Lecture 2

http://www.yale.edu/denglab/
xingwang.deng@yale.edu
Distinct features of Development

- **Animals**
  - Determinate
  - More fixed pattern
  - Totipotency unsure
  - Cell migration
  - Distinct mechanisms, shared some molecular components and features
  - Many distinct family of proteins not found in plant kingdom

- **Plants**
  - Indeterminate
  - Plastic development
  - All cell totipotent
  - No cell migration
  - Distinct mechanisms, shared some molecular components and features
  - Many distinct families of proteins not found in animal kingdom
Light VS. Plants

• **1.** Light provides the energy source for plants and thus all life forms

• **2.** Thus plants have evolved sophisticated mechanisms to optimize their capacity to harvest light energy.
Light Environment

- Quality
- Quantity
- Direction
- Duration/period
Light environment: sunlight

Visible light: 400-700 nm
or 0.4 -0.7 µm
Light quality variation during the day

The spectral distribution of radiant energy in direct sunlight at midday and at sunrise and sunset.
Light quality variation during the day

The Red and far-red light ratio change dramatically, which is critically important signal for plant response
Light quality variation due to location

The light quality alters drastically depending on the situation.

• Direct sunlight
• Skylight (blue sky!)
• Under shade
Light quantity variation in nature

The light intensity in nature varies at least ten orders of magnitude
Variations in light direction and photoperiod in nature

• Sun rises in east and sets in west, thus the direction of sunlight for an immobile plant changes constantly during a day.

• In both north or south hemispheres, the relative lengths of day and night changes with seasons
shade reflected light

directional light
direct light
cotyledon

towards light

cotyledon hypocotyl

shade avoiding characteristic photomorphogenic phototropic etiolated
darkness
Phytochromes

• Phytochrome photoreceptors exist in two different forms:

\[ \text{Pr} \rightleftharpoons \text{Pfr} \]

where Pr is the red absorbing form (660nm) and Pfr is the far-red absorbing form (735nm)
Pure Phytochrome in the Tube

- Phytochrome absorb red light, thus appears green
Absorption spectra of the two forms of Phytochrome
Phytochrome Chromophore

Red light
Far-red light or darkness
Light Environment & Arabidopsis seedling morphogenesis

Photomorphogenic development

Skotomorphogenic development or Etiolation

cotyledon
Hypocotyl
root
Seedling screens for light regulatory components

• Looking for etiolated or skotomorphogenic development in the light. Most of those called long hypocotyl mutants (note many light conditions can be used)

• Looking for photomorphogenic or de-etiolated development in darkness. Those are called de-etiolated \((det)\) or constitutive photomorphogenic \((cop)\) mutants
# Light control of Hypocotyl Elongation

<table>
<thead>
<tr>
<th></th>
<th>Blue</th>
<th>Red</th>
<th>Far-red</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hy1, hy2</em>:</td>
<td>Normal</td>
<td>Long</td>
<td>Long</td>
</tr>
<tr>
<td><em>phyB</em>:</td>
<td>Normal</td>
<td>Long</td>
<td>Normal</td>
</tr>
<tr>
<td><em>phyX</em>:</td>
<td>Normal</td>
<td>Normal</td>
<td>Long</td>
</tr>
</tbody>
</table>

1. Chromophore mutants (*hy1* and *hy2*) have long hypocotyl in both continuous red and far red light, while *phyB* (*hy3*) mutants only exhibit long hypocotyl in red (but not in far red).

