5) Photomorphogenesis

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

Handbook of Photosensory Receptors, Wiley-VCH

Photomorphogenesis in Plants and Bacteria, 3rd ed., Springer

Light and Plant Development
Blackwell Publishing
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.

Basic photomorphogenic responses:

- Inhibition of elongation
- Stimulation of chlorophyll synthesis
- Stimulation of leaf growth
For the process of photomorphogenesis, light is perceived by pigments that are a part of photoreceptors:

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

Phytochrome = protein pigment of blue light identified in 1959

Plant responses induced by phytochromes:

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

<table>
<thead>
<tr>
<th>TABLE 17.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical photoreversible responses induced by phytochrome in a variety of higher and lower plants</td>
</tr>
</tbody>
</table>

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

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)

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: $Pr : Pfr = 98% : 2%$
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
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 \textit{PHYA}  
Type II \textit{PHYB}  
\textit{PHYC}  
\textit{PHYD}  
\textit{PHYE}

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

\textbf{PMP}
PHYB - E expression is not affected by light => transcriptionally active in etiolated and green plants; proteins phyB - E are more stable

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

\[ \text{PHYB - E} \rightarrow \text{mRNA} \rightarrow \text{Pr} \xrightarrow{R} \text{Pfr} \rightarrow \text{Response} \]
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
Action spectrum of LFR for photoreversible stimulation and inhibition of *Arabidopsis* seed germination
Action spectrum of HIR for inhibition of elongation of etiolated hypocotyl
Action spectrum of HIR for inhibition of elongation of green hypocotyl

The more green plant, the less sensitive to FR

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

HIR of green plant is mediated by phytochrome phyB
c) Ecological functions of phytochromes

R/FR reversible pigment

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

Wavelengths R and FR = information for plant
R : FR in various environments

**TABLE 17.3**

Ecologically important light parameters

<table>
<thead>
<tr>
<th></th>
<th>Photon flux density (μmol m(^{-2}) s(^{-1}))</th>
<th>R/FR(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daylight</td>
<td>1900</td>
<td>1.19</td>
</tr>
<tr>
<td>Sunset</td>
<td>26.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Moonlight</td>
<td>0.005</td>
<td>0.94</td>
</tr>
<tr>
<td>Ivy canopy</td>
<td>17.7</td>
<td>0.13</td>
</tr>
<tr>
<td>Lakes, at a depth of 1 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Loch</td>
<td>680</td>
<td>17.2</td>
</tr>
<tr>
<td>Loch Leven</td>
<td>300</td>
<td>3.1</td>
</tr>
<tr>
<td>Loch Borralie</td>
<td>1200</td>
<td>1.2</td>
</tr>
<tr>
<td>Soil, at a depth of 5 mm</td>
<td></td>
<td>0.88</td>
</tr>
</tbody>
</table>


*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

R:FR = 1.2

R:FR = 0.8

Shade-avoidance response
- elongation
- reduction of leaf size
- decrease in chlorophyll
- reduction of sec. shoot formation
Circadian rhythms

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

They persist in the absence of exogenous factors

Requirement of endogenous stimuli (pacemakers)

**Endogenous oscillator**
- plants
- animals

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

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

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

*PHYB* is responsible for plant sensitivity to R and mediates photoreversible seed germination
PhyA is receptor continuous FR.

Mutant *phyA*:
- Does not respond to FR
- Develop tall and thin phenotype

\[ \text{Difficult to select mutants with a defect specifically in protein PHYA} \]

\[ \text{Phenotype of mutants with defect in chromophore or phyB} \]

\[ \text{Mutants with PHYA deficit} \]

\[ \text{Mutants with deficit in chromophore and/or in PHYB} \]
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

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

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

Interaction of phyA and phyB in shade-avoidance response

Direct sunlight:
Abundance of R => de-etiolation directed by phyB

Shade:
Abundance of FR => at the beginning de-etiolation mediated by phyA. PhyA is labile => later de-etiolation mediated by phyB.
d) Cellular and molecular mechanisms of phytochrome functions

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

Regulation of membrane potential and ion flux mediated by phytochromes

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

Leaf of *Mimosa*
Phytochrome regulates gene expression

Processes of photomorphogenesis and de-etiolation

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*

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

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

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

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

1) Regulation of gene expression directly by PfrB

2) Regulation of gene expression through PIF3

PIF3 (phytochrome interacting factor3)

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

- Interacting with C-terminal end of PfrB => PIF3 and PfrB form a complex
**Regulation of gene expression by phytochrome A**

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

**Dark:**  
Accumulation of COP1 in the nucleus  
Repression of expression of photomorphogenic genes – transcription factors (HY5, HFR1, LAF1,…) are ubiquitinated.

**Light:**  
Transport of COP1 from the nucleus to cytoplasm by ubiquitination of protein PfrA  
Restoration of expression of photomorphogenic genes by release of transcription factors (HY5, HFR1, LAF1,…)
**cop1** (constitutive photomorphogenesis 1) – etiolated plants show phenotype of plants growing in light

Nonmutated plant    Mutant *cop1*

Nonmutated (= functional) gene *COP1* – negative regulator of photomorphogenesis
**COP1** functions as E3 ubiquitin ligase – enzyme ensuring protein degradation in cell (proteolysis)

Proteolysis mediated by proteasome requires protein ubiquitin.

**Ubiquitination** – general mechanism of protein degradation in organisms
Regulation of transport of phyA into the nucleus

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

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

Transport of phyA into the nucleus – triggering of light responses (germination, flowering, etc.) + regulation of production of transcription factors **FHY3** and **FAR1** => feedback: phyA influences its own transport to the nucleus
Phosphorylation – important mechanism working in various signaling pathways, including phytochromes

Phosphorylation regulates activity of transcription factors (and other enzymes)

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

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

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

(B) Plant phytochrome

Red light

Phytochrome

Autophosphorylation

ATP

Chromophore

Kinase domain

COOH

H₂N

Ser

Phosphorylation of another protein
Factors involved in gene expression regulated by phytochromes

1. Red light converts PrA and PrB to their Pfr forms.
2. The Pfr forms of phyA and phyB phytochrome can autophosphorylate.
5. cGMP, calmodulin, and calcium may activate transcription factors (X and Y).
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 HYS.
11. In the dark, HYS is degraded with the assistance of the COP/D/DET/FUS proteasome complex.
12. In the light, COP1 interacts directly with SPA1 and is exported to the cytoplasm.

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