

Plant Growth Regulators - 1

Growth and development of plants, like all organisms, is regulated by a combination of genetic factors and environment influences.

- Plants have receptors that sense and respond to a number of environmental cues including photoperiod, temperature, pressure and moisture changes.
- Plant chemical growth regulators (hormones) mediate the effects of environmental cues.
- Genes code for the enzymes that catalyze the chemical reactions in plant growth and development.

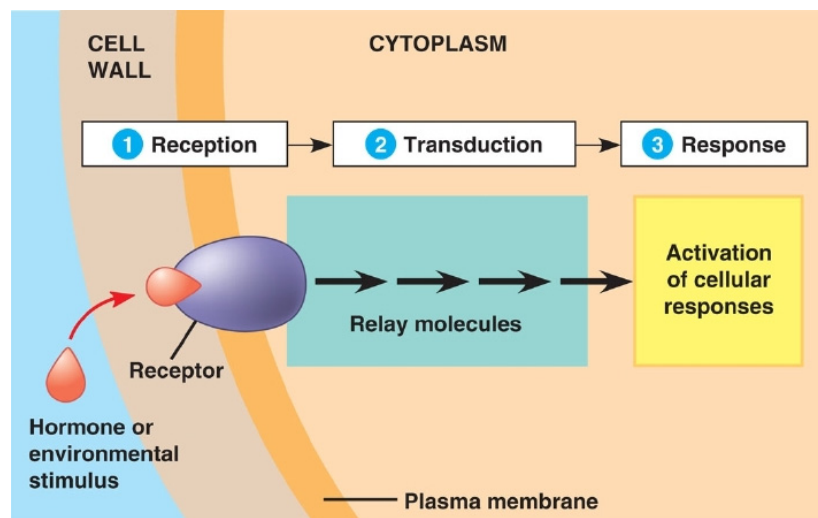
We are accustomed to studying the sensory systems of animals and the animal responses to sensory stimuli. A difference between plant responses and animal responses is that plant responses to the environment often involve differential growth patterns rather than behavioral activities in response to positive or negative stimuli.

In this section and the next we will be discussing some of the chemical plant growth regulators as well as typical plant responses to environmental cues and how those responses are internally signaled from the early embryo growth → seed and fruit development → seed dormancy → mobilization for germination → vegetative growth and development → flowering → senescence and death.

Signal Transduction Pathways in Plants

We learned in Biology 211 that hormones can function as signal molecules (or ligands) that trigger signal transduction pathways in cells. Such pathways often result in the synthesis of transcription factors that in turn promote synthesis of enzymes which facilitate chemical reactions within the cell (the response). In a similar fashion, a signal molecule may function to repress transcription.

Signal transduction pathways are equally important for chemical messaging in plants as in animals and both environmental cues and hormones serve as signal stimuli. We shall examine several examples of plant growth regulation involving signal pathways in this section.

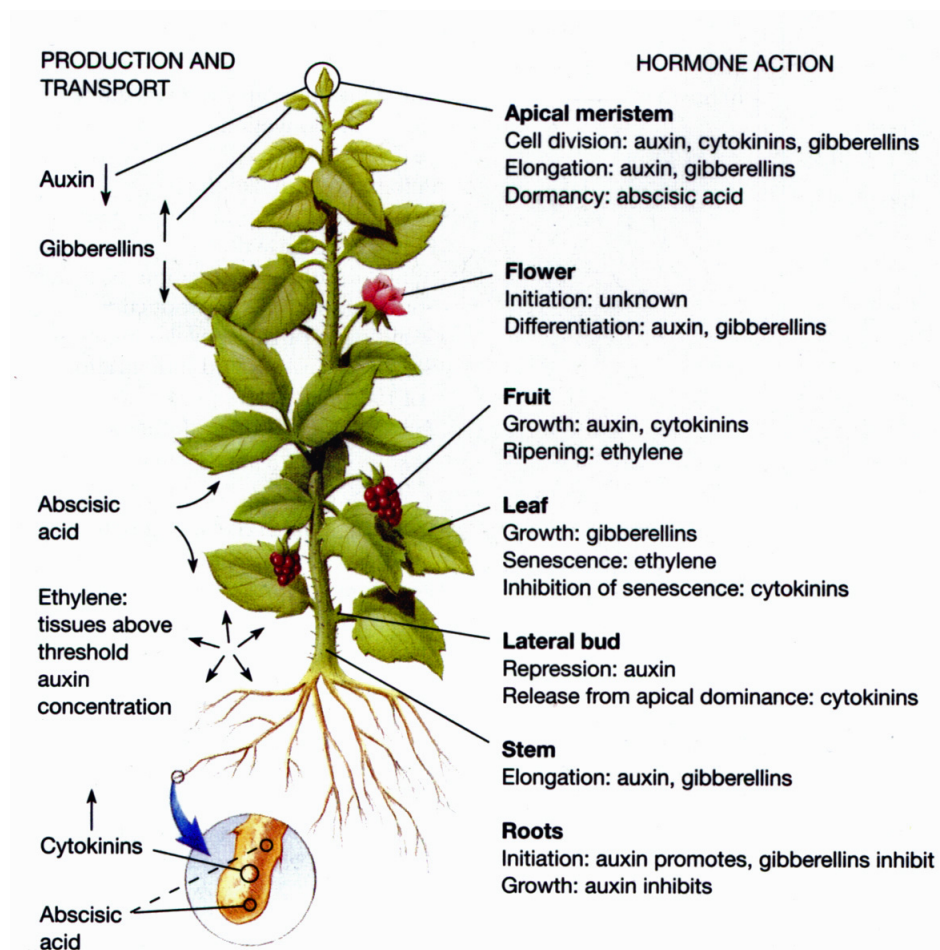


Plant Growth Regulators

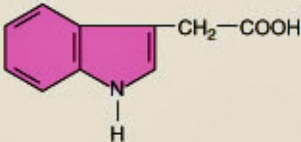
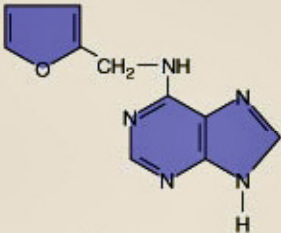
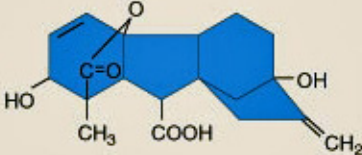
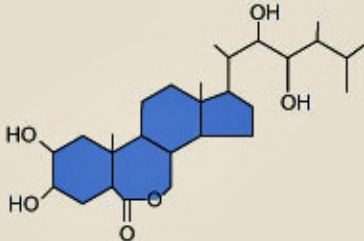
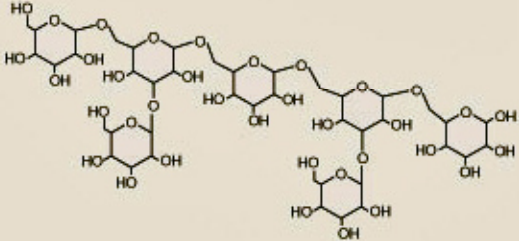
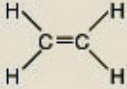
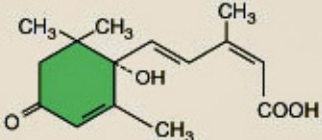
Plant hormones, or plant growth regulators, are chemicals produced by plants that alter growth patterns and/or maintenance of the plant. They can be found in many cells and tissues, although plant hormones seem to be concentrated in meristems and buds (which are dormant shoot meristems). Growth regulators control cell activities by sending chemical signals or messages to cells to do something or to not do something, including activating the genes that code for specific enzymes or blocking gene transcription. Plant hormones inhibit as well as promote cellular activities.

