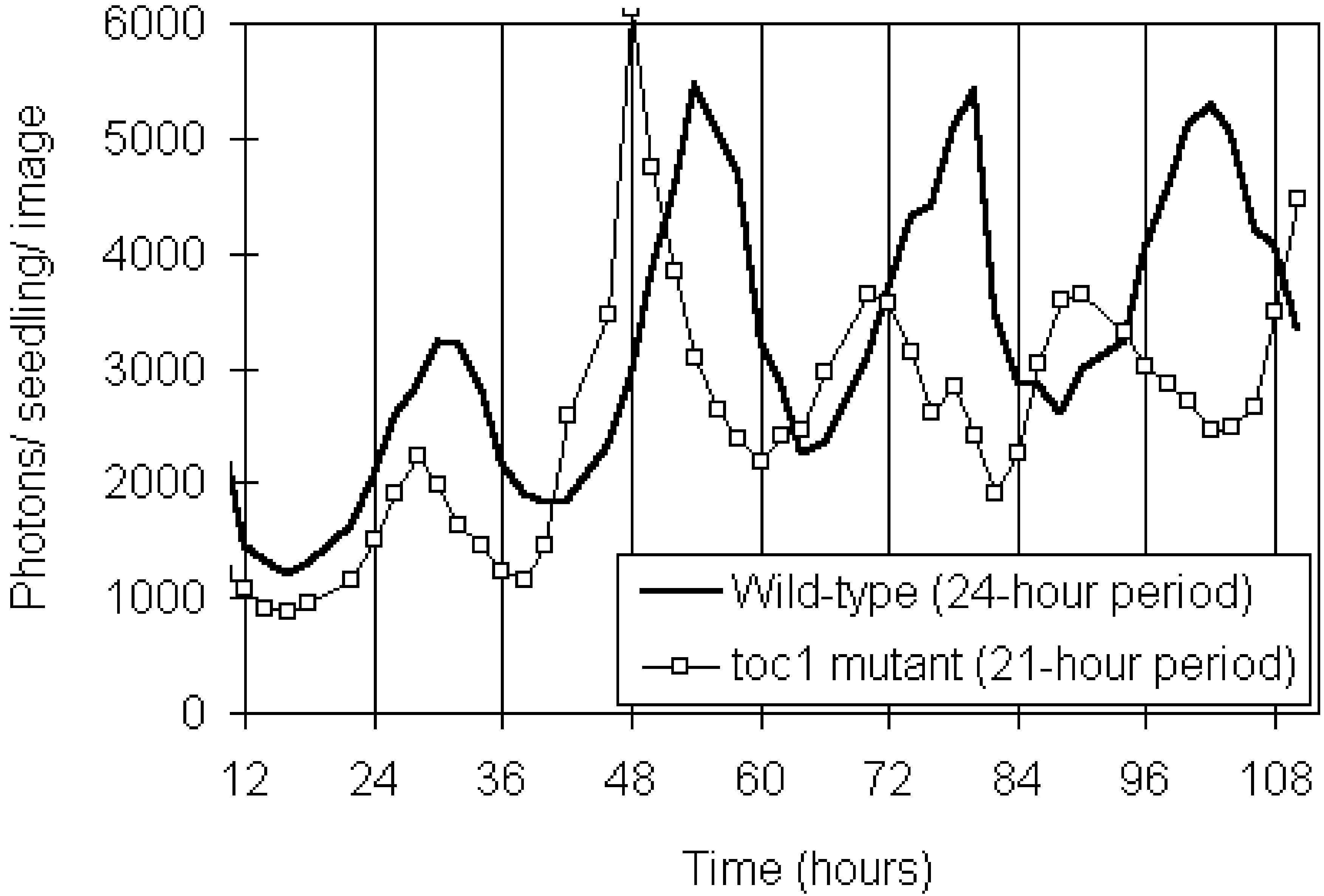


Photo-reactivation by blue light

Cryptochromes are involved in control of circadian rhythm in plants and animals

Input	Oscillator	Output
(e.g. CYP, PHY temperature, nutrients)	„Schrittmacher“ (e.g. PER, TIM, FRQ, TOC)	response (e.g. genes, K+ channels, phosphorproteins)



Photons/ seedling/ image
6000
5000
4000
3000
2000
1000
0
12
24
36
48
60
72
84
96
108
Time (hours)
Wild-type (24-hour period)
toc1 mutant (21-hour period)

circadian/diurnal control

<u>Length of period</u> (*peak* 1 > *peak* 2):

mutants in *Drosophila, Neurospora, Chlamydomonas, Arabidopsis*, mice)

per* gene in *Drosophila:

***perl, pers, pera* (long, short, arhythmic period)**

<u>Temperature compensation:</u> Q_{10} 0.8 - 1.3

***<u>Entrainment</u>*: Adjustment of light/dark rhythm by exogenous factors (e.g. sun light)**

General model

2 proteins in cytosol: period & timeless (cryptochrome)

Interaction via PAS domain

increasingly phosphorylated during the day

transloaction to nucleus

transcriptional repression of the transcription factor genes *clock & cycle*

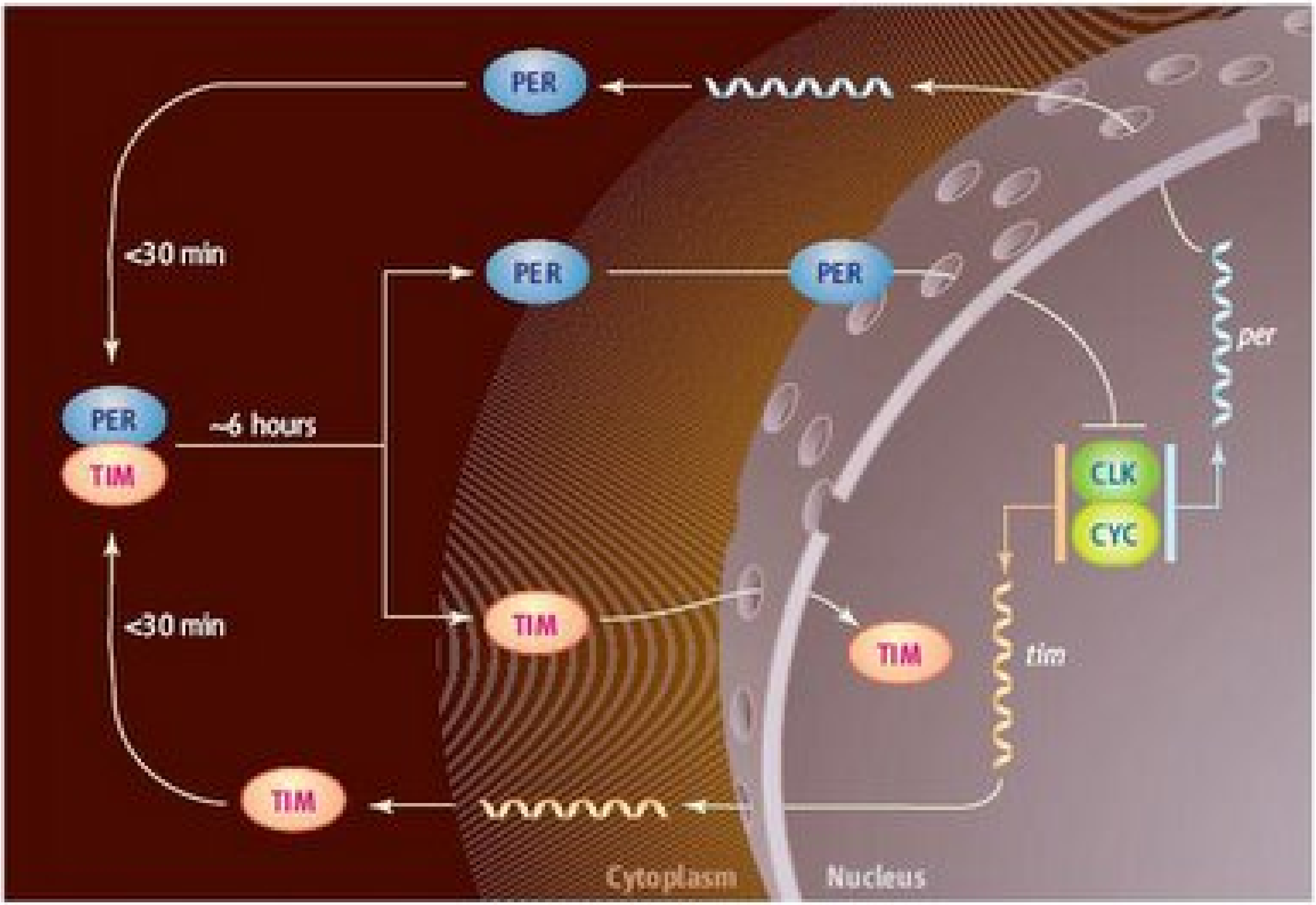
clock und cycle proteins are bHLH dimers via PAS domain: dimers binds to E box in promoter of *per* and *tim* genes and activate their transcription

Mechanism: *feedback loop*

Cryptochrome:

***Arabidopsis*/*Drosophila*: input signals**

mammalians: oscillator component



An interval timer influences the schedule of molecular events in the *Drosophila* circadian clock. Transcription of *per* and *tim* in the nucleus is driven by the combined action of transcription factors CLK and CYC. The resulting transcripts move to the cytoplasm where PER and TIM are made. These proteins rapidly associate into a heterodimer and remain as such for a long period of time. The duration of this interval, which contributes to the long time constant of the circadian clock, is set by a PER-influenced interval timer. Eventually PER and TIM enter punctate cytoplasmic foci (not shown) before their dissociation from each other and separate entry into the nucleus. PER then goes on to depress the activity of the CLK-CYC complex, thereby reducing expression of *per* and *tim*.

