Lecture 12: Ethylene, an olefin

$H_2C{=}CH_2$

Discovery

Biosynthesis

Physiological effects

Signal transduction

Discovery of ethylene

During the 19th century: coal gas used for street illumination
- trees in vicinity of street lamps defoliated more extensively than other trees → coal gas and air pollutants affect plant growth and development → ethylene identified as the active component of coal gas

1901: ***Dimitry Neljubov*** identifies that the "**triple response**" is caused by ethylene
- his observation: dark-grown pea-seedlings grown in the laboratory had the following symptoms:

reduced stem/hypocotyl elongation
increased lateral root growth (swelling), reduced root elongation
abnormal, horizontal growth/exaggeration of curvature of apical hook

by growing plants in fresh air, they regained their normal morphology and rate of growth

3-day-old Arabidopsis seedlings

Discovery of ethylene

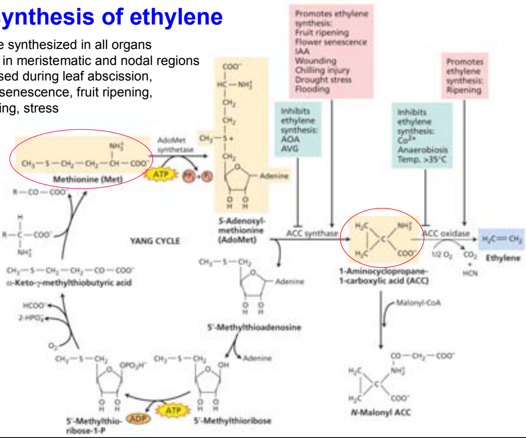
1910: **H. H. Cousins** identifies ethylene as a natural product of plant tissues
"emanations" from oranges stored in a chamber caused premature ripening of bananas when gas was passed through a chamber containing the fruit
Note: since oranges produce very little amounts of ethylene, it's likely that the oranges were infected with the fungus *Penicillium*, which produces copious amounts of ethylene

1934: **R. Gane** and others identified ethylene chemically as natural product of plant metabolism
ethylene was classified as a hormone

1959: introduction of gas chromatography → role of ethylene as plant hormone revisited

Biosynthesis of ethylene

- May be synthesized in all organs
- Mostly in meristematic and nodal regions
- Increased during leaf abscission, flower senescence, fruit ripening, wounding, stress



Properties of ethylene

MW = 28 g/mol

Flammable

Readily undergoes oxidation to ethylene oxide, C_2H_4O, which can by hydrolyzed to ethylene glycol

$C_2H_4O + H_2O \rightarrow HOCH_2CH_2OH$

Complete oxidation of ethylene

$C_2H_4 \rightarrow C_2H_4O \rightarrow HOOC\text{-}COOH \rightarrow 2\ CO_2$
Oxalic acid

Released from tissue and diffuses to gas phase through intercellular space and outside the tissue

$KMnO_4$ – an effective absorbent of ethylene
reduces concentration of ethylene in apple storage areas from 250 μL L^{-1}, extending the storage life of the fruit

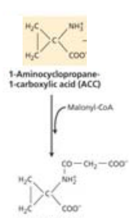
Catabolism and conjugation of ethylene

Catabolism

Metabolic breakdown products: CO_2, C_2H_4O, $HOCH_2CH_2OH$, glucose conjugate of ethylene glycol

Conjugation

- Not all ACC is converted to ethylene
- ACC conjugated to N-malonyl ACC, which does not break down and accumulates in tissue
- Control of ethylene biosynthesis



Factors affecting ethylene biosynthesis

Stimulation of ethylene biosynthesis by:

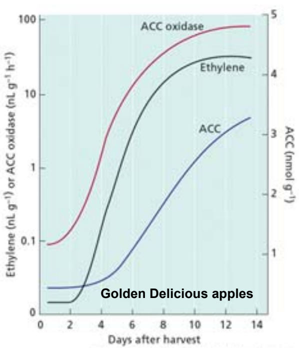
developmental state (fruit ripening)

environmental conditions (stress)

other plant hormones (auxin, cytokinin)

physical and chemical injury

Changes in ethylene and ACC content and ACC oxidase activity during fruit ripening