In contrast to animal hormones, which generally have very specific effects, the hormones identified in plants most often regulate division, elongation and differentiation of cells. Most hormones have multiple effects in plants. Plant hormones, as do animal hormones, work in very small concentrations.

In most cases, the effect plant hormones have on the plant depends on the location of and concentration of the hormone relative to other hormones in the specific tissues. Plant hormones often work in conjunction with each other, and have overlapping effects. They also work with environmental stimuli, as we shall discuss. There are several classes of plant hormones, including a number of recently “discovered” ones. We will look at each of these briefly.



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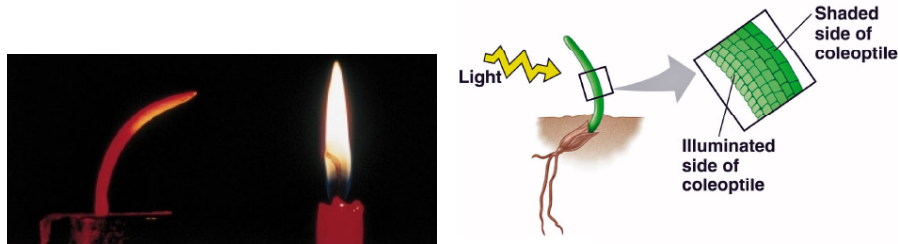
Hormone		Major Functions	Where Produced or Found in Plant
Auxins		Promotion of stem elongation and growth; formation of adventitious roots; inhibition of leaf abscission; promotion of cell division (with cytokinins); inducement of ethylene production; promotion of lateral bud dormancy	Apical meristems; other immature parts of plants
Cytokinins		Stimulation of cell division, but only in the presence of auxin; promotion of chloroplast development; delay of leaf aging; promotion of bud formation	Root apical meristems; immature fruits
Gibberellins		Promotion of stem elongation; stimulation of enzyme production in germinating seeds	Roots and shoot tips; young leaves; seeds
Brassinosteroids		Overlapping functions with auxins and gibberellins	Pollen, immature seeds, shoots, leaves
Oligosaccharins		Pathogen defense, possibly reproductive development	Cell walls
Ethylene		Control of leaf, flower, and fruit abscission; promotion of fruit ripening	Roots, shoot apical meristems; leaf nodes; aging flowers; ripening fruits
Absciscic acid		Inhibition of bud growth; control of stomatal closure; some control of seed dormancy; inhibition of effects of other hormones	Leaves, fruits, root caps, seeds

Also:

- Jasmonates
- Strigolactones
- Systemin
- Salicylic Acid

Auxins

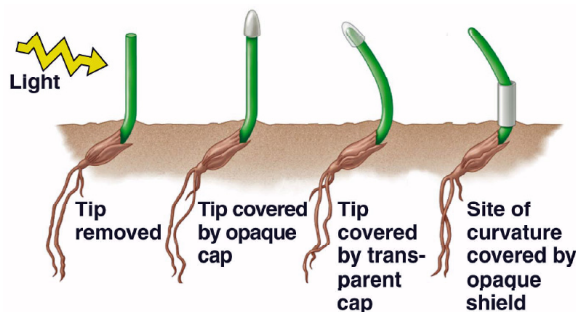
The concept of chemical messengers in plants was proposed in 1880 by Charles Darwin and his son Francis who spent time looking at the phenomenon of phototropism in grass seedlings. Plant shoots are positively phototropic. When a seedling is illuminated from the side, the shoot will bend towards the light. This directional growth makes sense, since plants need light for photosynthesis. The Darwin father and son did research on grass seedlings to try and determine what controlled this differential growth.



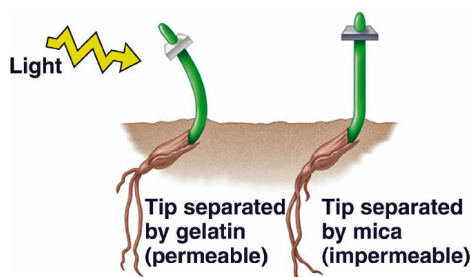
Interestingly, in their research, if the coleoptile of the wheat seedling was removed, the plant no longer curved towards light. They did a number of experiments and determined that a chemical located in the coleoptile traveled to the region of elongation and effected a differential elongation of cells furthest from the light sources.

In 1913 Peter Boysen-Jensen affirmed that the signal for bending was mobile. When he placed a mica slice between the coleoptile tip and the rest of the shoot, no bending occurred.

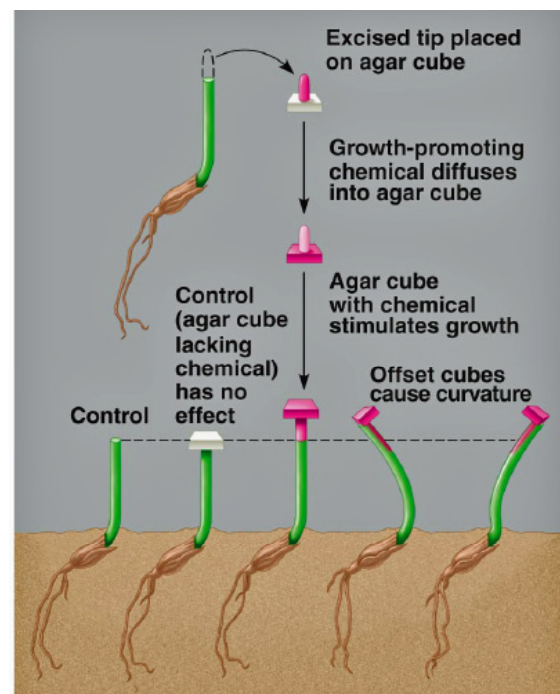
The mobile chemical was subsequently studied and named **auxin** by Frits Went in 1926.