4. UV-B photoreceptor (UVR8)

anthocyanin

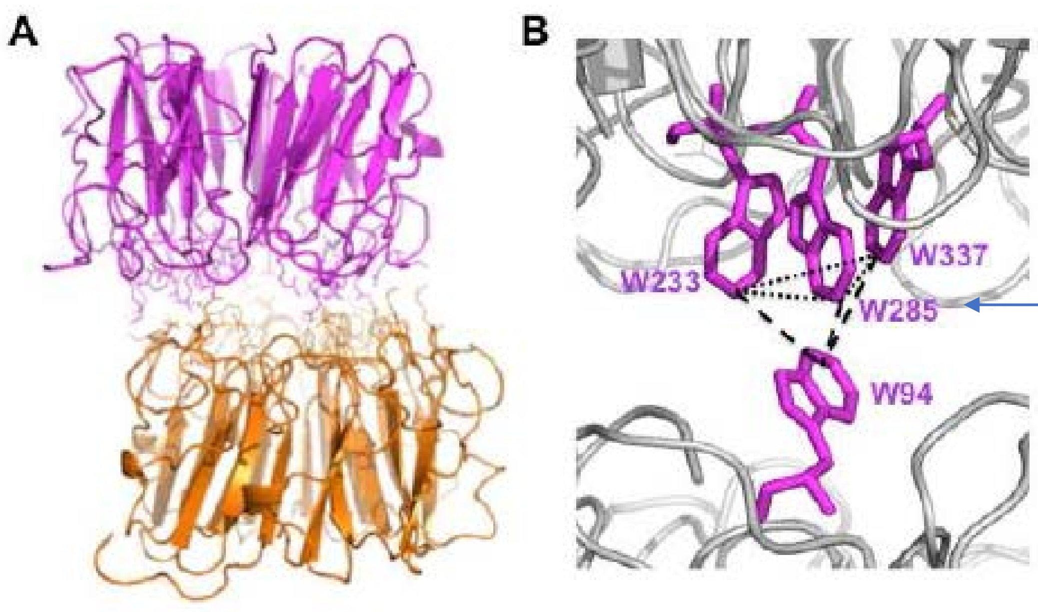
- Protection against UV-B light
- Activation of genes for enzymes involved in flavonoid biosynthesis
- Pigments accumulate in vacuole and absorb UV-B

- UVR8 absorbs 280-315 nm, max. 285 nm
- no prosthetic chromophore; light sensing (intrinsic to the molecule) with Try-285.
- Orthologous genes in all land plants

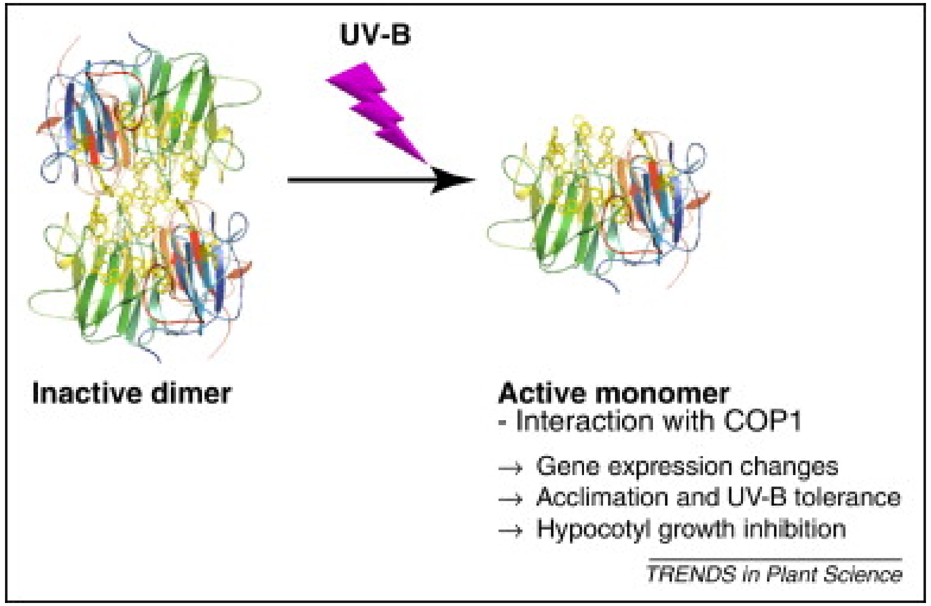
- No UV: UVR8 protein as dimer in the cytosol
- With UV: monomers that travel to the nucleus
- The monomer interacts with COP1 in the nucleus (like phytochrome and cryptochrome)

UVR8 structure & function

dimer monomer



Try-285



complex with COP1 ’ signal transduction in nucleus ’ complex interacts with HY5 (specific for UV-B stress) ’ transcription of genes involved in UB-B stress tolerance

