Lecture 16: Plant hormone signal mechanisms

1. Ethylene
2. brassinosteroid
## Five classical plant hormones

<table>
<thead>
<tr>
<th>AUXIN</th>
<th>GIBBERELLIN*</th>
<th>CYTOKININ</th>
<th>ETHYLENE</th>
<th>ABSCISIC ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td>NATURALLY-OCCURRING</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Indoleacetic Acid (IAA)" /></td>
<td><img src="image" alt="GA₃ (Gibberellic Acid)" /></td>
<td><img src="image" alt="Zeatin" /></td>
<td><img src="image" alt="Ethylene" /></td>
<td><img src="image" alt="Abscisic Acid" /></td>
</tr>
<tr>
<td>SYNTHETIC</td>
<td></td>
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</tr>
<tr>
<td><img src="image" alt="Indolebutyric Acid (IBA)" /></td>
<td><img src="image" alt="a-Naphthaleneacetic Acid (NAA)" /></td>
<td><img src="image" alt="6-Furfurylmino Purine (kinetin)" /></td>
<td><img src="image" alt="6-Benzylamino Purine (BA)" /></td>
<td><img src="image" alt="Abscisic Acid" /></td>
</tr>
</tbody>
</table>

*There are 52 or more forms of gibberellin, all of which slightly differ only in structure from the GA₃ shown here.*
Brassinolide is a recently rediscovered hormone
$C_2H_4$ leads to:

**Responses to Ethylene**

- Fruit ripening
- Senescence of leaves, flowers
- Abscission of leaves, flowers, fruits
- Altered geotropism in roots and stems
- Promotion of seed germination
- Inhibition/promotion of cell division and cell elongation
- Induction of phytoalexins and other disease resistance factors
- Initiation of roots
- Inhibition/promotion of flowering
- Epinasty of leaves
- Bud dormancy release
- Sex shifts in flowers
Ethylene Biosynthesis

Methionine (Yang) cycle

5-Methylthioribose 1-phosphate

5-Methylthioribose

Methylthioribose

MTR kinase

ATP

ADP

Ethylene

N-Malonyl-ACC

ACC N-malonyltransferase

ACC oxidase

1-Aminocyclopropane-1-carboxylic acid (ACC)

$\frac{1}{2}O_2 \rightarrow CO_2 + HCN + H_2O$
Triple responses to ethylene

- inhibition of stem elongation
- radial swelling of the stem
- apical hook formation
Two types of mutants in ethylene mutants

Type 1: constitutive response
eto1 (ethylene over-producer)
ctr1 (constitutive ethylene response)

Type 2: ethylene insensitive
etr1 (ethylene resistance)
ein2, 3, 4, 5 (ethylene insensitive)

eto1: phenotype can be blocked by ethylene synthesis inhibitors
ctr1: phenotype is unaffected by ethylene synthesis inhibitors
Epistasis pathway established by double mutant analyses

- **ctr1**: constitutive responses
- **eto1**: constitutive responses:
- **etr1**: ethylene insensitive
- **ein2**: ethylene insensitive
- **etr1 ctr1**: constitutive response
- **ein2 ctr1**: ethylene insensitive
- **eto1 etr1**: ethylene insensitive
- **eto1 ein2**: ethylene insensitive
Two component system

Sensor

Response regulator

Signal → Input domain → Histidine kinase

P

Output signal
Arabidopsis ethylene receptor family

Subfamily 1

<table>
<thead>
<tr>
<th>ETR1</th>
<th>ERS1</th>
<th>Ethylene binding</th>
<th>GAF</th>
<th>Histidine kinase</th>
<th>Receiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>78%</td>
<td>64%</td>
<td>64%</td>
<td></td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>44-54%</td>
<td>38-41%</td>
<td>16-29%</td>
<td>32%</td>
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</tbody>
</table>

Subfamily 2

<table>
<thead>
<tr>
<th>EIN4</th>
<th>ETR2</th>
<th>ERS2</th>
<th>Histidine kinase</th>
<th>Receiver</th>
</tr>
</thead>
<tbody>
<tr>
<td>58%</td>
<td>54%</td>
<td>55%</td>
<td>38%</td>
<td>53%</td>
</tr>
<tr>
<td>52%</td>
<td>40%</td>
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</tbody>
</table>

Histidine kinase
GAF
Ethylene binding
Receiver
-*etr1* is a dominant mutation
- loss of *etr* and its redundant receptors gives rise to constitutive phenotypes

A. No ethylene

B. Yes ethylene
Ethylene

ETR1  ERS1  ETR2  EIN4  ERS2

Cytoplasm

CTR1 (MAPKK?) (MAPK?)

EIN2

Nucleus

Transcription Factors

EIN3, EIL1, EIL2

ERF1 (other EREBPs?)