Darwin and Darwin 1880



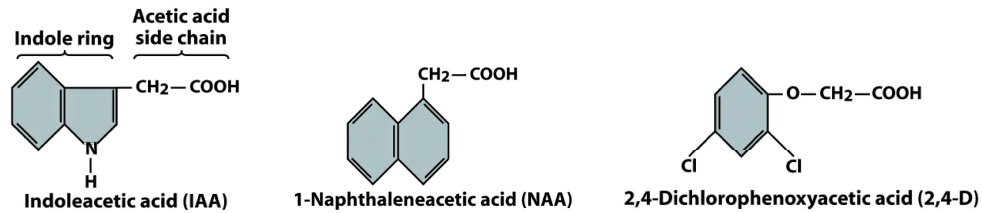
Boysen- Jensen 1913



Went's Experiment Confirming Chemical Control of Coleoptile Curvature

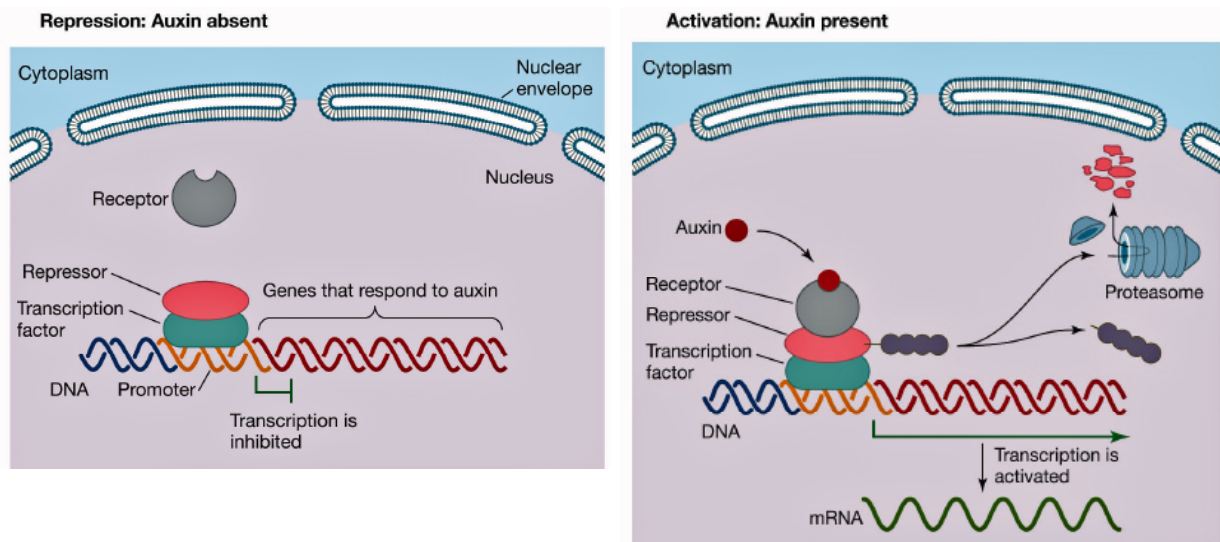
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Chemically, auxin is indoleacetic acid (IAA), which is synthesized from indole or tryptophan. There are a number of synthetic “auxins” too. Auxins promote growth in molar concentrations of 10^{-3} to 10^{-8} .



Auxin and Gene Activity

The genes that control activities activated by auxin are normally repressed. Auxin binds to the repressor, which activates ubiquitin to bind to the auxin-repressor complex, dragging the complex to proteasomes for degradation. Once the repressor is removed, gene transcription for the appropriate activity occurs.



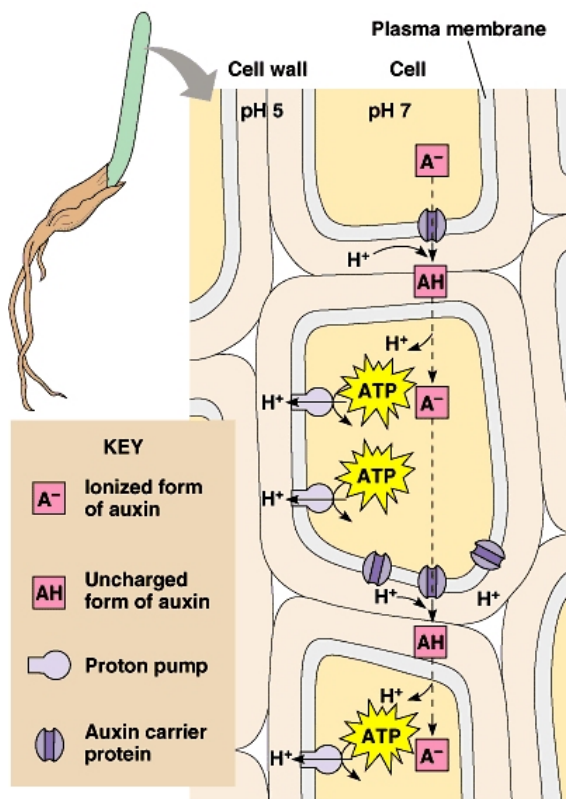
Auxins are synthesized in shoot apical meristems, young leaves, seeds and fruits. Auxin always moves down the stem parenchyma cells towards roots by **polar (charged) transport**. Auxin becomes negatively charged, using proton pumps, an ATP requiring process.

For auxin transport to be successful, the following conditions must be met:

- Diffusion of a polar molecule across the plasma membrane
- Carrier molecules for auxin located only at the basal (bottom) end of the cell
- Proton pumps must be present to remove H^+ from the cell, altering the pH gradient
- Weak acid ionization (Indole Acetic Acid, IAA, aka auxin (A), is a weak acid: $\text{A}^- + \text{H}^+ = \text{AH}$)

Auxin enters cells as IAAH passively, or as IAA^- via active cotransport. IAAH dissociates within the cytosol and special auxin transport proteins in the basal end of the cell are needed to carry auxin through the plasma membrane to the top of the adjacent cell. Auxin destined for root tissue, however, moves through phloem sieve tubes.

