# Cytokinin

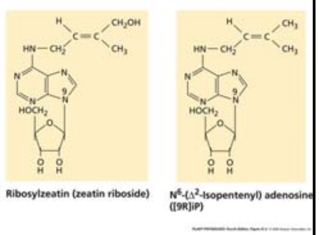
Abundant in young, dividing cells

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

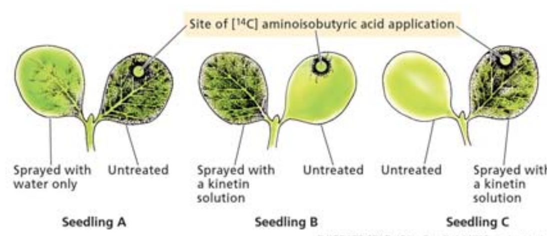
**Cytokinin is localized at the root tip.**

Cytokinin-induced gene *(ARR5) expression* is localized to the root tip.

*In situ hybridization*

*ARR5 mRNA*

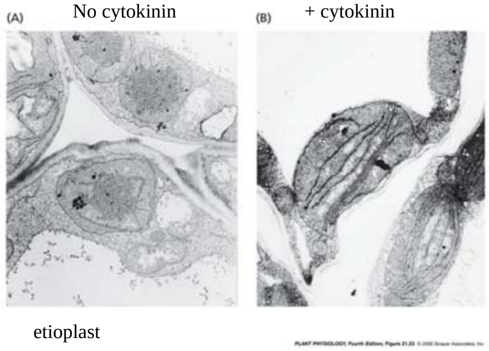
*21- 22. Cytokinin promote movement of nutrients*




*Nutrients accumulate in cytokinin treated tissue.*
*Aminoisobutyric acid = Non-hydrolyzable amino acid*

F 21-23. Cytokinin stimulates chloroplast development in the dark

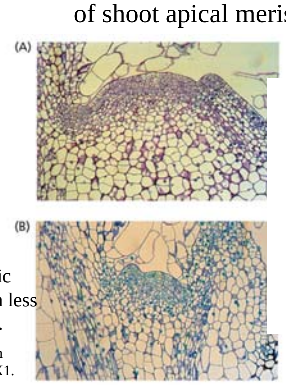
(A) No cytokinin (B) + cytokinin



etioplast

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

Wt



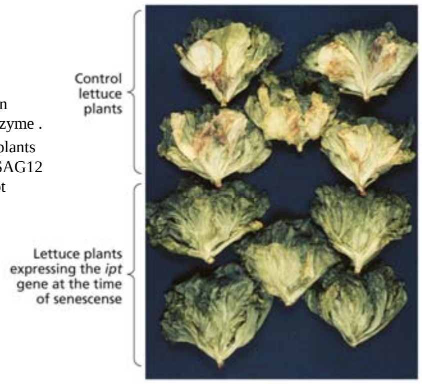

Transgenic plant with less cytokinin.

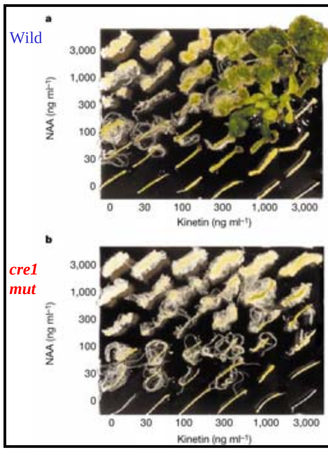
OE cytokinin oxidase CKX1.