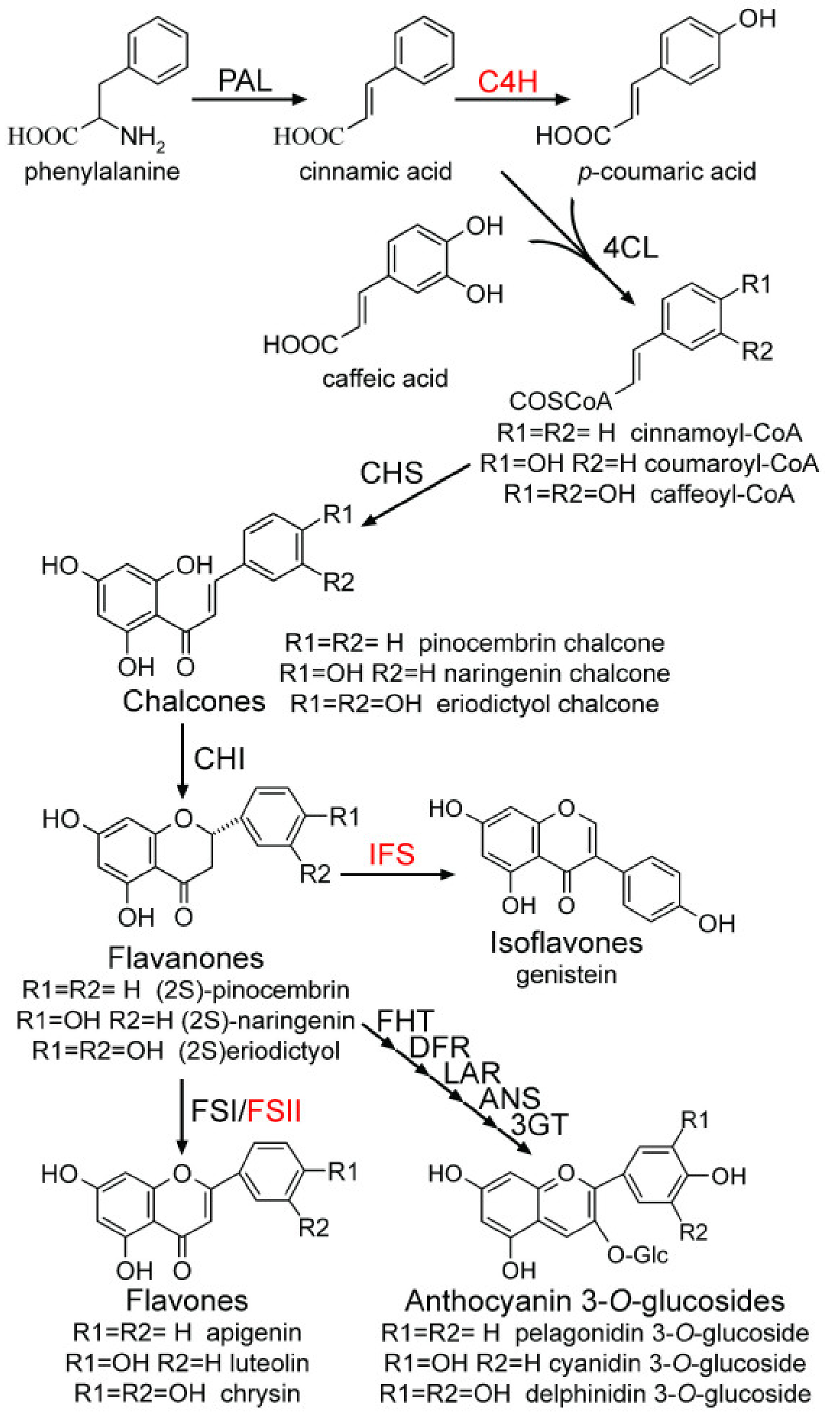

PAL
C4H
HOOC
NH_2
phenylalanine
cinnamic acid
OH
p-coumaric acid
4CL
caffeic acid
R1
R2
COSCoA
R1=R2= H cinnamoyl-CoA
R1=OH R2=H coumaroyl-CoA
R1=R2=OH caffeoyl-CoA
CHS
HO
O
R1=R2= H pinocembrin chalcone
R1=OH R2=H naringenin chalcone
R1=R2=OH eriodictyol chalcone
Chalcones
CHI
IFS
Isoflavones
genistein
Flavanones
R1=R2= H (2S)-pinocembrin
R1=OH R2=H (2S)-naringenin
R1=R2=OH (2S)eriodictyol
FHT
DFR
LAR
ANS
3GT
FSI/FSII
O-Glc
Flavones
R1=R2= H apigenin
R1=OH R2=H luteolin
R1=R2=OH chrysin
Anthocyanin 3-*O*-glucosides
R1=R2= H pelagonidin 3-*O*-glucoside
R1=OH R2=H cyanidin 3-*O*-glucoside
R1=R2=OH delphinidin 3-*O*-glucoside

Abb. 17.6. Ausbildung von „Jugendanthocyan" bei der Rose (*Rosa spec.*). Die Rotfärbung der jungen Blätter vieler Pflanzen geht auf die Akkumulation von Anthocyanen in der Vacuole der Epidermiszellen zurück. Kälte und hoher Lichtfluß fördern diese Reaktion. Anthocyane absorbieren kurzwelliges Licht und UV und schützen dadurch das empfindliche Assimilationsparenchym während des Aufbaus des Photosyntheseapparats. Anschließend wird das Pigment wieder abgebaut. Auch die lichtinduzierte Anthocyansynthese von Keimlingen ist als eine solche **Lichtschutzreaktion** zu deuten

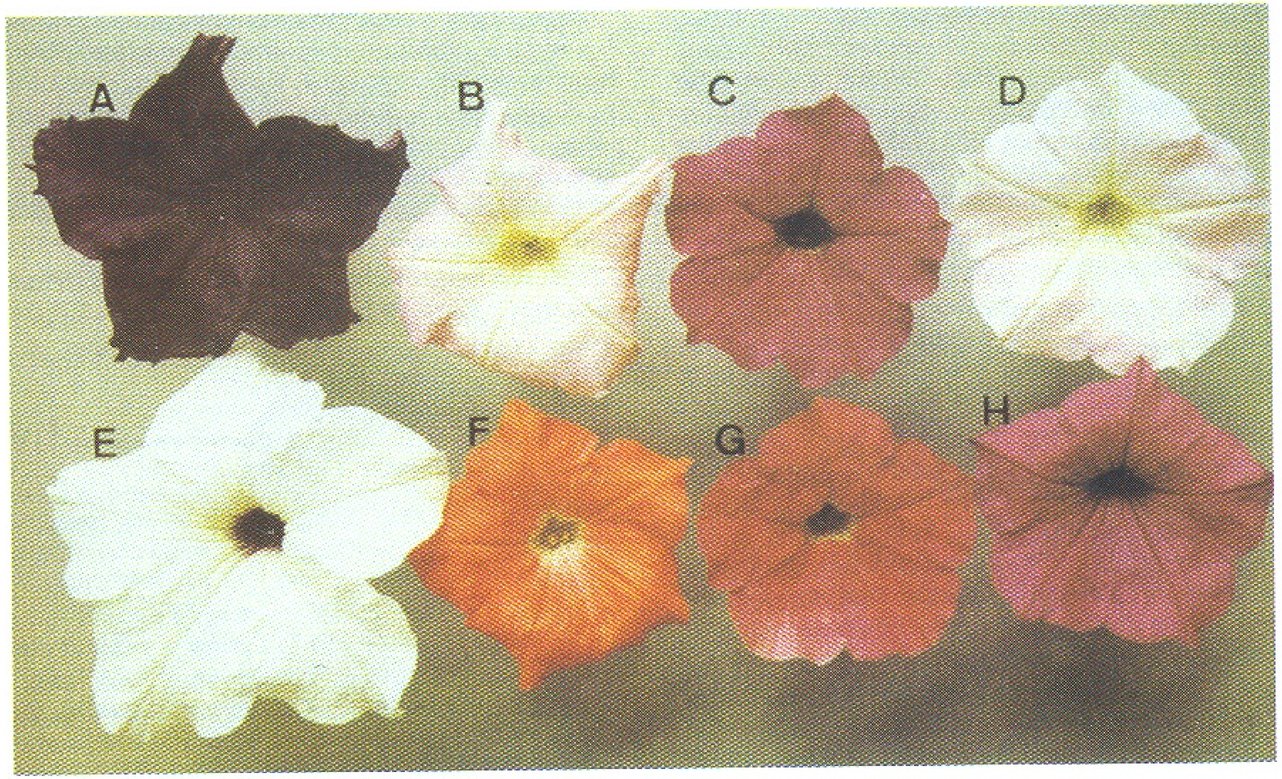
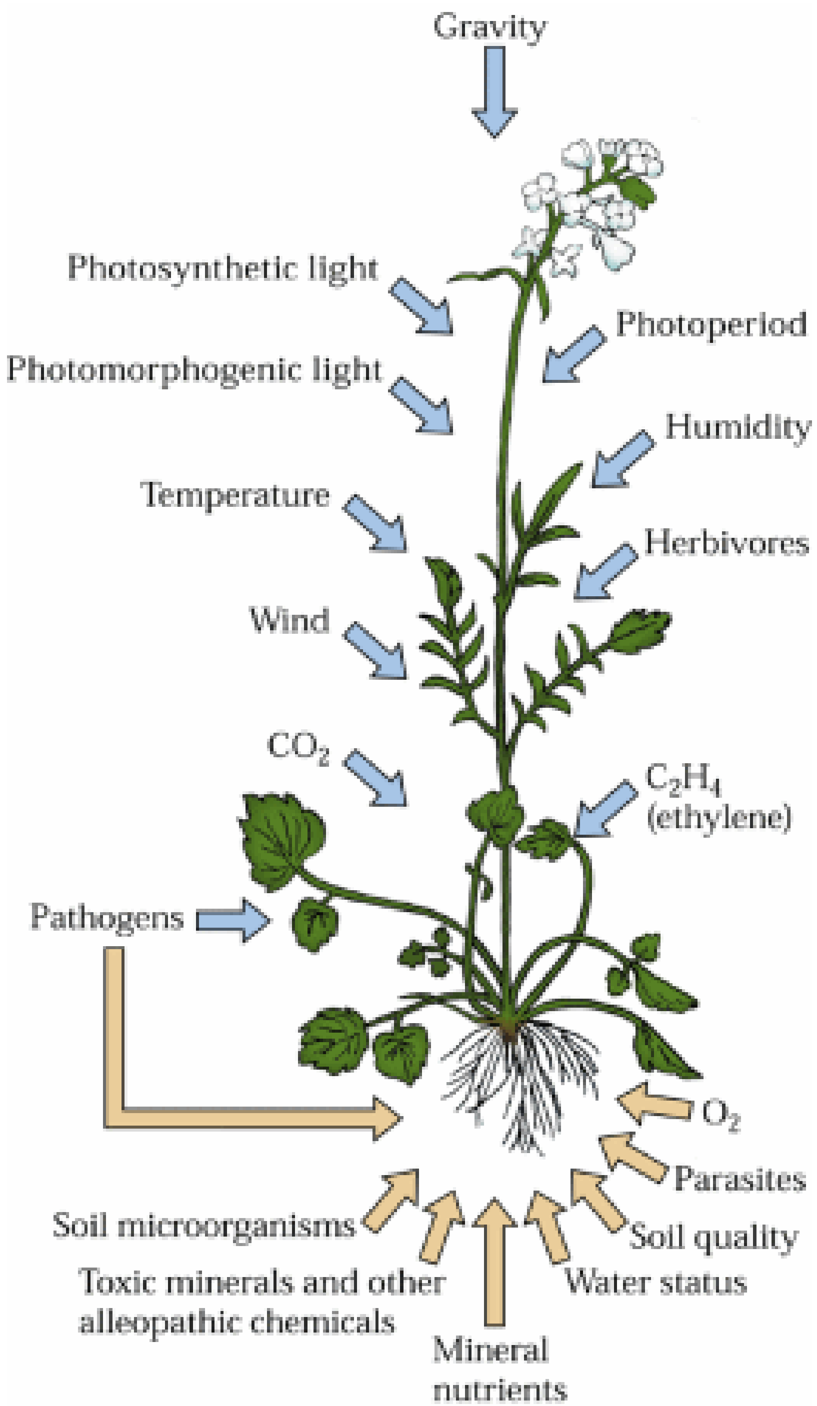