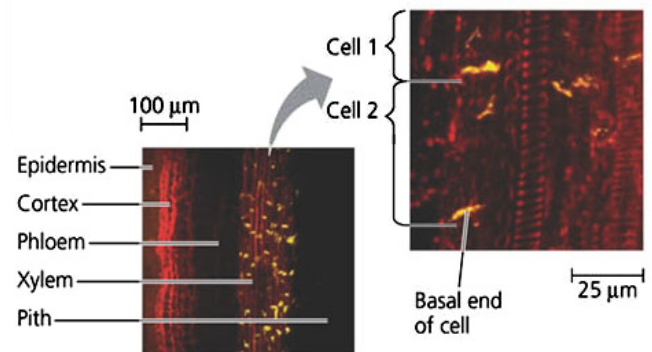
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Auxin polar transport



Auxin synthesis in leaf

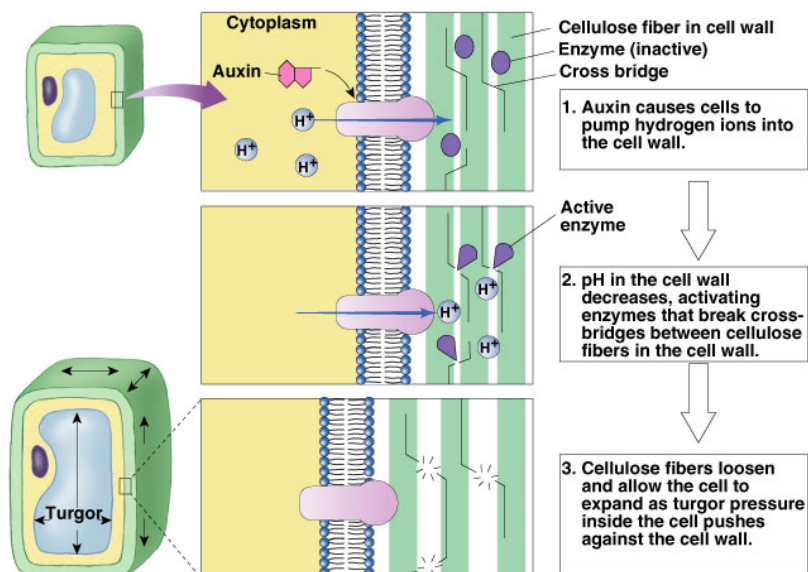


Auxin transport protein (yellow)

Auxin Functions

- Auxin promotes elongation and cell enlargement

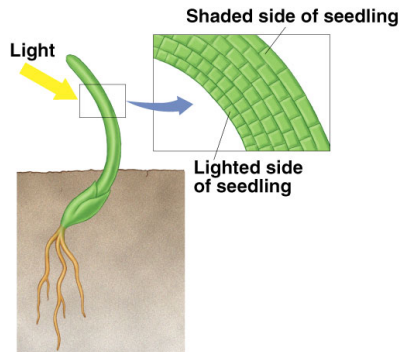
For cell elongation, auxins initiate expression of proton pump genes, which result in the activation of proton pumps in the plasma membrane. The proton pumps increase the H^+ concentration in cell walls, which stimulates **expansins**, proteins that disrupt hydrogen bonds and break cross linkages in cellulose. This facilitates wall elongation when cells take in more water.



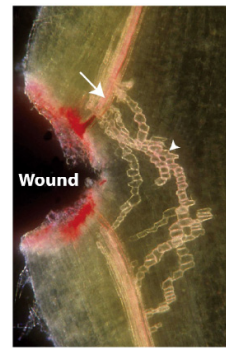
Auxin role in cell wall expansion

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- Auxins are involved in **tropic responses**. Both phototropism and geotropism, to be discussed later, result from unequal elongation induced by unequal concentrations of auxin within the elongating cells.
 - In phototropism, auxins migrate away from light, which accounts for the uneven elongation of cells on the shaded side of a plant, as studied by the Darwin.
 - In root gravitropism, auxin migrates toward gravity

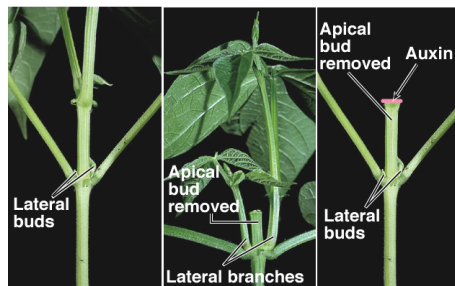


Phototropism uneven elongation



Xylem regeneration in wound

- Auxin stimulates the production of **secondary growth** by stimulating cambium cells to divide and secondary xylem to differentiate. **Wound** tissue repair is initiated by auxin when portions of vascular bundles are damaged.
- Apical Dominance:** Auxin produced in apical buds tends to inhibit the activation of buds lower on the stems. This is known as **apical dominance**. Auxin promotes synthesis of **strigolactones**, growth regulators that repress lateral bud growth. This effect lessens with distance from the shoot tip. **Cytokinins** (another group of plant hormones moving upward from roots) counter the apical dominance effect of auxins and promote lateral bud development. There is also evidence that electrical signals may promote lateral bud formation in some plants.



- Auxins also promote **lateral and adventitious root** development



Auxin applied to shoots on left and center

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- In addition, auxins:
 - Promote other hormone production, especially ethylene, when auxin concentration changes
 - Promote flower initiation
 - Reduced auxin initiates leaf abscission by promoting ethylene synthesis
 - Fruit development requires auxin produced by the developing seed. Auxin pastes applied to developing ovaries can promote **parthenocarpy** (fruit development in the absence of viable seeds). Parthenocarpy is also induced by **abscisic acid**.



Normal Fruit

No fruit with seeds removed

One row of seeds removed

Auxins are toxic in large concentrations. These concentrations affect mostly eudicots but not monocots. Monocots seem to be able to rapidly degrade the synthetic auxins. Synthetic auxin herbicides include 2-4-D and 2-4-5-T. (Agent orange of the Vietnam era contained synthetic auxins.)

- Used as defoliants
- Used as weed killers

While "poorly" documented, adverse effects on humans are also asserted from the use of synthetic auxins in high concentrations.

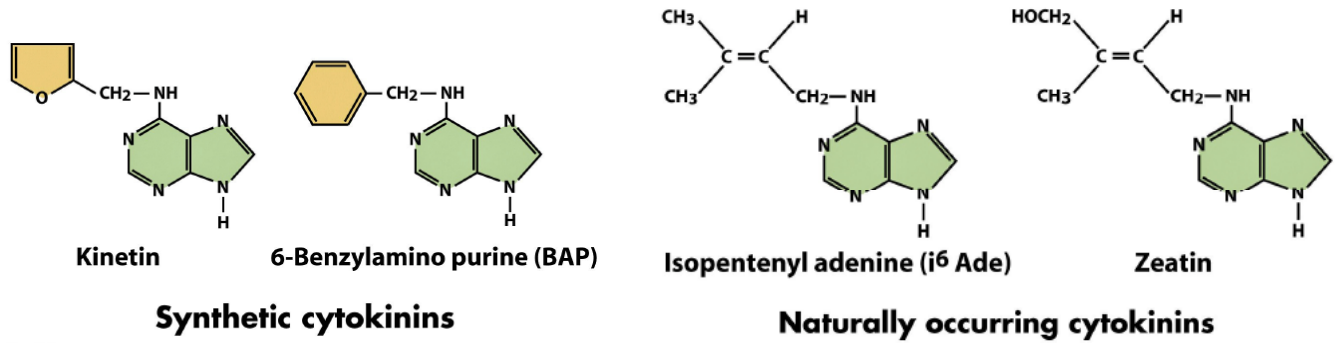
Cytokinins

Cytokinins are a group of phenyl urea derivatives of **adenine**, one of the purines found in nucleotides. The first cytokinin was chemically isolated in 1913 and cytokinins were studied using coconut endosperm for a number of years starting in the 1940's. This isolate was shown to be a potent growth promoter and was used in tissue culture and embryo development studies.