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:*

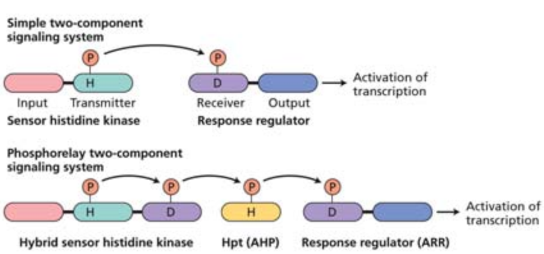
*CRE1, AHK2, AHK3*

21.27 Phenotypes of Arabidopsis plants harboring mutations in cytokinin receptors

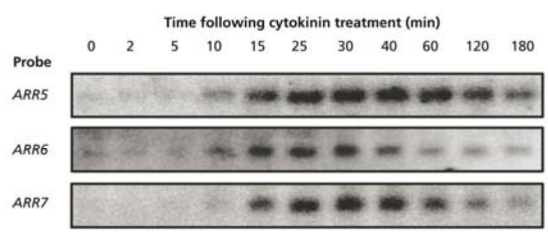

## Evidence CRE1 is a cytokinin receptor

- 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

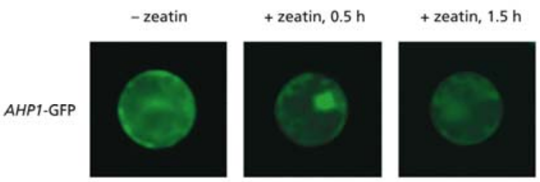



## 21.29 Induction of some type-A *ARR* genes in response to cytokinin



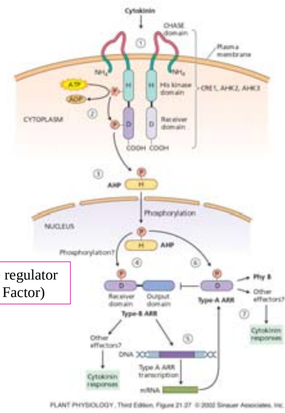

*ARR are early response genes.*

21.30 Cytokinin induces the transient movement of some AHP proteins into the nucleus




*cytosol* *nucleus*

21-31 cytokinin signaling

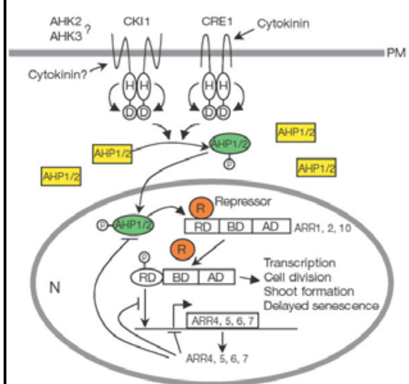



~p of response regulator (Transcription Factor)

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

# Summary

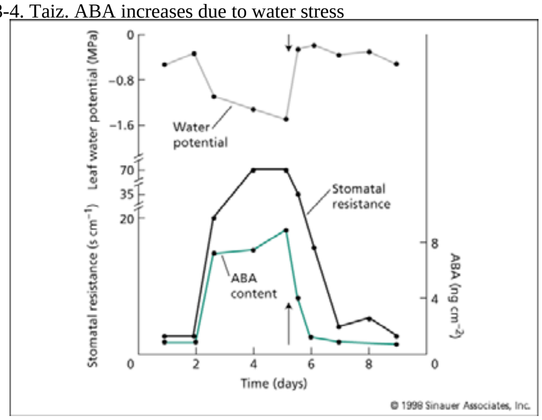
# ABA: stress hormone

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.*

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 dessication tolerance.*

*ABA maintains mature embryo in dormant state.*

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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.

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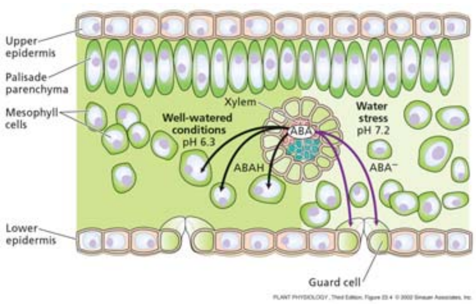