As fruits mature, rate of ACC and ethylene biosynthesis increases

mRNA levels and activities of ACC oxidase and ACC synthase increase

Factors affecting ethylene biosynthesis

Auxin-induced ethylene production

- auxin promotes ethylene biosynthesis by enhancing ACC synthase activity
- inhibitors of protein synthesis block both ACC and IAA-induced ethylene synthesis
- Transcription of several ACC synthase genes elevated by exogenous IAA

Cytokinins promote ethylene biosynthesis in some tissues

- e.g. application of exogenous cytokinin causes rise in ethylene production, resulting in triple-response phenotype in Arabidopsis
- Effect due to increased stability and/or activity of one isoform of ACC synthase

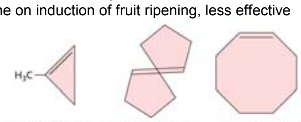
Ethylene inhibitors

Use of inhibitors useful to distinguish between different hormones that have identical effects

Example: ethylene mimics high concentrations of auxin by inhibiting stem growth and causing epinasty (downward curvature of leaves)

Inhibitors of ethylene action:

- Ag^+ applied as $AgNO_3$ or $Ag(S_2O_3)_2^{3-}$ -> inhibitor of ethylene action
- 5-10% of CO_2 inhibits effect of ethylene on induction of fruit ripening, less effective than Ag^+
- *trans*-cyclooctene (volatile): strong competitive inhibitor of ethylene binding probably to receptor
- MCP binds irreversibly to receptor



1-Methylcyclopropene (MCP) | *trans*-Cyclooctene | *cis*-Cyclooctene not active

Inhibitors of ethylene synthesis:

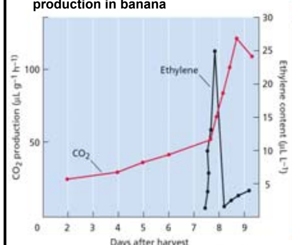
Aminoethoxy-vinylglycine (AVG)
Aminooxyacetic acid (AOA)
} block conversion of AdoMet to ACC

Ethylene promotes ripening of some fruits

Fruit ripening: tissue softening (enzymatic breakdown of cell walls)
starch hydrolysis
sugar accumulation
disappearance of organic acids and phenolic compounds (tannins)

plant: seeds are ready for dispersal

Climacteric rise in CO_2 and ethylene production in banana

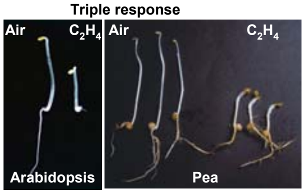


Climacteric and nonclimacteric fruits

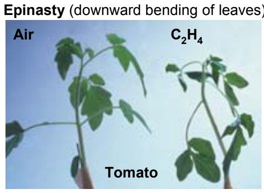
Climacteric	Nonclimacteric
Apple	Bell pepper
Avocado	Cherry
Banana	Citrus
Cantaloupe	Grape
Cherimoya	Pineapple
Fig	Snap bean
Mango	Strawberry
Olive	Watermelon
Peach	
Pear	
Persimmon	
Plum	
Tomato	

Some physiological effects of ethylene

Triple response



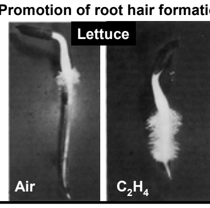
Epinasty (downward bending of leaves)



Inhibition of flower senescence

STS = Silver thiosulfate

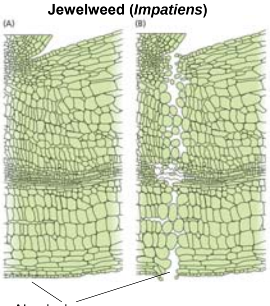
Promotion of root hair formation



Roles of ethylene and auxin during leaf abscission

Abscission = the shedding of leaves, fruits, flowers…

Jewelweed (*Impatiens*)