Folke Skoog and Carlos Miller spent several years attempting to isolate this growth substance. They finally succeeded in isolating a breakdown product of DNA that promoted cell division. They named the substance **kinetin** and the related group of growth regulators, **cytokinins**, because they were involved in cell division. However natural cytokinins were not chemically identified until 1963, when **zeatin** was identified in corn.

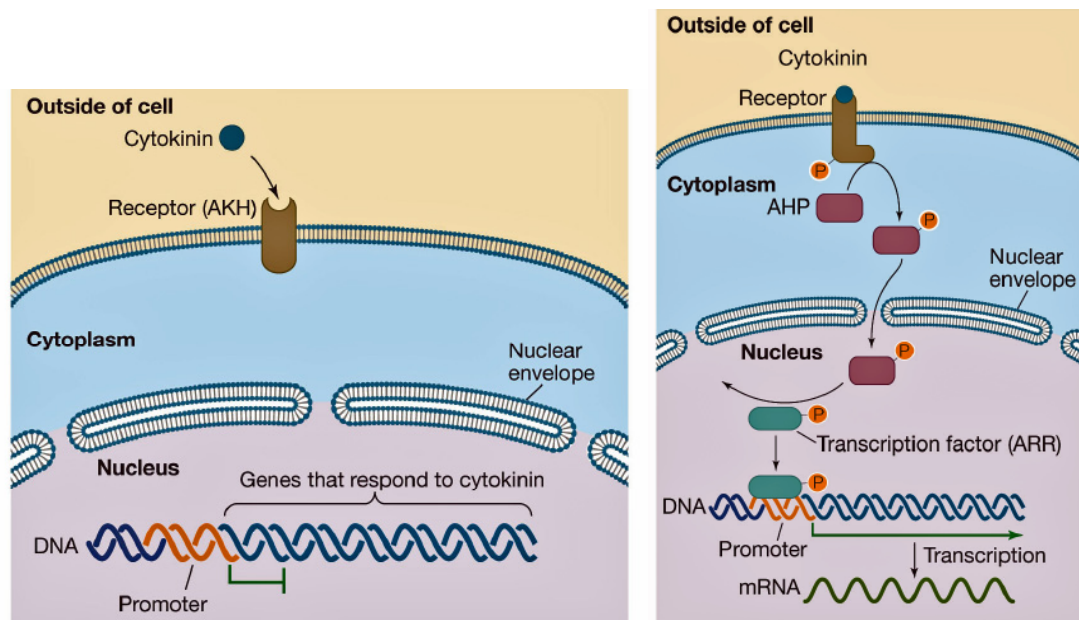
Cytokinins are found in actively dividing tissues of seeds, fruits, leaves and root tips, and wound tissue sites. Studies indicate that root tips are most likely the location for cytokinin production and cytokinins are transported through xylem to the rest of the plant. However, localized cytokinins are needed to release buds from dormancy.

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Cytokinins and Gene Activity

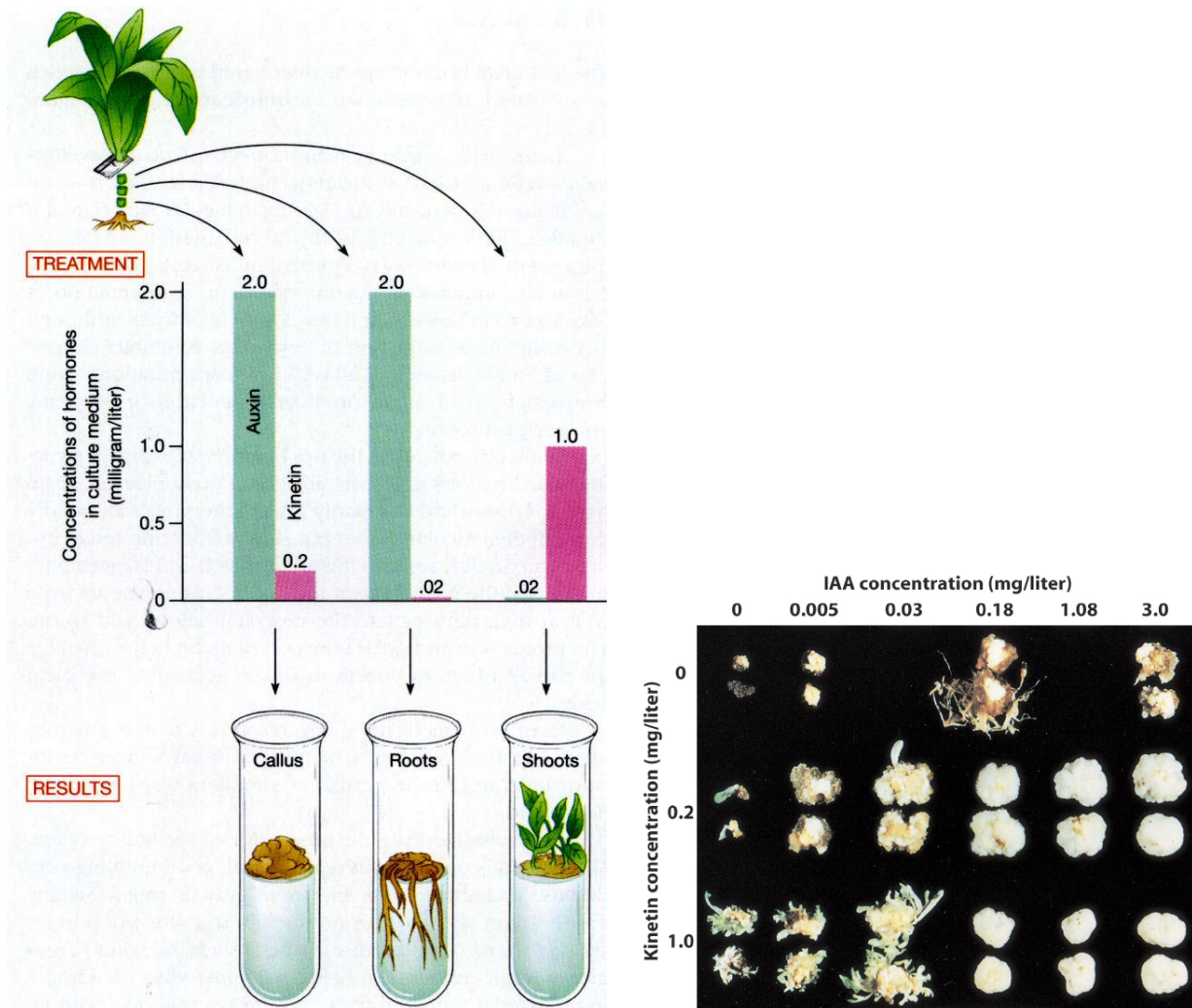
Cytokinins are the signal molecules for a receptor (AKH) that activates a protein kinase transduction pathway that ultimately phosphorylates a transcription factor that binds to the promoter region of over 20 different cytokinin response genes.