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## Signal transduction

*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*

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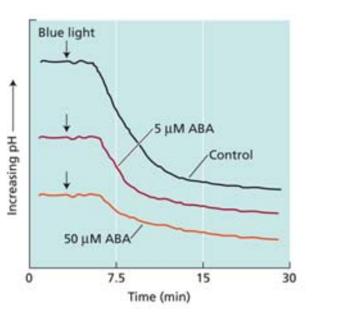
ABA inhibits blue-light stimulated H+ pumping




*acid*

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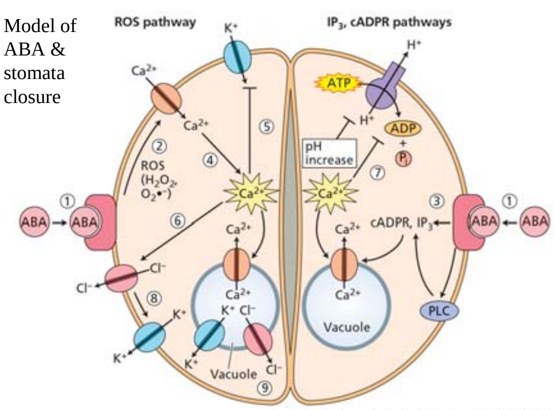
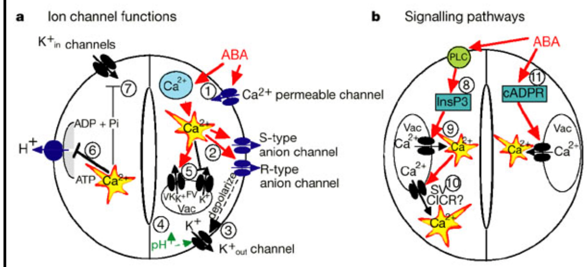
Model of ABA & stomata closure



Box 1 Components and pathways of guard cell ABA signalling
a, 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 (R-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 activity44. Overall, the long-term efflux of both anions and K+ from guard cells contributes to the loss of guard cell turgor, leading to stomatal closing3. 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 vacuole8. 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 (7). 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 closing8.
From Schroeder and Allen 2001. Nature.

the long-term efflux of both anions and K+ from guard cells contributes to the loss of guard cell turgor, leading to stomatal closing.

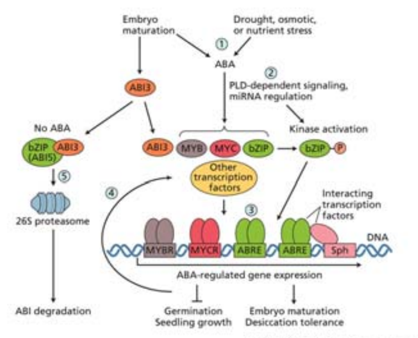



From Schroeder and Allen. 2001.
Nature

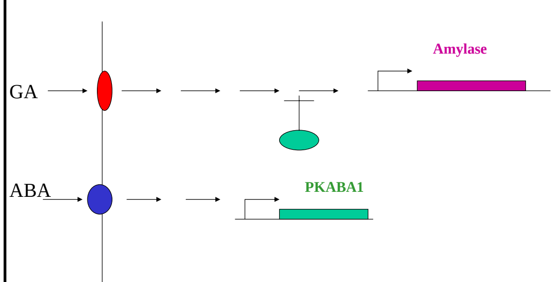
ABA regulates gene expression of Transcription Factors

a. Seed dormancy
b. Water stress

## ABA regulated gene expression




ABA induce seed dormancy and inhibit GA action




From Lovegrove & Hooley 2000 TiPS

## Summary & Questions

# Ethylene

Ethylene synthesis is stimulated by:

$H_2C = CH_2$

*Fruit ripening*

*Flower Senescence*

*Stress: drought, chilling, mechanical,*
*Wounding e.g. from Elicitor/pathogen*

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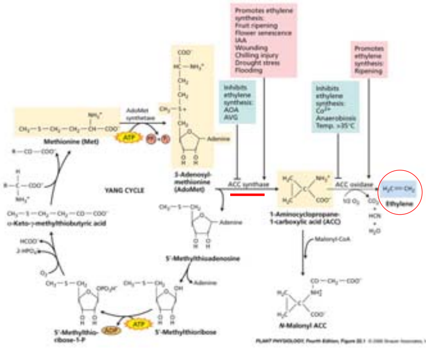
22-1 Taiz