Abb. 17.7. Farbvarianten von Petunienblüten (*Petunia atkinsiana*), die durch konventionelle Züchtung (*A–D*) oder gentechnische Transformationen (*E–H*) erzeugt wurden; z. B. wurde die Transformante *E* durch Einbau eines *antisense*-Gens für Chalconsynthase (→ Abb. 17.5) in die Sorte *A* erhalten. (Nach Holton u. Cornish 1995)

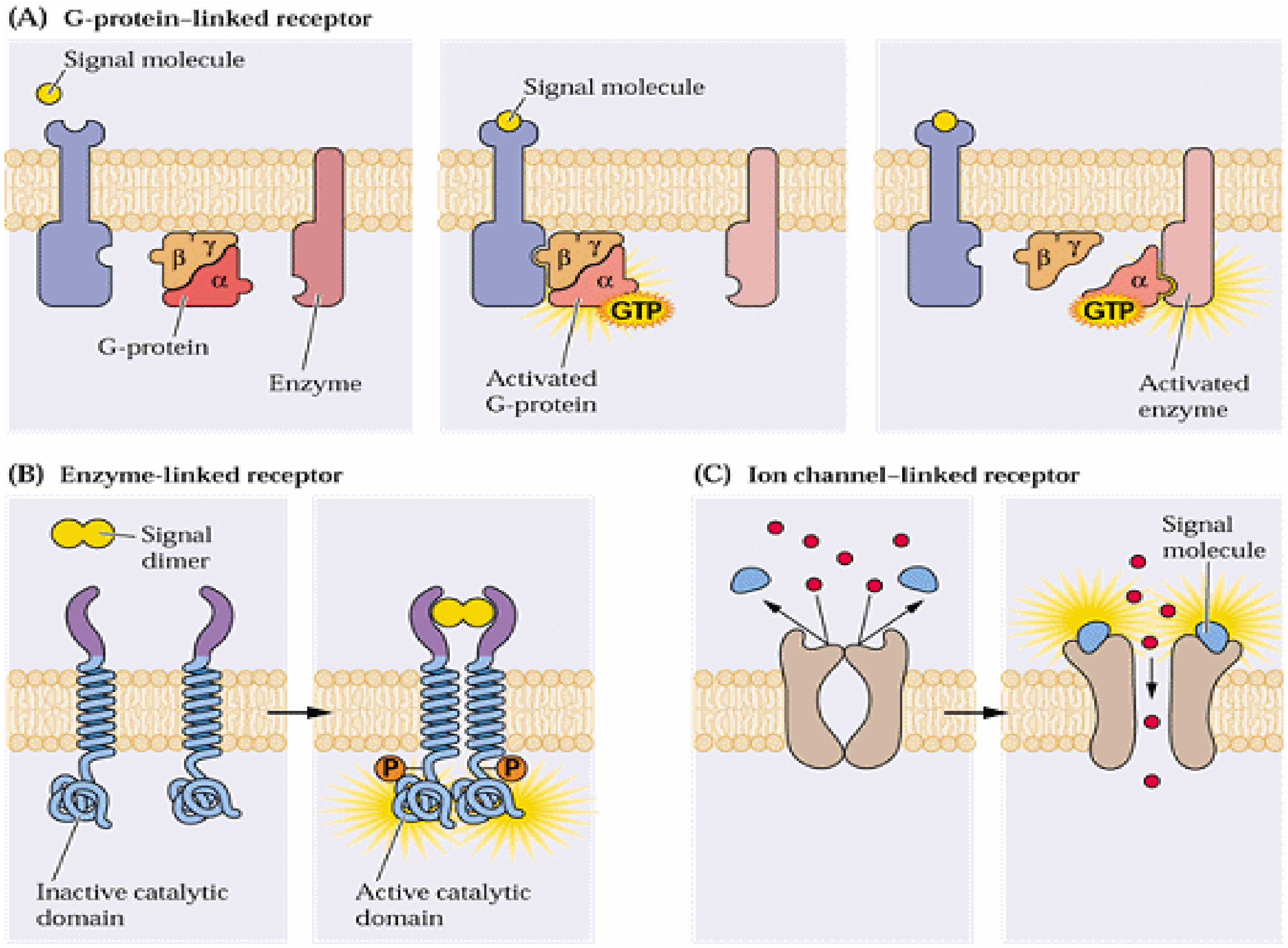
Basics in plant signal transduction

-general mechanisms
- differences plants/animals

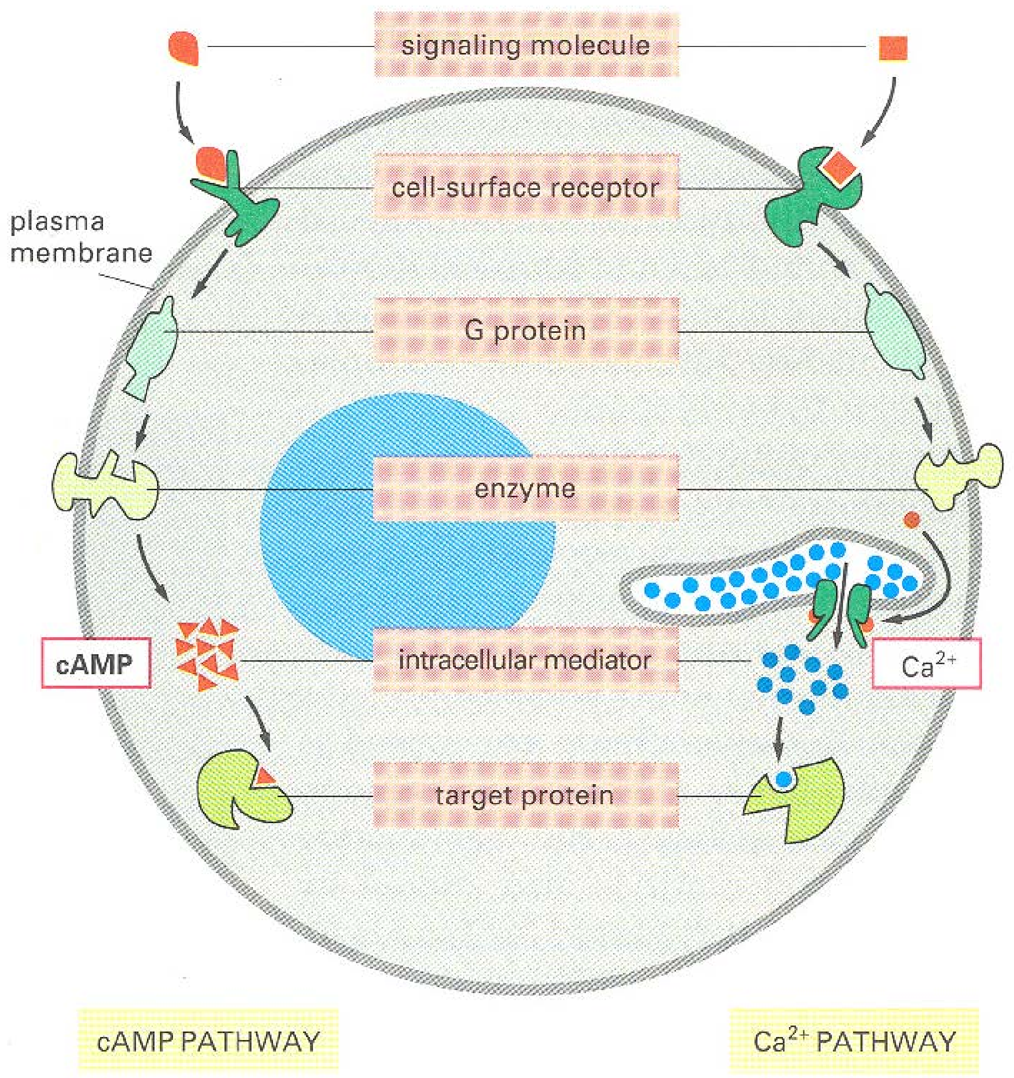