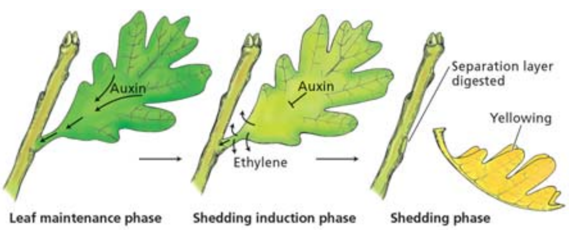


(A)
- 2 or 3 layers in abscission zone undergo cell wall breakdown

(B)
- Resulting protoplasts round up and increase in volume, pushing apart the xylem cells, facilitating separation of leaf from stem

Roles of ethylene and auxin during leaf abscission

Model of hormonal control of leaf abscission



High auxin from leaf reduces ethylene sensitivity of abscission zone and prevents leaf shedding.

A reduction in auxin from the leaf increases ethylene production and ethylene sensitivity in the abscission zone, which triggers the shedding phase.

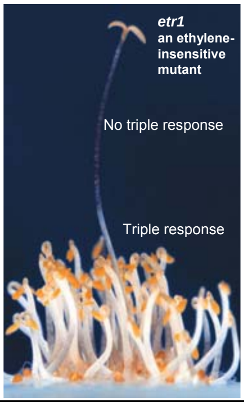
Synthesis of enzymes that hydrolyze the cell wall polysaccharides, resulting in cell separation and leaf abscission.

Effect of ethylene on abscission in birch

Transgenic: birch plant transformed with mutated version of Arabidopsis ethylene receptor, ETR1

Transgenic plants do not drop their leaves when sprayed with 50 ppm of ethylene

Ethylene receptors



Isolation of mutant plants that:

fail to respond to exogenous ethylene (**ethylene-resistant or ethylene-insensitive**)

display the response even in the absence of ethylene (**constitutive mutants**)

etr1 = ***e****thylene* ***r****esistant 1*; first ethylene-insensitive mutant isolated
ETR1 encodes two-component histidine kinase, first eukaryotic histidine kinase isolated
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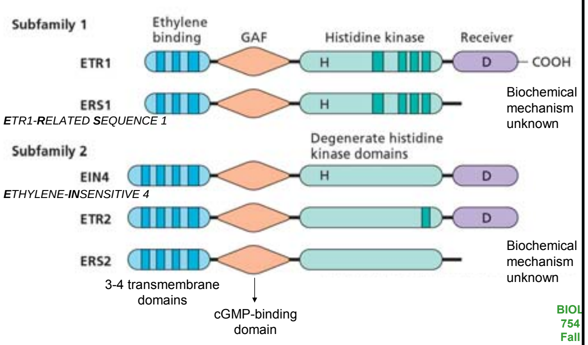
ETR1 might be an ethylene receptor

Arabidopsis seedlings grown in the presence of ethylene

Ethylene receptors in Arabidopsis

Arabidopsis genome encodes four additional proteins similar to ETR1, which all bind ethylene



High affinity binding of ethylene to its receptor requires a copper cofactor

Ethylene binds to its receptor via copper or zinc
predicted because of the high affinity of olefins for these metals

Evidence for copper binding comes from identification of
RAN1 (***R****ESPONSIVE TO* ***AN****TAGONIST 1*)
ran1 mutations block formation of functional ethylene receptors
RAN1 transfers copper ion to ethylene receptor

Unbound ethylene receptors are negative regulators of the response pathway

- In Arabidopsis, tomato (and others?), ethylene receptors are encoded by multigene family

- The 5 Arabidopsis ethylene receptors are functionally redundant, i.e.
  - inactivation of one gene → no effect
  - inactivation of all five genes → constitutive ethylene response