Cytokinin Functions

- As stated, cytokinins promote cytokinesis, so their location in root meristems, embryos and fruits is not surprising. Cytokinins migrate from roots to the shoot systems of plants in xylem tissue.
- Cytokinins can promote germination in some seeds in the absence of light, when the seeds normally require light for germination
- The effects of cytokinins are often studied in tissue culture. Normally, cytokinins work in conjunction with auxins, which promote elongation and cell expansion. When auxin is added to a tissue culture medium, cells in the absence of cytokinins enlarge, but division does not take place. When kinetin and IAA are both added to a tissue culture medium, cells divide rapidly producing many small cells. The ratio of cytokinins to auxin controls what tissues differentiate in the tissue culture.

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- Cytokinins can promote axillary (lateral) bud growth by over-riding the apparent inhibiting effect of auxin. This is one of the ways in which plants balance root and shoot growth. Shoot tip auxins inhibit lateral bud activation. Cytokinins produced in root meristems travel upward in xylem and if in higher concentration counter the inhibition of auxins to activate lateral buds, resulting in more shrubby appearance with many branches.
- Cytokinins can also promote lateral expansion in both stems and roots, particularly in storage roots.
- Cytokinins also retard leaf senescence, probably by stimulating RNA and protein synthesis and delaying degradation of chlorophyll.



Cytokinin treated Leaf (R)



Cytokinin treated plant (L)



Crown gall tumor

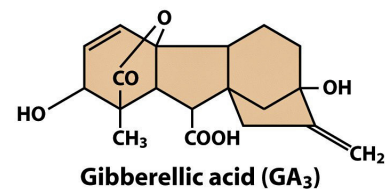
- Some pathogens that promote tumor formation, called galls in plants, take advantage of cytokinin function. The bacteria that form these tumors contain genes for the synthesis of cytokinins, resulting in rapid undifferentiated cell growth in the infected part of the plant. *Agrobacterium tumefaciens*, a common vector in plant biotechnology contains such genes.

Gibberellins

Ewiti Kurosawa is credited with the discovery of gibberellins when he determined that a fungus was responsible for abnormal rice seedling growth, called the "foolish seedling" disease. The fungus secreted a chemical that caused the rice plants to grow abnormally long, and then collapse from weakness. The fungus was *Gibberella fujikuroi*, hence the hormone name.

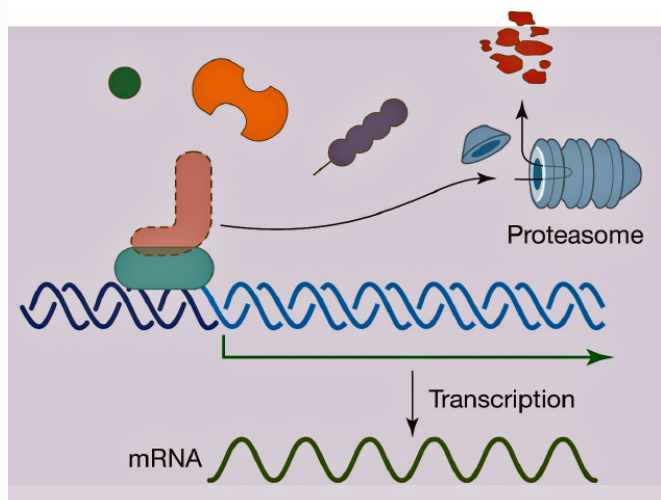
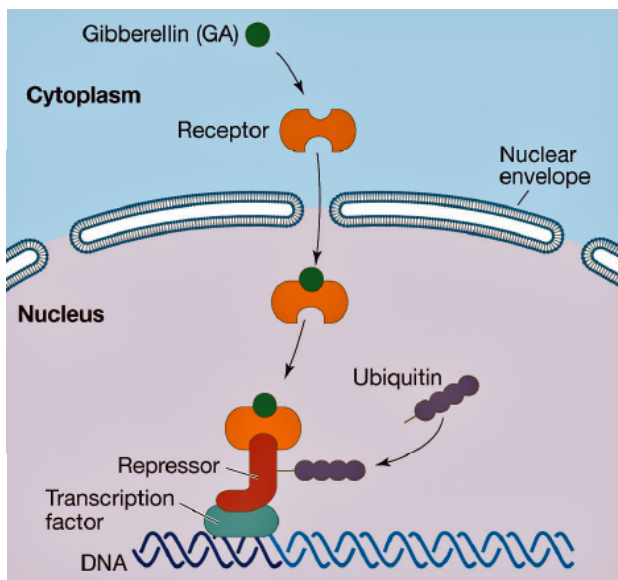


Normal and foolish rice



Gibberellin Gene Activity

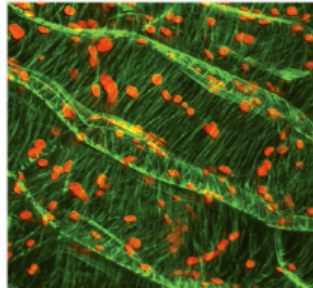
As does auxin, gibberellin stimulates transcription by activating a receptor molecule to first bind to a gene repressor molecule and tag the repressor with ubiquitin, which will direct the repressor molecule to a proteasomes for degradation.



Many seeds contain a variety of different gibberellins. Over 100 different gibberellins (organic acids synthesized from mevalonic acid) are known. Gibberellins are produced in roots and younger leaves, but have their highest concentration in seeds. Most effects of gibberellins are shown only in concert with auxins.

Gibberellin Functions

- Gibberellins work with auxins to promote rapid elongation and division of stem tissue. Gibberellins determine microtubule alignment in the preprophase band that determines the plane of cellulose expansion.



