Hormone signaling
Ethylene and GA

“BIG PICTURE”:
Historical perspective
Practical applications
Experimental approaches
Key insights
Themes

Caren Chang
Feb. 28, 2007

Ethylene Responses

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

“One bad apple spoils the whole barrel…”

Apple slices induce ripening of persimmons

8 days in bag with apple slices
Controls, kept 8 days outside of bag

Wounding induces ethylene production
Ethylene causes senescence

Can block ethylene receptors with silver thiosulfate
Practical importance

You need Ethylene Control!

Practical Importance

Fresh Produce - A Matter of Science

With a Sachet

Without a Sachet

These two opened apples were held in a home refrigerator for 3 months.

Ethylene Control puts you in control

Historical background

- Ethylene used unwittingly throughout history

Gashing promotes ripening in figs (4 days later)

Wood-burning fires promote synchronous flowering in pineapple

Historical background

- In the late 19th-early 20th century, illuminating gas was found to cause detrimental effects on plants (premature senescence and defoliation)

Historical background

- 1901 Neljubov - discovered that ethylene is the biologically active agent in illuminating gas

Ethylene Biosynthesis Pathway - 1960s

- 1934 Gane - discovered that plants produce ethylene
**Ethylene Biosynthesis Pathway - 1960s**

**Ethylene Biosynthesis:**

- ACC Synthase gene family

**Ethylene**

**Biosynthesis:**

- Regulation of ACC Synthase proteins

**Ethylene signaling:**

- the basic problem

**Arabidopsis in the 1980’s**

- The life cycle is short—about 6 weeks from germination to seed maturation.
- Seed production is prolific and the plant is easily cultivated in restricted space.
- Self-fertilizing, but can also be out-crossed by hand.
- Small compact genome with a low amount of repeat sequences

**1988 - Genetic map of molecular markers provided a link to DNA**

*A genetic map of molecular markers on the chromosome allows one to clone any gene for which there is a mutant phenotype*
Chromosome walking (prior to map-based cloning today)

Ethylene signaling: the basic problem

Proteins
Genes

Physiology

SIGNAL → ? → RESPONSE

Arabidopsis “triple response” - grad student Tony Bleecker

Genetic screen: to dissect the ethylene signaling pathway at the molecular level

Ethylene Responses in Arabidopsis

Ethylene-Response Mutants in Arabidopsis

Ethylene-insensitive mutants
etr1 etr2 ein4 (dominant)
ein2 ein3 ein5 (recessive)
ein6 ein7 “Positive Regulators”

Constitutive-response mutants
ctr1 (recessive)
eto1 “Negative Regulators”
Genetic EPISTASIS to order the genes in the pathway

```
etr1 + ctr1 = etr1 ctr1
```

**ethylene**

```
ETR1 → CTR1 → responses
```

Ethylene-Response Mutants in *Arabidopsis*

- Ethylene-insensitive mutants
  - \(etr1\) \(etr2\) \(ein4\) (dominant)
  - \(ein2\) \(ein3\) \(ein5\) (recessive)
  - \(ein6\) \(ein7\)
- “Positive Regulators”

- Constitutive-response mutants
  - \(ctr1\) (recessive)
  - \(eto1\)
- “Negative Regulators”

```
C_2H_4 → ETR1 ERS1 ETR2 EIN4 ERS2 (insensitive - dominant) → CTR1 (constitutive) → EIN2 EIN3 EIN5 EIN6 EIN7 (insensitive - recessive) → responses to ethylene
```

**Arabidopsis ethylene receptor family**

<table>
<thead>
<tr>
<th>Subfamily 1</th>
<th>ETR1</th>
<th>ERS1</th>
<th>ETR2</th>
<th>EIN4</th>
<th>EIN5</th>
<th>EIN6</th>
<th>EIN7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylene binding</td>
<td>78%</td>
<td>64%</td>
<td>64%</td>
<td>38-41%</td>
<td>16-29%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Subfamily 2</td>
<td>EIN4</td>
<td>EIN5</td>
<td>EIN6</td>
<td>EIN7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETR2</td>
<td>59%</td>
<td>54%</td>
<td>59%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERS2</td>
<td>55%</td>
<td>55%</td>
<td>55%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How do the receptors function?

- GENETICS
- Protein-Protein interactions
- Biochemical analyses
What is basis for dominant mutant?

To answer this: Examine a null mutant to understand ethylene receptor action (positive or negative regulator?)

Ethylene-Response Mutants in Arabidopsis

Ethylene-insensitive mutants

\[ \text{etr1 etr2 ein4 (dominant)} \]
\[ \text{ein2 ein3 ein5 (recessive)} \]
\[ \text{ein6 ein7} \]

“Positive Regulators”

Constitutive-response mutants

\[ \text{ctr1 (recessive)} \]
\[ \text{eto1} \]

“Negative Regulators”

The Ethylene Receptors Are Negative Regulators of Ethylene Responses

Receptor action

Ethylene response

Receptor action

Ethylene response

Ethylene-Binding Domain
Expressing Arabidopsis etr1-1 gene in tomato and petunia

EBF1 and EBF2 discovered from microarrays and protein-protein interactions

What have we learned?

- Pathway consists of a unique combination of signaling components
- Negative regulation!
- Post-translational regulatory mechanisms important
- Interactions with other signals (sharing of components)
- Pathway components are conserved with other plant species
**Future directions in ethylene signaling**

- Continue elucidating regulatory mechanisms and new components
- Mechanistic insights into how the pathway components signal to each other
- Understanding complex networks of crosstalk with other signaling pathways
- Understanding divergence of responses at the tail end of the pathway
- Does signaling always involve changes in gene expression?

**Genetic dissection summary**

- Need to identify a phenotype that is specific to the process you are interested in studying
  - Sometimes limitations to a genetic screen
    - Specificity of the phenotype
    - Gene redundancy
- Design appropriate screen for mutant
- Genetic analysis (e.g., epistasis)
- Isolate gene by map-based cloning
- Investigate at cell biological and biochemical level

**Current status of Arabidopsis as a model system**

- Complete 125 Mb genome has been sequenced and annotated.
- Extensive genetic and physical maps of all 5 chromosomes are available.
- Transformation is simple using a dipping method with Agrobacterium tumefaciens.
- Mutant collections available for nearly every gene
- A large number of genomic resources, including databases for gene expression analyses, multinational projects, etc.
- Useful “reference plant” for other plant species

**Effects of Gibberellic Acid**

- Increasing growth
- Overcoming seed dormancy
- Inducing flowering
- Increasing fruit set
- Frost protection
- Inhibition of root formation

**A Model of GA Signaling in Rice**


**Future research in GA signaling**

- Identifying proteins that interact with GID1 and DELLA/SLR
- Identifying regulators of DELLA/SLR
- Finding gene targets of DELLA/SLR
- Elucidating crosstalk with ethylene and auxin, which both delay DELLA degradation
Some comparisons of GA and ethylene pathway research

- Receptor gene mutations
  - Ethylene receptor genes identified through dominant gain-of-function mutations
  - GA receptor gene identified by loss-of-function mutant in rice
- Negative regulators
  - Post-translational regulation by proteosome pathway
    - Degradation of transcriptional activator in absence of ethylene signal
    - Degradation of transcriptional repressor in presence of GA signal

Some themes in Hormone Response

- Protein turn-over an important regulatory mechanism
- Negative regulation is highly prevalent in signaling
- Negative and positive feedback mechanisms exist
- Gene families may provide enhanced sensitivity and/or diversity
- Pathways are conserved among plant species