2. Clearly, other phytochrome or phytochromes are important for regulating hypocotyl length in far-red light.
The complementary roles of PhyA and PhyB
Phytochrome function specificity during early Arabidopsis development

<table>
<thead>
<tr>
<th>Phytochrome</th>
<th>Light</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>phyA</td>
<td>Far-red</td>
<td>De-etiolation (major)</td>
</tr>
<tr>
<td>phyB</td>
<td>Red</td>
<td>De-etiolation (major)</td>
</tr>
<tr>
<td>phyC</td>
<td>Red</td>
<td>Primary leaf expansion</td>
</tr>
<tr>
<td>phyD</td>
<td>Red</td>
<td>De-etiolation (minor)</td>
</tr>
<tr>
<td>phyE</td>
<td>Red</td>
<td>Internode suppression</td>
</tr>
</tbody>
</table>

NOTE: Those responses listed above only apply to high irradiance response.
Structure-Function of Phytochromes

- N-terminal half of phytochromes dictating the light specificity, C-half for transducing the signal.
Shade Avoidance Response

1. Sense red vs far red light Ratio;

2. phyB, together with phyD, and phyE, mediates This response.

3. The responses include: Promoting elongation growth, Early flowering, shoot vs root ratio
Two types of blue light receptors

phototropin

NH₂  LOV1  LOV2  kinase  COOH

cryptochrome

NH₂  PHR  DAS  COOH

DQXVP  E/D  STAES
## Light control of Hypocotyl Elongation

<table>
<thead>
<tr>
<th></th>
<th>Blue</th>
<th>Red</th>
<th>Far-red</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hy1, hy2:</strong></td>
<td>Normal</td>
<td>Long</td>
<td>Long</td>
</tr>
<tr>
<td><strong>hy3/phyB:</strong></td>
<td>Normal</td>
<td>Long</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>phyA:</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Long</td>
</tr>
<tr>
<td><strong>X:</strong></td>
<td>Long</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Long Hypocotyl Mutants  
(Arabidopsis white light screen)

*hy1, hy2*: defective in genes for phytochrome chromophore biosynthesis. They affect all phytochromes (Koornneef et al., 1980)

*hy3*: specifically defective in phyB apoprotein gene (Koornneef et al., 1980; Reed et al., 1993)

*hy4*: specifically defective in a blue light receptor CRY1 (Koornneef et al., 1980; Ahmad and Cashmore, 1994)

*hy5*: defective in a component downstream of multiple photoreceptors (Koornneef et al., 1980; Ang and Deng, 1994).
The roles of two phototropin blue light receptors

- **WT**
- **phot1**
- **phot2**
- **phot1 phot2**
A phenotype screen for phototropin 2 mutant
Photo2 mutant has CT accumulation even in strong blue light
phototropin 2 mutant affect leaf color after strong blue light
<table>
<thead>
<tr>
<th>Light Receptor</th>
<th>Function Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cryptochrome 1</strong></td>
<td>De-etiolation (Major in High Intensity)</td>
</tr>
<tr>
<td><strong>Phototropin 1</strong></td>
<td>Phototropism (Low light)</td>
</tr>
<tr>
<td></td>
<td>Chloroplast accumulation at low light</td>
</tr>
<tr>
<td></td>
<td>Stomatal opening (redundant)</td>
</tr>
<tr>
<td><strong>Cryptochrome 2</strong></td>
<td>De-etiolation (Major in low intensity)</td>
</tr>
<tr>
<td></td>
<td>&amp; Flowering time</td>
</tr>
<tr>
<td><strong>Phototropin 2</strong></td>
<td>Phototropism (High light)</td>
</tr>
<tr>
<td></td>
<td>Chloroplast avoidance at high light</td>
</tr>
<tr>
<td></td>
<td>Stomatal opening (redundant)</td>
</tr>
</tbody>
</table>
Arabidopsis Seedling Development

- Light
- Dark

- Cotyledon
- Hypocotyl
A Genetic Approach

Dark Light Dark Light
Wild Type cop1 Mutant
The Pleiotropic COP/DET/FUS Genes

- All mutations are recessive and loss-of-functional, thus they act as repressor of photomorphogenic development.
The COP9 signalosome (CSN) subunits & the COP/DET/FUS genes

