Cytokinin

Abundant in young, dividing cells
Shoot apical meristem
Root apical meristem

Synthesized in root tip, developing embryos, young leaves, fruits
Transported passively via xylem into shoots from roots.

Cytokinin effects and the mode of action

Effects of cytokinin:
1. Stimulate Cell division
2. Promote greening, chloroplast development
3. Controls shoot formation
4. Promote nutrient movement to ‘sink’
5. Delay senescence

Cytokinin is localized at the root tip.
Cytokinin-induced gene (ARR5) expression is localized to the root tip.
In situ hybridization
ARR5 mRNA

21-22. Cytokinin promote movement of nutrients
Nutrients accumulate in cytokinin treated tissue.
Aminoisobutyric acid = Non-hydrolyzable amino acid

F 21-23. Cytokinin stimulates chloroplast development in the dark
No cytokinin + cytokinin
etiolast

Fig. 21-9. Cytokinin needed for growth of shoot apical meristem

Transgenic plant with less cytokinin.
OE cytokinin oxidase CKX1.

Wt OE
21.25 Leaf senescence is retarded in transgenic lettuce plants expressing ipt

- Ipt, cytokinin synthesis enzyme.
- Transgenic plants expressing SAG12 promoter::ipt

Searching for a cytokinin receptor

Figure 1 Callus growth of the cytokinin-resistant mutant cre1-1 in different auxin and cytokinin concentrations. From Nature 2001 Feb. Inoue et al. Kakimoto

3 related cytokinin receptors in Arabidopsis:
CRE1, AHK2, AHK3

21.27 Phenotypes of Arabidopsis plants harboring mutations in cytokinin receptors

Wild type
Columbia Ws
ahk2 cre1
ahk2 cre1
ahk2 cre1
ahk2 cre1
ahk2 cre1

Evidence CRE1 is a cytokinin receptor

- Yeast His Kinase mutant is rescued by AtCRE1 in a cytokinin-dep manner.
- Cytokinin binds to CRE1 expressed in yeast membrane
- cre1 mutant plant is insensitive to CK.
- cre1 mutant protein cannot bind to CK.
- In vitro CRE1 can phosphorylate AHP if CK is present.
- AHP can then phosphorylate B-type ARR transcription factors.
- B-type ARR then activate transcription of cytokinin primary response genes.
- All 3 receptors have overlapping, distinct, roles in cytokinin signaling

21.26 Simple versus phosphorelay types of two-component signaling systems

Simple two-component signaling system
Input Transmitter Sensor histidine kinase Receiver Output Response regulator Activation of transcription

Phosphorelay two-component signaling system
Hybrid sensor histidine kinase Hpt (AMP) Response regulator (ARR) Activation of transcription

21.29 Induction of some type-A ARR genes in response to cytokinin

<table>
<thead>
<tr>
<th>Probe</th>
<th>Time following cytokinin treatment (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ARR5</td>
<td>ARR5</td>
</tr>
</tbody>
</table>

ARR are early response genes.
21.30 Cytokinin induces the transient movement of some AHP proteins into the nucleus

ABA: stress hormone

1. ABA increases when plant is water stressed.

ABA is synthesized from carotenoids in chloroplast/plastids. ABA is transported in vascular tissues.

2. [ABA] levels decreases upon watering.
   ABA is degraded
   ABA is exported
   Synthesis is decreased.

Summary

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21-31 cytokinin signaling

1. Receptor senses CK and -p itself
2. Signal transduction
3. Response: gene expressed

Cytokinin signaling

1. Cytokinin binds R
2. R is phosphorylated
3. Active R then phosphorylates AHP1/2,
4. AHP1/2 enters nucleus
5. AHP1/2 phosphorylates RD
6. RD-p activates other genes ARR4-7
   Activate cell division etc.
Hwang & Sheen Sept. 2001 Nature

23-4. Taiz. ABA increases due to water stress
Physiological effects

*ABA* regulates seed maturation
*ABA* inhibits premature germination.
*ABA* promotes seed storage reserve accumulation and desiccation tolerance.
*ABA* maintains mature embryo in dormant state.

Water stress is sensed first in the root

*ABA* is synthesized in root
*ABA* moves up via xylem to the leaf

Guard cells are in the leaf.