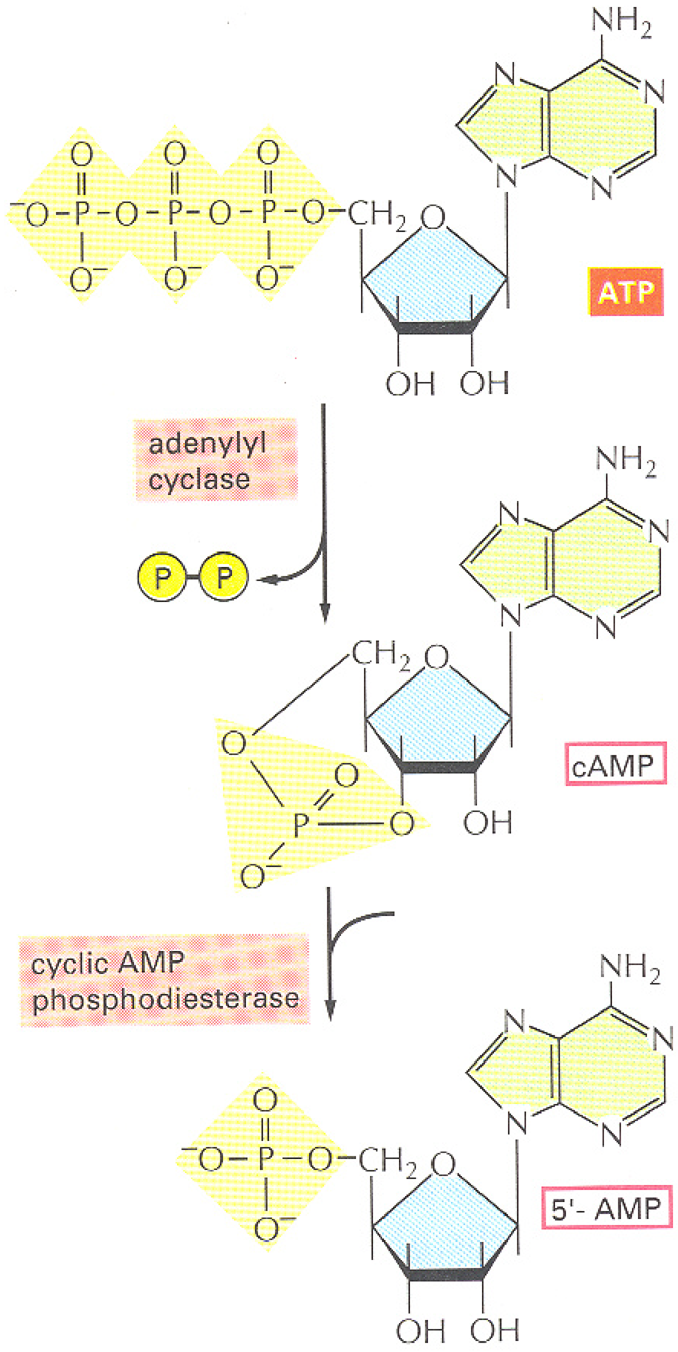
Gravity
Photosynthetic light
Photoperiod
Photomorphogenic light
Humidity
Temperature
Herbivores
Wind
CO_2
C_2H_4 (ethylene)
Pathogens
O_2
Parasites
Soil microorganisms
Soil quality
Toxic minerals and other alleopathic chemicals
Water status
Mineral nutrients

Three types of receptors regulate signaling accross the plasma membrane



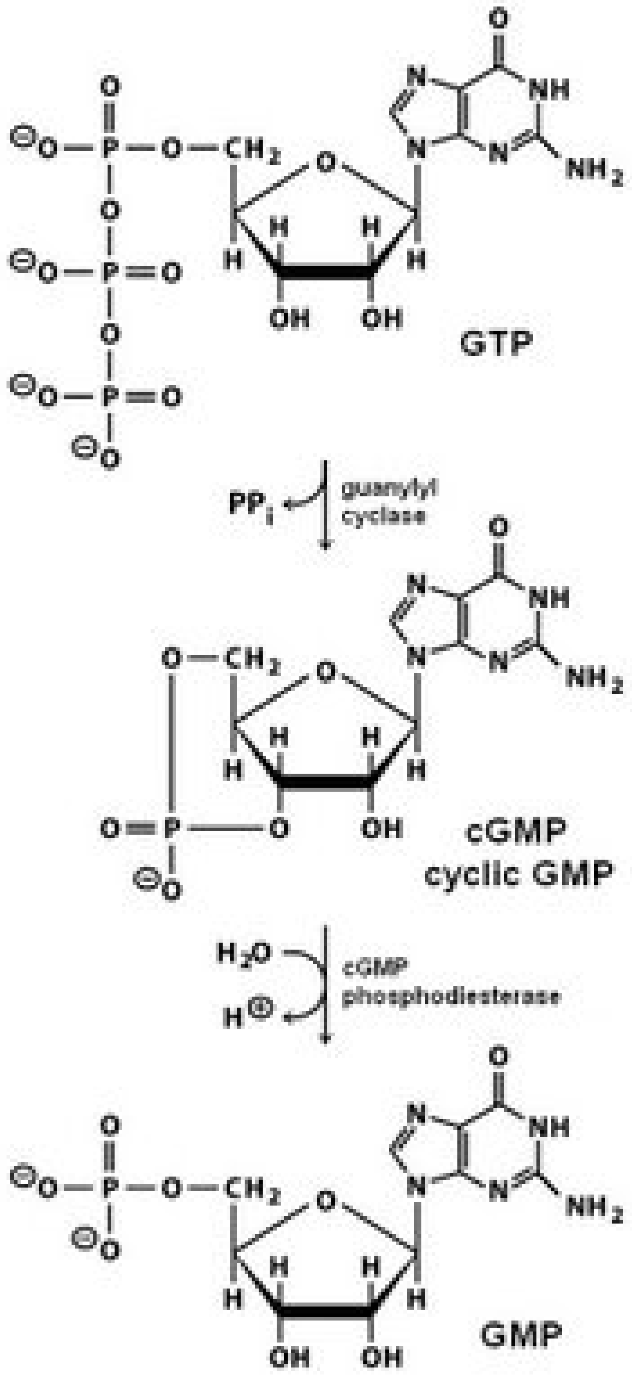
Signaling depends on calcium and cAMP in animals and cGMP in plants