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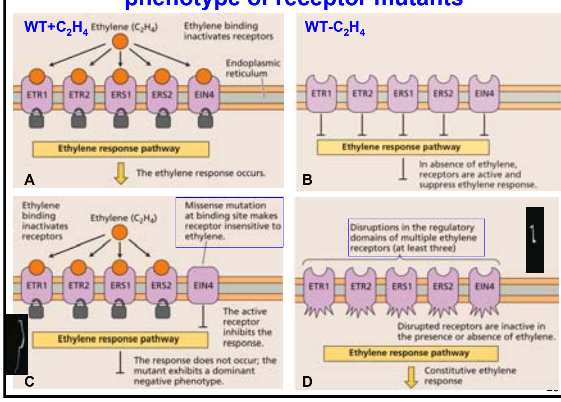
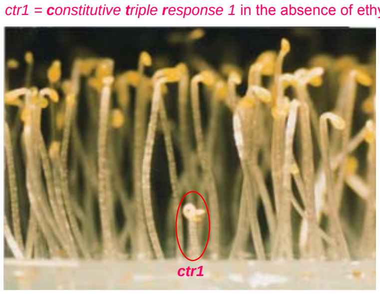
Observation that ethylene response, such as triple response, become constitutive when receptors are disrupted → indication that **receptors are normally "on", i.e. in an active state**

Model of ethylene receptor action based on the phenotype of receptor mutants



A serine/threonine kinase is also involved in ethylene signaling

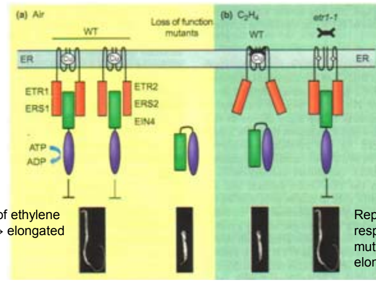
ctr1 = ***c****onstitutive* ***t****riple* ***r****esponse 1* in the absence of ethylene



CTR1 – related to RAF-1, a MAPKKK serine/threonine protein kinase (**m**itogen-**a**ctivated **p**rotein **k**inase **k**inase **k**inase)

Arabidopsis seedlings grown in dark in air for 3 d

Proposed signaling mechanism of ethylene receptors and CTR1 at the ER



Repression of ethylene responses → elongated hypocotyl

Repression of ethylene responses due to mutation in receptor → elongated hypocotyl

- in absence of ethylene -> receptors in active state
- receptors interact with CTR1
- Loss of function of multiple receptors -> dissociation of CTR1
- result: ethylene responses occur → short hypocotyl

- presence of ethylene causes conformational change -> inactive receptors
- CTR1 is released and becomes inactivated
- result: ethylene responses occur: short hypocotyl
- dominant mutation in receptor that disrupts ethylene binding -> constitutive receptor-CTR1 interaction and repression of downstream components

Other components in the ethylene signaling pathway

- Ethylene affects mRNA level of numerous genes, e.g. cellulase, ripening-related genes, ethylene-biosynthetic genes

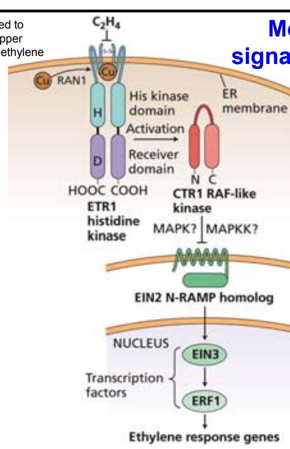
EIN3 transcription factors = key components in mediating ethylene effect on gene transcription

in response to ethylene, homodimers of EIN3 bind to promoter of ***ERF1*** (*__E__THYLENE __R__ESPONSE __F__ACTOR 1*) and activate its transcription

*ERF*s encode proteins belonging to ***ERE*** (*__E__THYLENE __R__ESPONSE __E__LEMENT*)-binding proteins, a family of transcription factors

Model of ethylene signaling in Arabidopsis

RAN1 required to assemble copper cofactor into ethylene receptor



In **absence of ethylene**, ETR1 and other receptors activate CTR1 kinase → repression of ethylene responses through MAPK cascade

In **presence of ethylene**, binding of ethylene to receptors results in their inactivation, causing CTR1 to become inactive

Inactivation of CTR1 allows EIN2 to become active.

Activation of EIN2 turns on EIN3 transcription factors, which induce expression of ERF1.
→ changes in gene expression
→ alterations in cell functions