<table>
<thead>
<tr>
<th>Subunit</th>
<th>COP/DET/FUS Gene</th>
<th>Affect the Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>FUS6/COP11</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>FUS12/COP12</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>FUS11/COP13</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>COP8/FUS4</td>
<td>COP/DET/FUS genes do not affect the COP9 Signalosome accumulation:</td>
</tr>
<tr>
<td>S5</td>
<td>AJH1 &amp; AJH2</td>
<td>COP1/FUS1, COP10/FUS9, DET1/FUS2</td>
</tr>
<tr>
<td>S6</td>
<td>CSN6A/CSN6B</td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>FUS5/COP15</td>
<td></td>
</tr>
<tr>
<td>S8</td>
<td>COP9/FUS7/FUS8</td>
<td></td>
</tr>
</tbody>
</table>

Wei, Chamovitz, Staub, Kwok, Peng, Schwechheimer, Serino, 1992-2002
1. Distinct receptors for different light regions.

2. Each photoreceptor system has their own signaling pathways.

3. All pathways converge to negative regulate common repressors, COP/DET/FUS.

4. The common repressors Negatively regulate transcription factors.
A Biochemical Model for the Common Repressors

E1 \[\text{Ub}\] \rightarrow \text{COP10 Complex (UEV)} \[\text{Ub}\] \rightarrow \text{CSN (Base and 20S core)} \rightarrow \text{Degradation}

The COP9 Signalosome Proteasome

COP1/CIP8 complex as E3 ligase

HY5 & HYH etc
Light Control of Plant Development

Seed → Seedling → Development Arrested

Seedling:
- Light: Photomorphogenesis
- Darkness: Skotomorphogenesis

Adult: WT_L → WT_D

Light Control of Plant Development
Blue/UV-A light

CRY1  CRY2

Via direct Contacting COP1

COP10, CSN COP1, DET1

HY5, HYH, etc

Genome Expression & Photomorphogenesis
Far-red light

phyA

SPA1, EID1

FIN2, PHY3, PHY4, FAR1, FIN2, HFR1, FHY1, LAF1, LAF6, PAT1

COP10, CSN, COP1, DET1

HY5, HYH, etc

Genome Expression & Photomorphogenesis

Blue/UV-A light

CRY1, CRY2

Via direct Contacting COP1
phyA

Red light

Red

phyB etc

Red light

Blue/UV-A light

CRY1 CRY2

Via direct Contacting COP1

COP10, CSN COP1, DET1

HY5, HYH, etc

Genome Expression & Photomorphogenesis
What are the whole genome expression profile defining photomorphogenesis achieved in individual organ types

Cotyledon

Hypocotyl

Root
Arabidopsis 70mer array for 26,090 genes
Organ-specific Genome Expression

Cotyledon
- 11065 (43%)  11425 (44%)
- Overlap: 89%

Hypocotyl
- 6398 (25%)  5586 (21%)
- Overlap: 83%

Root
- 11486 (43%)  11142 (44%)
- Overlap: 93%

All 3 organs express ~5000 common genes in darkness and light
## Organ-specific Light Regulation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Percentage</th>
<th>Value Before Light</th>
<th>Value After Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon</td>
<td>30%</td>
<td>1593</td>
<td>997</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>36%</td>
<td>1197</td>
<td>1097</td>
</tr>
<tr>
<td>Root</td>
<td>12%</td>
<td>848</td>
<td>567</td>
</tr>
</tbody>
</table>
Only small overlaps in light regulated genes in three organs

Genes highly expressed in the light

Genes highly expressed in darkness
Light Regulated Transcription Network and Cascades

1. Focus on Blue and Far red light effect on dark-grown seedlings
   Whole genome array will be used to define kinetic pattern of the genome expression changes in wild type and null mutants of key transcription factors

2. Define dynamic genome binding sites of key transcription factors
   The genomic binding sites at key time points after blue or far red light treatments will be revealed using chip-chip assay
Arabidopsis ChIP-Chip

- Arabidopsis 3.8K promoter array. In collaboration with professor Qu of Peking University, promoter fragments from selected 3.8K genes have been amplified to produce a promoter microarray.

- Arabidopsis whole genome tiling chip is available through collaboration.