Microtubule alignment in hypocotyls (Red dots are chloroplasts)

The effects of gibberellins on elongation are seen in:

- Bolting of biennials, to produce flowers during the first growing season
- Reversal of genetic dwarfism



GA and flower bolting



GA and bolting cabbage



GA on dwarf tomato

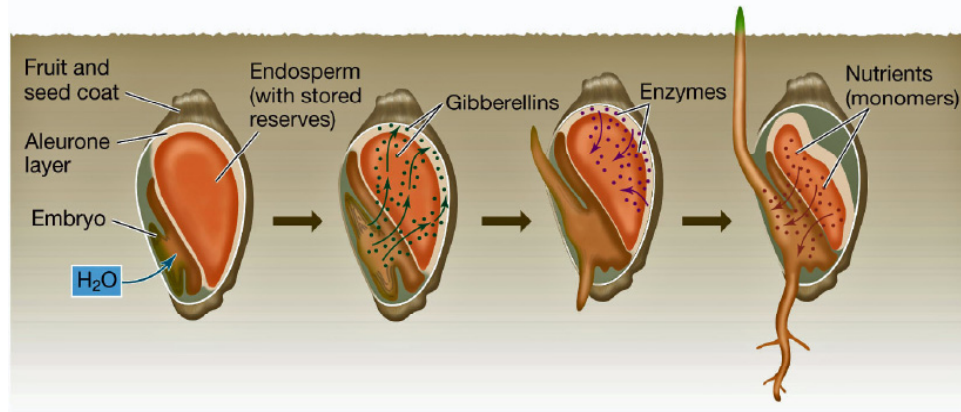
- Gibberellins are important to breaking dormancy after imbibition of water by the seed coat. Gibberellins signal germination activities.

Application of gibberellins to seeds will counter the normal environmental cues, such as exposure to low temperatures. Gibberellins stimulate synthesis of enzymes that convert stored nutrients (starches) to sugars needed for rapid cell respiration during germination.

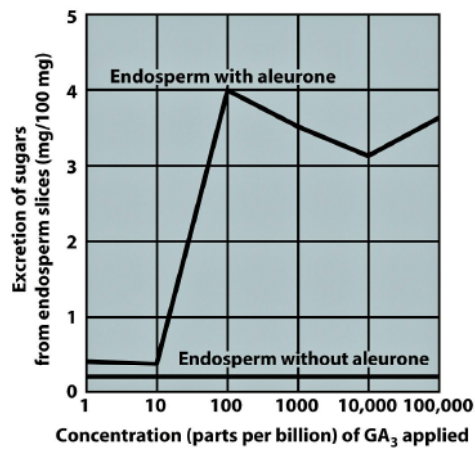
Gibberellins secreted by embryos at the start of germination (imbibition of water) typically activate a set of genes found in the aleurone layer of seeds for this conversion. The malting industry uses gibberellins to enhance the barley's starch reserves conversion to malt and then to glucose.

Absciscic acid can counter the effect of gibberellin to keep seeds dormant.

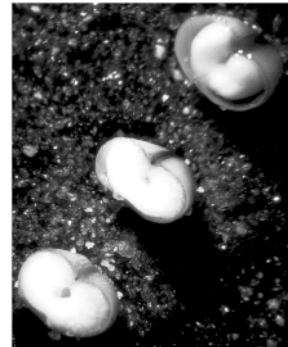
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Gibberellin mobilizing nutrients



Sugar Excretion and Gibberellin



Sugar production in seed with GA application

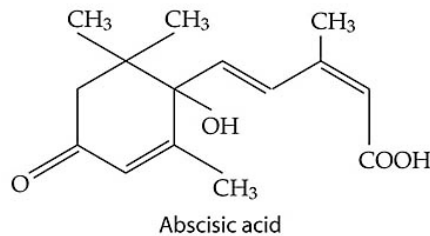
- Gibberellins can, as can auxins, promote **parthenocarpy** in many plants, which has commercial applications, although the most significant use in the grape industry, where grapes grow larger, and with longer internodes.



- In horticulture, farmers may use gibberellin inhibitors to reduce stem elongation so that flowers are produced on shorter stems. Such flowers may have greater appeal to the consumer.

Abscisic Acid

Absciscic acid (ABA) is a hormone that functions by inhibiting growth activities in times of environmental stress rather than by promoting growth. It often serves as an antagonist to the other growth promoting hormones in plants. Absciscic acid, which is also synthesized from mevalonic acid, got its name from the erroneous belief that it promoted the formation of abscission layers in leaves and fruits. It does not, although leaf abscission accompanies dormancy in many plants.



Absciscic Acid Functions

- ABA promotes **seed dormancy** activities. ABA levels are high when seeds mature, promoting lowered metabolism and synthesis of proteins needed to withstand the dehydration associated with dormancy. Seeds germinate when ABA is degraded by some environmental action. Desert seeds must have the ABA washed out of the seed coat, as do plants of marshy areas; temperate area plants have ABA degraded by light-stimulated enzymes. In other cases breaking dormancy is relative to the ratio of ABA (which keeps seeds in dormancy) and gibberellins (which promote germination).

Low levels of ABA in maturing seeds promote premature germination.

Mangrove seeds naturally have low levels of ABA and germinate while still attached to the shoot system. Certain mutations affecting ABA synthesis may result in seeds germinating prematurely.



Mangrove germination Premature corn germination

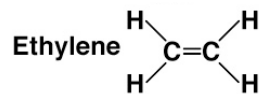
- Similar to seed dormancy, ABA promotes winter bud scale formation on woody plants in preparation for winter dormancy. ABA derivatives, called dormins, are used in commercial nurseries to keep materials to be shipped in dormant conditions. The dormancy can be reversed with gibberellins.



- ABA is also referred to as the **stress** activity hormone. For example, ABA promotes stomata closure during leaf water deficit conditions by activating K^+ ion transport out guard cells. This involves signal transduction pathways with calcium secondary messengers. ABA in this case originates in roots, and detects low water level in root tissues. ABA moves upward into leaves and activates stomatal closure.

Ethylene

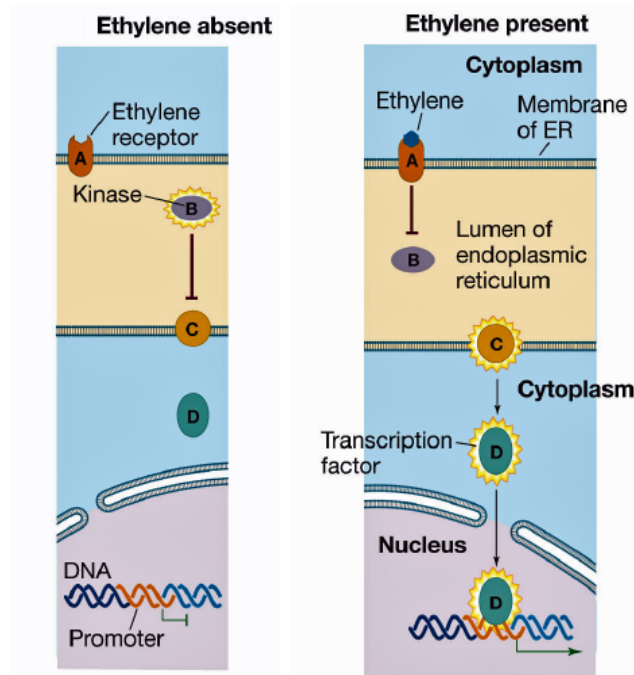
The small hydrocarbon, ethylene, is the sole plant growth regulator known that is a gas. (You will recall that nitric oxide gas is involved in signal transduction pathways affecting smooth muscle.)