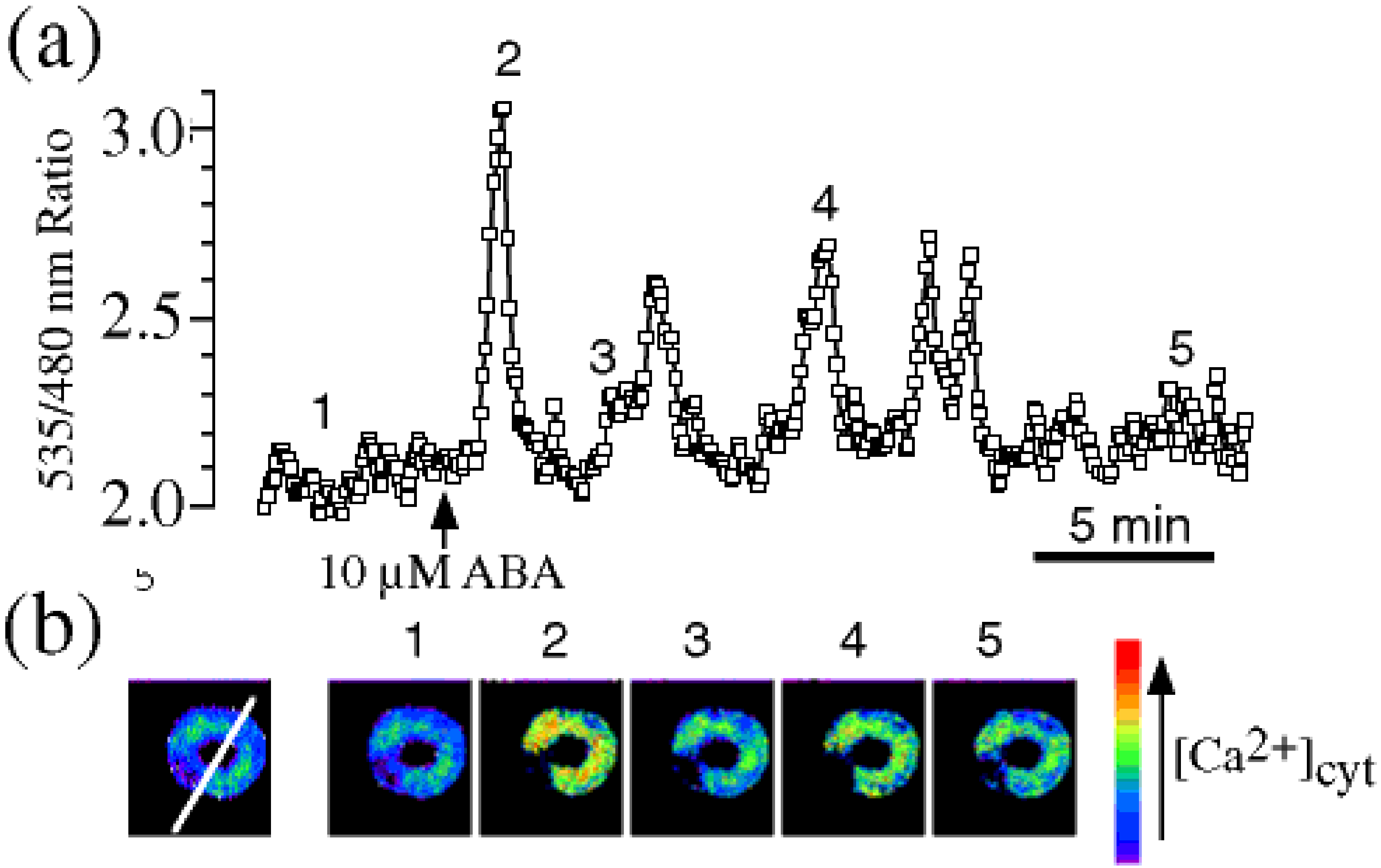


cAMP is replaced by cGMP

as second messanger in plants



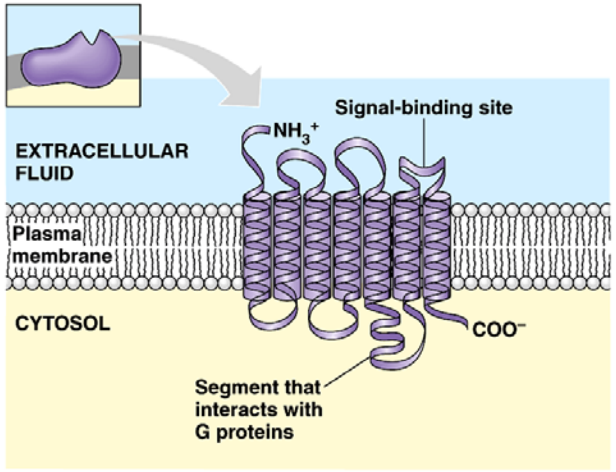
Ca signature determines response patterns

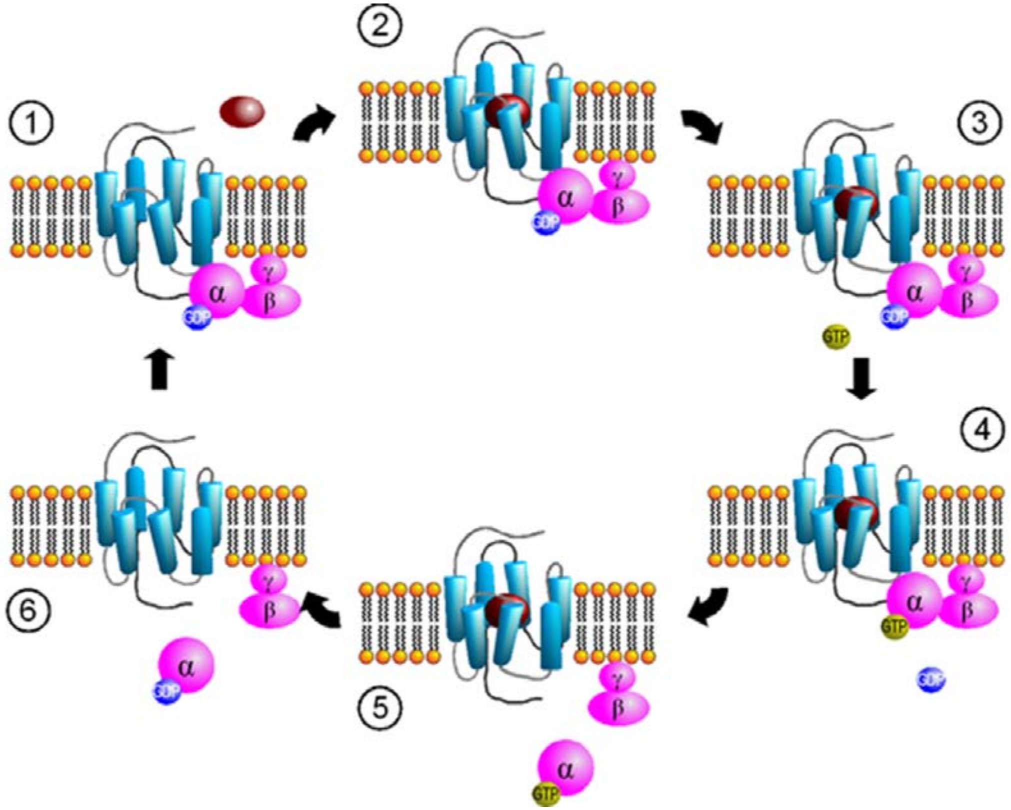


Ca signaling is very complex in plants

- Source of Ca (external, internal stores)
- CDPK
- more than 100 Ca-binding pregulatory proteins (network)
- CaCaMK are located in cytoplasm and nucleus
- Differences in Ca signatures determine specifity

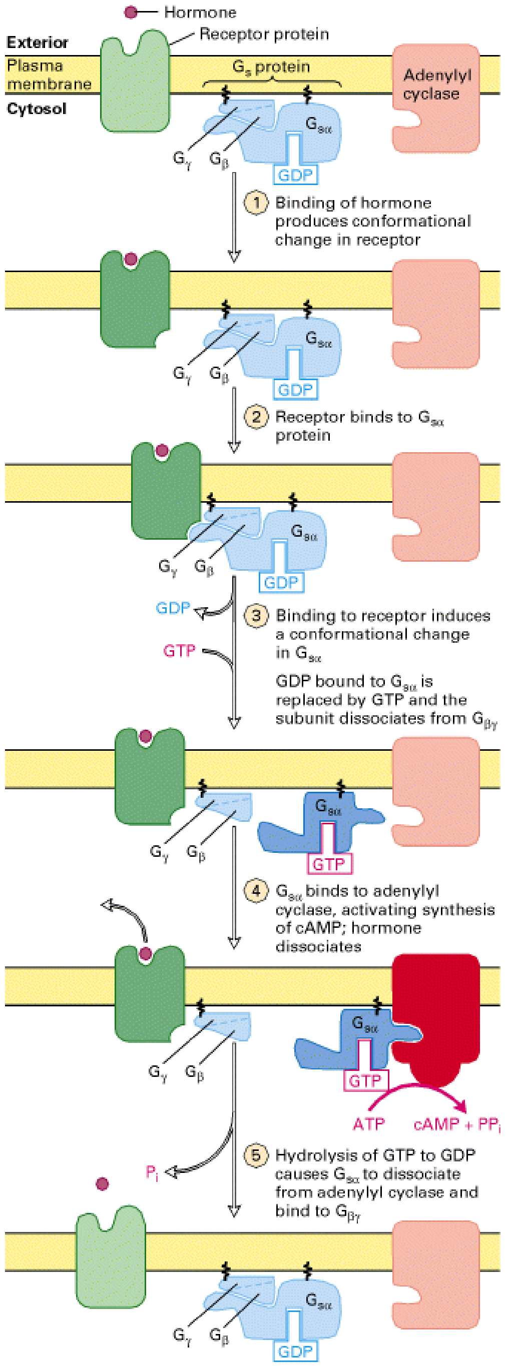
Heterotrimeric G-proteins play a major role in animals, but are less important in plants