Ethylene Synthesis




*Met --> SAM ---[ACC synthase]-->ACC –[ACC oxidase]--> ethylene*

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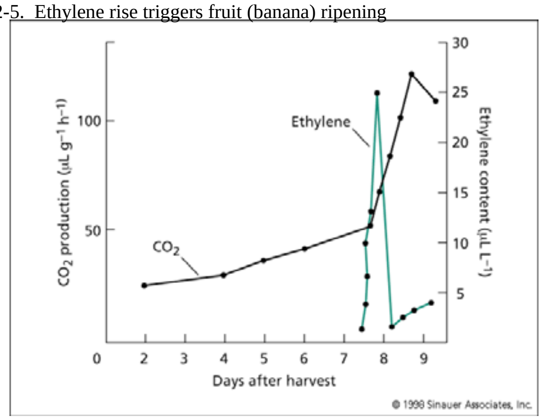
22-5. Ethylene rise triggers fruit (banana) ripening

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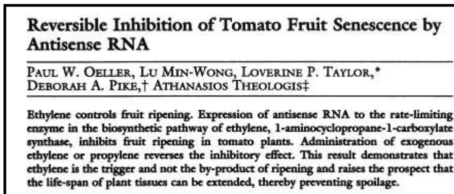

**Reversible Inhibition of Tomato Fruit Senescence by Antisense RNA**

PAUL W. OELLER, LU MIN-WONG, LOVERINE P. TAYLOR,* DEBORAH A. PIKE,† ATHANASIOS THEOLOGIS‡

**Ethylene controls fruit ripening. Expression of antisense RNA to the rate-limiting enzyme in the biosynthetic pathway of ethylene, 1-aminocyclopropane-1-carboxylate synthase, inhibits fruit ripening in tomato plants. Administration of exogenous ethylene or propylene reverses the inhibitory effect. This result demonstrates that ethylene is the trigger and not the by-product of ripening and raises the prospect that the life-span of plant tissues can be extended, thereby preventing spoilage.**

Wt

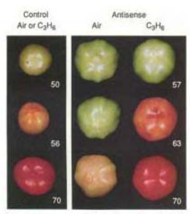


**Fig. 3.** Phenotype of the fruits used in Fig. 2A. The numbers indicate the age of the fruits in days.

Antisense ACC synthase

Slowing fruit ripening by inhibiting ethylene synthesis.
Science 1991

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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*

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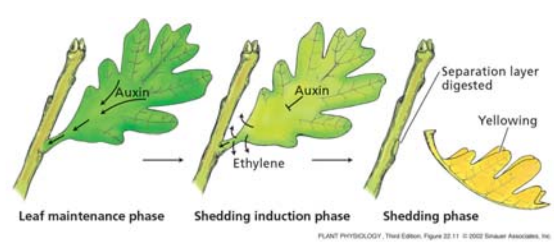
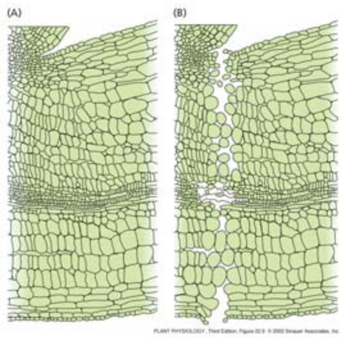
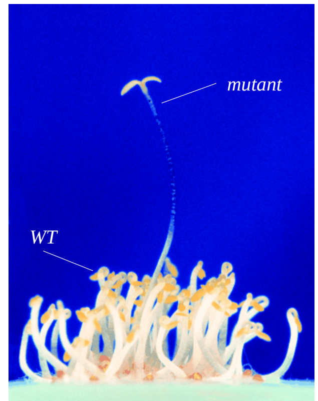
22-8. Role of auxin and ethylene in leaf abscission



22-9 Abscission zone: cell walls breakdown in 2-3 rows of cells.