Ethylene-response gene induction
Figure 18.22, WT: wild-type.
14079 (TR): transgenic plant expressing ETR1-1
Brassinosteroid (BR)

(A) BR biosynthetic mutants

DET2 encodes steroid 5a reductase

det2 is defective in biosynthesis of BR
det2 phenotype can be rescued by BR

BR-defective mutants: dark green (light grown), small cells, reduced apical dominance.
reduced male fertility. dark grown plants have similar phenotype to cop/det/fus, a role in photomorphogenesis
(B) BR signaling components

(I) BRI1 (BR receptor):
Receptor like (serine/threonine) kinase (RLK)
leucine-rich repeat (LRR) extracellular domains
localizes to plasma membrane (thus differs from animals)
(II) BIN2: member of a 10-member family glycogen synthase kinase

bin2: semidominant
phenotype similar to bri (BR receptor mutation)
negative regulator of BR signaling

(III) BEZ1 and BZR1: highly similar in protein seq (89% identity)
6 member gene family
nuclear protein (upon BR treatment)
no DNA-binding domain
novel protein

Bez1 and bzc1: dominant mutant
constitutive response phenotype
A model of BR signaling pathway
How do you distinguish mutants defective in BR synthesis (det2) from mutants defective in BR perception (bri) and signal transduction (bin2-D)?
Lecture 17: Plant hormone signaling mechanism II

GA, Auxin, cytokinin, and ABA

**Gibberellic Acid**
- floral induction
- germination
- hypocotyl elongation
- leaf expansion
- apical dominance
- floral development
- fruit maturation
- internode elongation
GA biosynthetic mutants

GA1: encodes for ent-CDP synthase

gal-3: loss-of-function mutant of GA1
deficient for GA,
do not germinate,
short and dark green leaves
dwarf
reduced apical dominance
can be rescued by GA application
GA signaling mutants

(I) RGA, RGL1, GAI all encode GRAS (GAI RGA Scarecrow) transcription factor and serve as negative regulators of GA responses

gai: **dominant allele**
mimics the effect of GA-deficiency
harbors high levels of GA
can’t be rescued by GA application, defective in GA signaling

lose-of function allele

tall plants
insensitive to GA biosynthesis inhibitor paclobutrazol
Epistasis with GA biosynthetic mutants

\[ \text{GA1} \quad \text{RGA} \quad \text{GAI} \quad \text{GA response} \]
RGA-GFP localization
(II) SPINDLY (SPY)

Loss-of-SPY exhibits constitutive GA response

Encode OGT, which modifies other proteins in a manner similar to phosphorylation. Ie. SPY may add an O-GlcNAc moiety to other proteins to regulate their activities.
GA functions as an inhibitor of inhibitors

GA deficient cell

WT cells

Proposed GA receptor, not yet found

active form
Auxin: the growth hormone, required for viability

- phototropism
- gravitropism
- germination
- delay leaf abscission
- promote cell elongation
- promote cell division
- promote formation of lateral & adventitious roots
- required for apical dominance
- vascular differentiation

Polar transport of auxin: synthesized in shoot tip
Auxin signaling mechanism

ABP1: auxin binding protein 1 = Auxin receptor?

knockout mutants are lethal
Genes identified in auxin signaling pathway

**ARF: auxin-response factors**
- 23 member family in Arabidopsis
- has DNA binding domains
- can be either activators or repressors

**AUX/IAA: 29 member in Arabidopsis**
- Has protein binding domain
- heterodimerize with ARFs
- rapid auxin-dependent turn-over of AUX/IAA
- is dependent on ubiquitin-proteasome-pathway
Auxin signaling pathway

IAA  IAA  IAA
    ↓    ↓    ↓
     IAA

Degradation via SCF$^{TIR1}$ Complex

AUX/IAA  ARFs

AuxRE

TGTCTC

Auxin-response gene

1. AUX/IAA
2. SAUR
3. GH3
4. GST (glutathion-S-transferase)
5. ACC synthase
ABA: Seed maturation and anti-stress signal

Initiation and maintenance of seed and bud dormancy acquire desiccation tolerance by inducing LEA promote seed storage proteins prevent preharvest sprouting (vivipary)
Response to stress (water stress) close stomata in response to water stress
No ABA receptor has been identified

In Stomatal guard cells:

ABA binding to receptor

\[ \text{ROS (Reactive Oxygen Species)} \]

Increased cytosolic \( \text{Ca}^{++} \)

\( \text{Ca}^{++} \) influx through Plasma-membrane

\( \text{Ca}^{++} \) release from vacuoles

Increase cellular PH (7.67 to 7.94)

Membrane depolarization

\[ \text{Stomata closing} \]
SCHEMATIC MODEL OF ABA Pathway

ABA

↓

IP3 & other second messengers

ABI1 | P
ABI2

↓

Tanscription factor: ABI3, ABI4, and ABI5

↓

STRESS RESPONSES (RD29A expression)

ABI1 and ABI2 encode phosphatase
Cytokinin: regulators of cell division

Cytokinin-over producer:

Shoot apical meristem produce more leaves
Leaves have higher chlorophyll levels & greener
Adventitious shoot may form from unwounded leaf
Leaf senescence is retarded
Apical dominance is reduced
Root growth rate is reduced

Morphogenesis in cultured tissues:

High auxin:cytokinin ratio: root
Low auxin/cytokinin ratio: shoot
Intermediate levels: callus
Agrobacteria causes crown-gall

tms: auxin synthesis (tms)
tmr: cytokinin synthesis

LB, RB: 25 bp repeat, left and right border

tms: tumor morphology shoot

tmr: tumor morphology root

ori: origin of replication

vir: virulence region

Ti-plasmid
Receptor:
CKI1: (over-producers that confer cytokinin-independent growth)
CRE1: (mutants failed to produce shoots in response to cytokinin)
AHK2
AHK3

All encode two component signaling system

Sensor

Response regulator
ARR: Arabidopsis Response Regulator
AHP: Arabidopsis Histidine Phosphotransfer