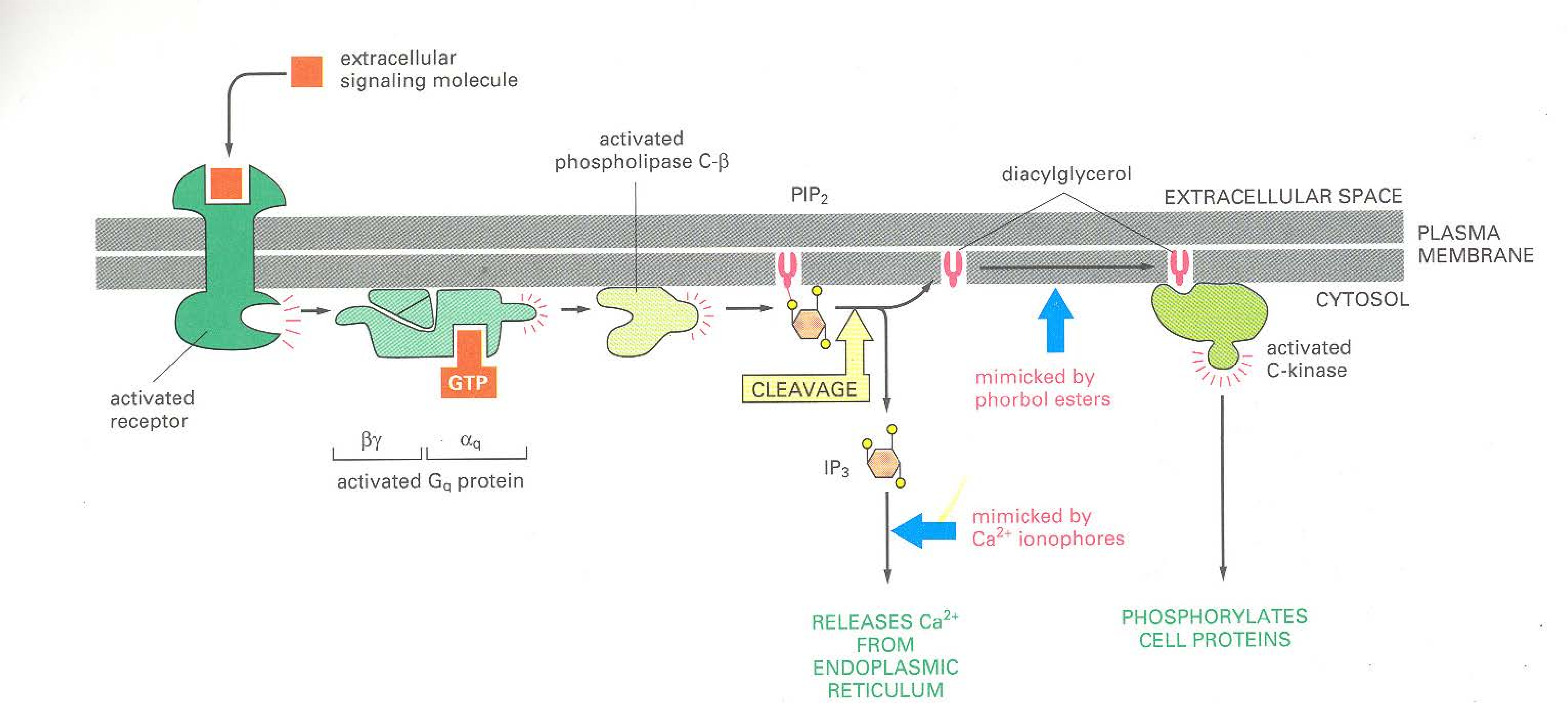
Plants: 1 receptor gene, 1 gene for ±,² ,³ subunits ’ little changes for specificity in signaling
Animals: more genes for the proteins ’ more protein combinations ’ higher specificity

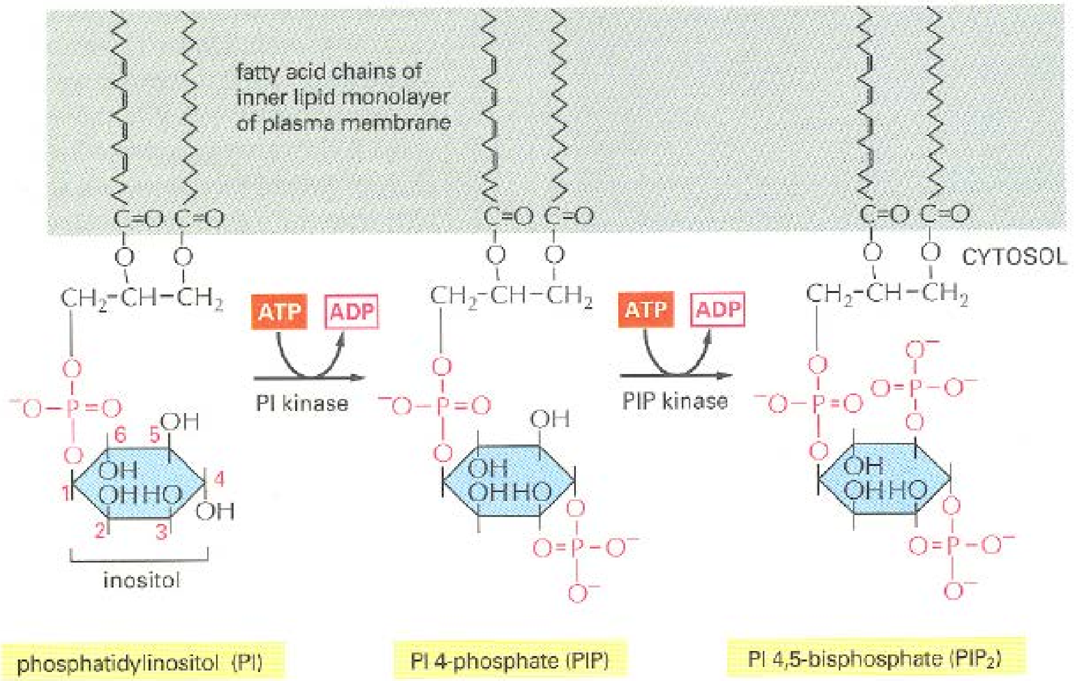
Heterotrimeric G-proteins activate the adenylate cyclase in animals, and a guanylate cyclase in plants

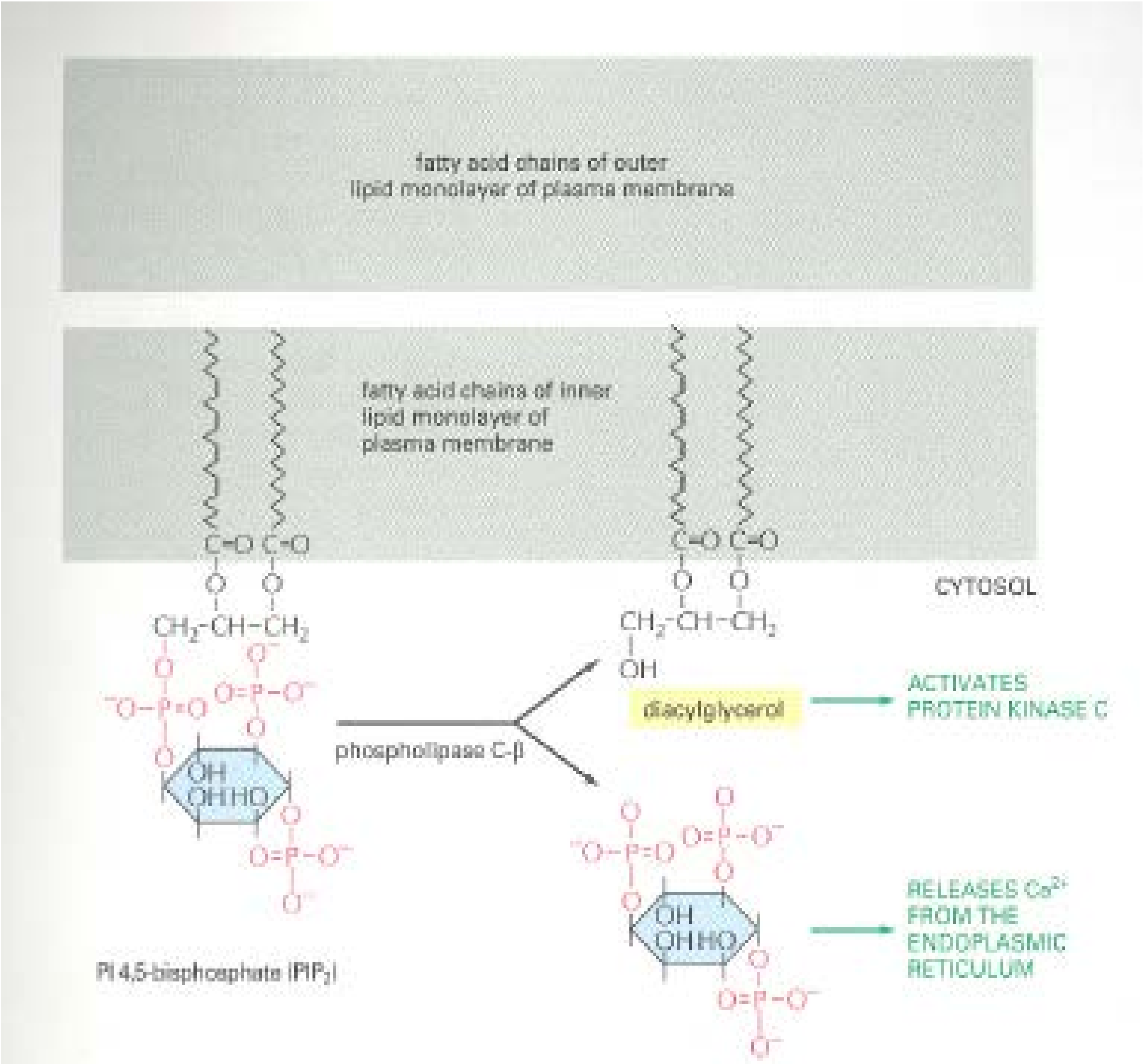


Plant phospholipid signaling is quite different to animals, (phosphatidic acid, PLC and PLD, no IP_3 receptor at ER)