*C2H4 on Wt vs etr1-1*




Receptor of ethylene has been identified.

22-12 Taiz. **Ethylene resistant mutant (etr1).** Seedlings WT germinated in ethylene show triple response:

-curved apical hook
-inhibition of hypocotyl
-horizontal growth

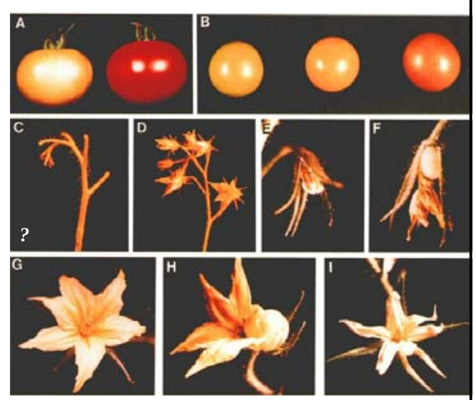
(From T. Bleecker)

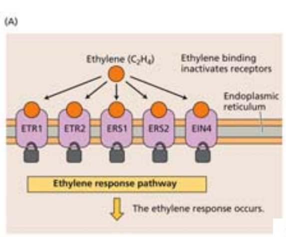
## Evidence ETR1 is a receptor

- Mutant insensitive to ethylene
- ETR1 protein expressed in yeast bind $C_2H_4$.
- Etr1-1 mutants cannot bind $C_2H_4$ et
- 4 related proteins also act as $C_2H_4$ receptors. Mutants of 4 genes are $C_2H_4$ insensitive.

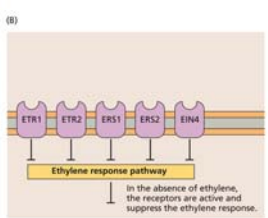
Mut Wt *NR/NR mut* -/+ +/+

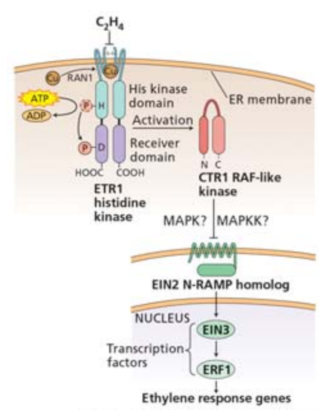
- **Tomato**: Never-ripe has a mutation in tomato ETR1.
- C-D. Flower Ethylene treated.
- G, H. flower did not senescence
- Lanahan et al Klee 1994. Plant Cell




Ethylene receptors (empty) are negative regulators. Ethylene binding shuts off the inactivation.




Cellulase

Abscission

Ripening

22-16

## Questions

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

## Summary of Hormones

| | | | |
|---|---|---|---|
| **Auxin** | • Stimulate cell elongation<br>• Phototropism<br>• Gravitropism<br>• Inhibit root growth | How?<br>• Stimulate acid secretion;<br>• Stimulate auxin-response gene expression | PM H+-ATPase |
| **GA** | • Stimulate stem elongation<br>• Promote germination | Induce expression of enzymes to degrade stored food for seed germination | a-amylase |
| **Cytokinin** | • Induce cell division<br>• Delay senescence<br>• Initiate chloroplast development | Regulates expression of Tx factors and genes needed for cell division or greening | |

## Summary of ABA & Ethylene

| | | | |
|---|---|---|---|
| **ABA** | Stress Hormone<br>Seed development | • Stomatal closure due to regulation of ion transporters<br>• Regulates gene expression | |
| **Ethylene** | Fruit ripening<br>Leaf abscission | Regulates gene expression of enzymes for ripening | Cellulase<br>Polygal'ase |