Signal transduction

*How? Two modes of action:*

a. Guard cell: *ABA* causes increase in Ca influx, close stomates

b. *ABA* changes gene expression of TF. Induces seed dormancy

*ABA* inhibits blue-light stimulated H+ pumping

Model of *ABA* & stomata closure
Box 1 Components and pathways of guard cell ABA signalling

1. ABA is detected by as yet unidentified receptors (right guard cell) and induces cytosolic Ca2+ elevations (1) through extracellular Ca2+ influx and release from intracellular stores (for reviews see refs 2, 8, 9). (2) Ca2+-cyt elevations activate two types of anion channel that mediate anion release from guard cells: slow-activating sustained (S-type) or rapid transient (T-type) anion channels3, 8. (3) Anion efflux causes depolarization, which activates outward-rectifying K+ (K+out) channels and results in K+ efflux from guard cells2, 3. (4) ABA causes an alkalization of the guard cell cytosol, which enhances K+out channel activity4, 44. Overall, the long-term efflux of both anions and K+ from guard cells contributes to the loss of guard cell turgor, leading to stomatal closing. Over 90% of the ions released from the cell during stomatal closing must be first released from vacuoles into the cytosol. (5) At the vacuole, Ca2+-cyt elevation activates vacuolar K+ (VK) channels, which are thought to mediate Ca2+-induced K+ release from the vacuole. In addition, fast vacuolar (FV) channels can mediate K+ efflux from guard cell vacuoles at resting Ca2+-cyt45. ABA also inhibits ion uptake, which is required for stomatal opening (left guard cell). (6) Ca2+-cyt elevations inhibit the electrogenic plasma membrane proton-extruding H+-ATPases46 and K+ uptake (K+in) channels2, 3, 44. Initiation of ion efflux (1–5, right guard cell) and inhibition of stomatal opening processes (6, 7, left guard cell) provide a mechanistic basis for ABA-induced stomatal closing.


ABA regulates gene expression of Transcription Factors

a. Seed dormancy
b. Water stress

From Lovegrove & Hooley 2000 TiPS

ABA induced seed dormancy and inhibit GA action

From Lovegrove & Hooley 2000 TiPS

Summary & Questions
Ethylene synthesis is stimulated by:

- Fruit ripening
- Flower Senescence
- Stress: drought, chilling, mechanical, Wounding e.g. from Elicitor/pathogen

\[ H_2C = CH_2 \]

22-1 Taiz
Ethylene Synthesis

Met $\rightarrow$ SAM $\rightarrow$ ACC synthase $\rightarrow$ ACC $\rightarrow$ ACC oxidase $\rightarrow$ ethylene

22-5. Ethylene rise triggers fruit (banana) ripening

- CO$_2$ production (mg kg$^{-1}$ h$^{-1}$)
- Ethylene concentration (ppm)

Biochemical changes of fruit ripening

1. Tissue softening
2. Fruit becomes sweet
3. Brightly colored anthocyanins & carotenoids accumulate
4. Aroma increase
5. Respiratory rise e.g. banana, tomato

Control ripening with biotechnology

Reversible Inhibition of Tomato Fruit Senescence by Antisense RNA

Paul W. Qiller, Lu Ming-Wong, Lorenz P. Taylor, Deborah A. Free, A. Thiemann Theobald

Ethylene controls fruit ripening. Expression of antisense RNA to the rate-limiting enzyme in the biosynthetic pathway of ethylene, 1-aminoacycpropane-1-carboxylate oxidase, decreases ethylene production and delays ripening. Slowing fruit ripening by inhibiting ethylene synthesis.

Wt Antisense
ACC synthase

Slowling fruit ripening by inhibiting ethylene synthesis.
Science 1991

22-8. Role of auxin and ethylene in leaf abscission
Evidence ETR1 is a receptor

- Mutant insensitive to ethylene
- ETR1 protein expressed in yeast bind C₂H₄
- Etr1-1 mutants cannot bind C₂H₄ et
- 4 related proteins also act as C₂H₄ receptors. Mutants of 4 genes are C₂H₄ insensitive.
Questions

• Ripening- is caused by what biochemical changes.
• Abscission – ? Possible target genes?

Summary of Hormones

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Effects</th>
<th>How?</th>
<th>How?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auxin</td>
<td>Stimulate cell elongation, Phototropism, Gravitropism, Inhibit root growth</td>
<td>PM H+-ATPase</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>Stimulate stem elongation, Promote germination, Induce expression of enzymes to degrade stored food for seed germination</td>
<td>a-amylase</td>
<td></td>
</tr>
<tr>
<td>Cytokinin</td>
<td>Induce cell division, Delay senescence, Initiate chloroplast development</td>
<td>Regulates expression of Tc factors and genes needed for cell division or greening</td>
<td></td>
</tr>
</tbody>
</table>

Summary of ABA & Ethylene

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<tr>
<th>Hormone</th>
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</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td>Seed development, Stomatal closure due to regulation of ion transporters, Regulates gene expression</td>
<td>Ethylene Fruit ripening, Leaf abscission, Regulates gene expression of enzymes for ripening, Cellulase Polygal’ase</td>
</tr>
</tbody>
</table>