Ca^{2+} is mainly taken up from the cell wall and less from internal stores

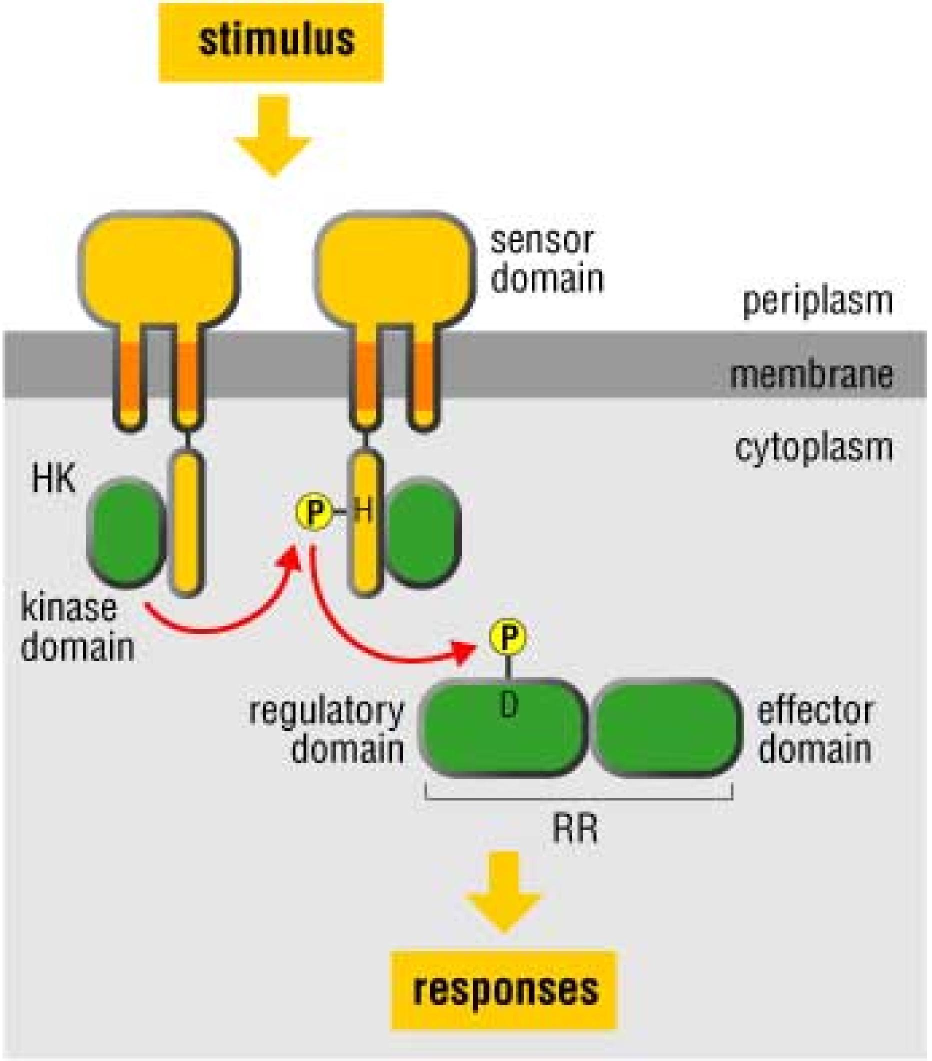



fatty acid chains of inner lipid monolayer of plasma membrane
CYTOSOL
ATP
ADP
PI kinase
PIP kinase
inositol
phosphatidylinositol (PI)
PI 4-phosphate (PIP)
PI 4,5-bisphosphate (PIP2)


fatty acid chains of outer lipid monolayer of plasma membrane
fatty acid chains of inner lipid monolayer of plasma membrane
CYTOSOL
phospholipase C-β
diacylglycerol
ACTIVATES PROTEIN KINASE C
RELEASES Ca2+ FROM THE ENDOPLASMIC RETICULUM
PI 4,5-bisphosphate (PIP2)

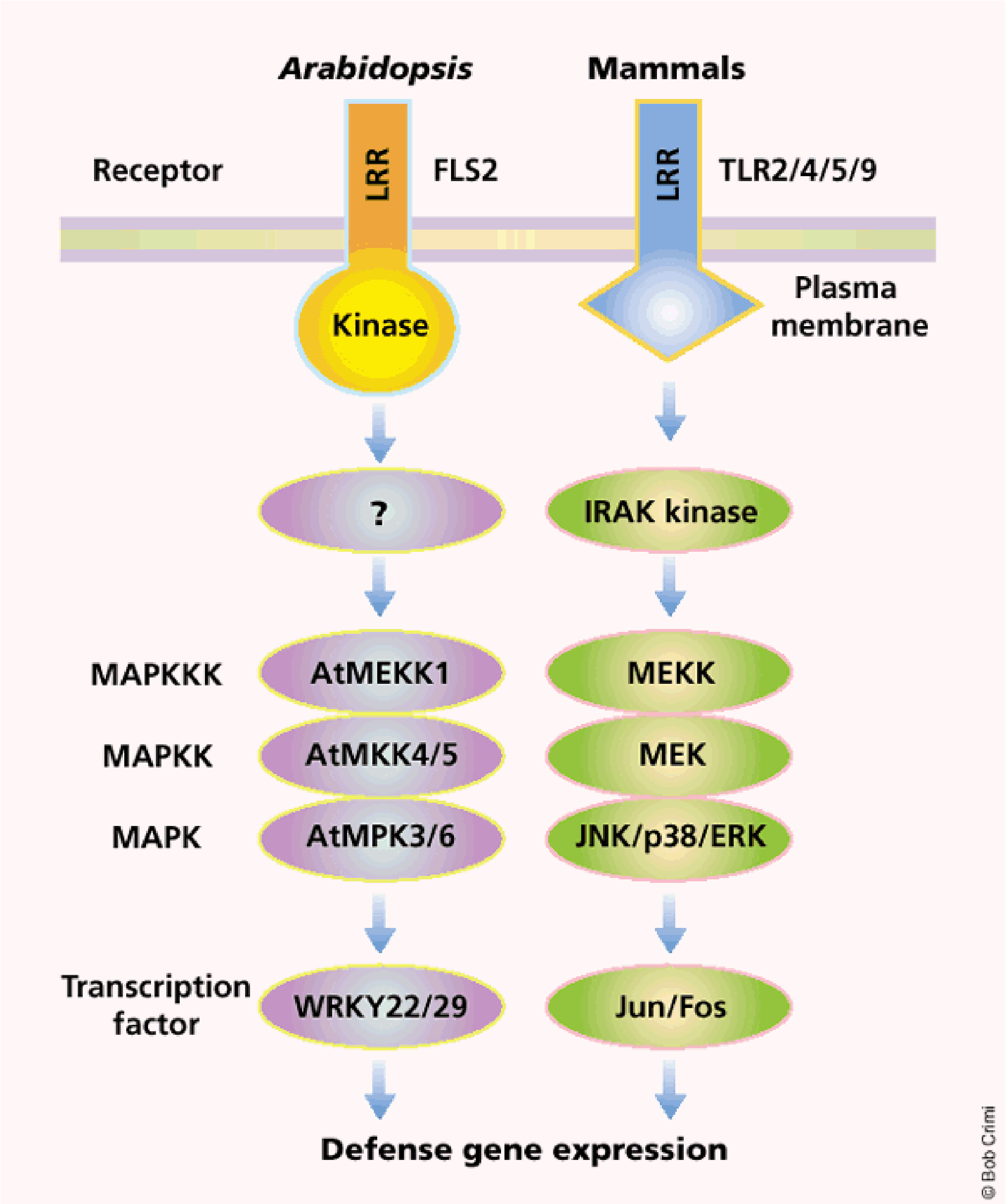
The two component system plays an important role in plant hormone signaling (histidine kinase & response regulator), but is not present in animals

histidine kinase



response regulator

MAPKs play important roles in plant defense



- Genetic approaches to identify signaling processes in plants
vs.
- Biochemical analyses of signaling processes in animals