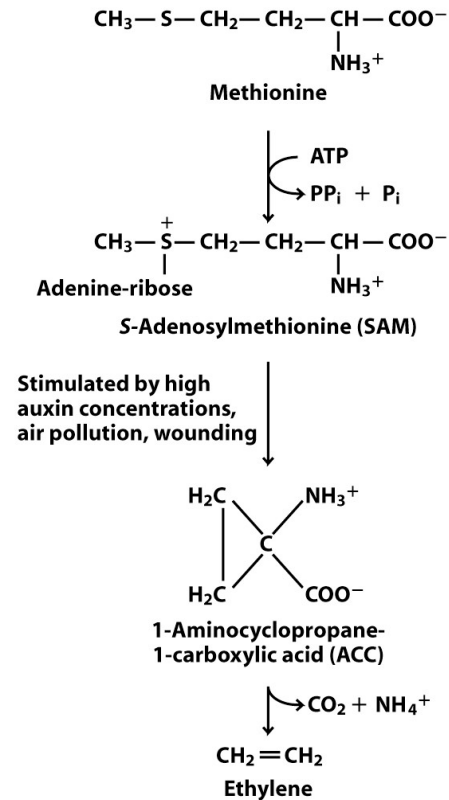
Ethylene Gene Activity

Ethylene activates a sequence of four proteins that ultimately promote transcription.

- In the absence of ethylene, its membrane receptor (A) located on the endoplasmic reticulum membrane phosphorylates a kinase (B) within the endoplasmic reticulum of the cell which blocks a second ER protein (C).
- In the presence of ethylene, its ER membrane protein (A) does not phosphorylate the internal ER protein (B), which results in phosphorylating the second ER membrane protein (C). The phosphorylation cascade activates protein (D), which moves into the nucleus activating appropriate transcription factors.
- Ethylene is the signal molecule for signal transduction pathways that activate a number of tissue growth and development activities throughout the plant, including fruit maturation, leaf abscission and senescence.



Ethylene Signal Transduction Pathway



Ethylene Synthesis

Ethylene is produced in many plant organs, though its effects are most studied in fruits.

Ethylene is synthesized from the amino acid **methionine**. High auxin concentrations promote an intermediate step in this pathway that activates an enzyme in the tonoplast (vacuole membrane) to convert the intermediate into ethylene. Toxic substances such as air pollutants also trigger the intermediate step.

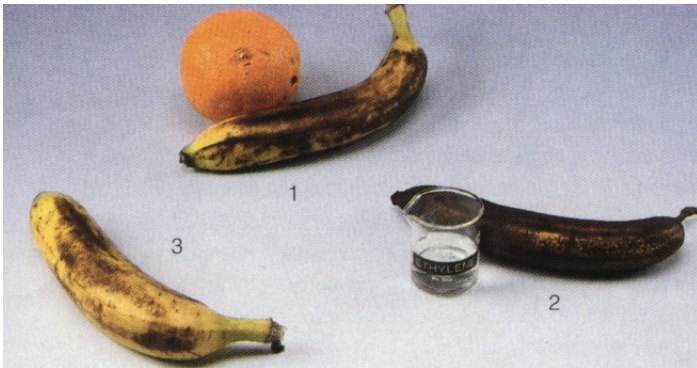
Ethylene is also produced in wounded or bruised tissues. The benefit of this is unknown; it may be an artifact of the wounding response.

Ethylene Functions

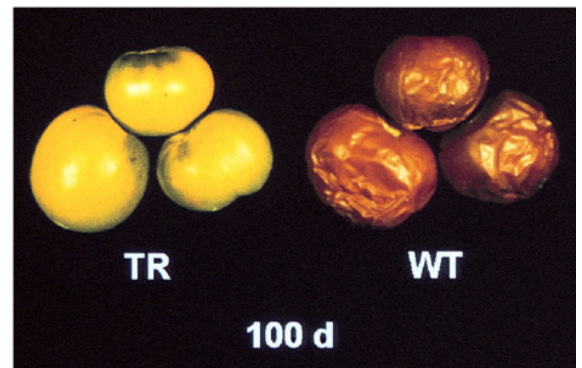
- **Fruit Maturation** The benefits of ethylene as a fruit ripener were known for centuries prior to it being identified as a plant product in the early 1900's. Chinese gardeners knew centuries ago that fruits ripened better in rooms with burning incense. Citrus growers used kerosene stoves in the rooms in which they ripened their fruit. During the era of gas lamps, leaking lamps along city streets often promoted leaf abscission. Today, grocer warehouses have ethylene rooms that are used for ripening most of our produce, which is shipped unripe. Immature fruits are firmer and less subject to damage. Thanks to ethylene rooms, we have "mature green tomatoes".

Ethylene activates ripening in fruits by signaling chemical reactions that degrade the pectins of the middle lamella, softening fruit, and promoting the conversion of stored starches and/or oils into sugars that attract seed dispersers. Some fruit ripening involves a significant increase in the rate of cell respiration with concurrent high O₂ uptake.

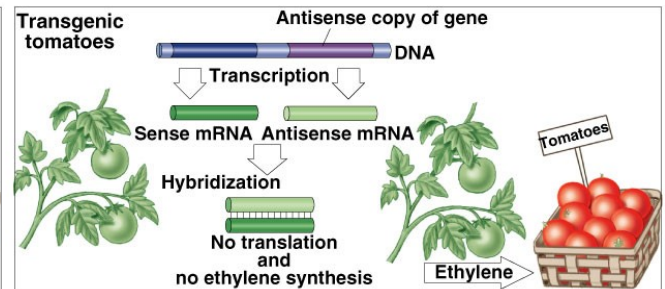
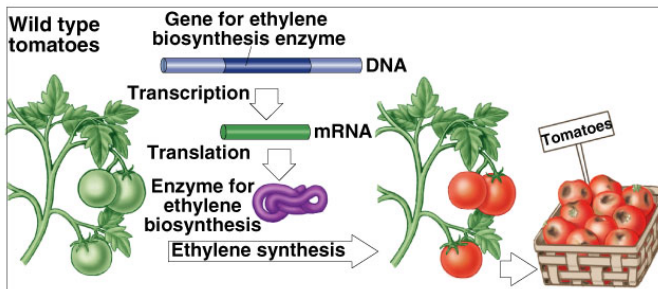
This function of ethylene (fruit ripening) has extensive agricultural impact. Biotechnology has been used to alter sensitivity to ethylene in some crops to facilitate harvest and shipping of fruits. Using gene technology, tomatoes lacking ethylene receptor genes have fruits with a shelf life of 100 days!



Ethylene promoting ripening



Tomatoes on left lack ethylene receptor genes



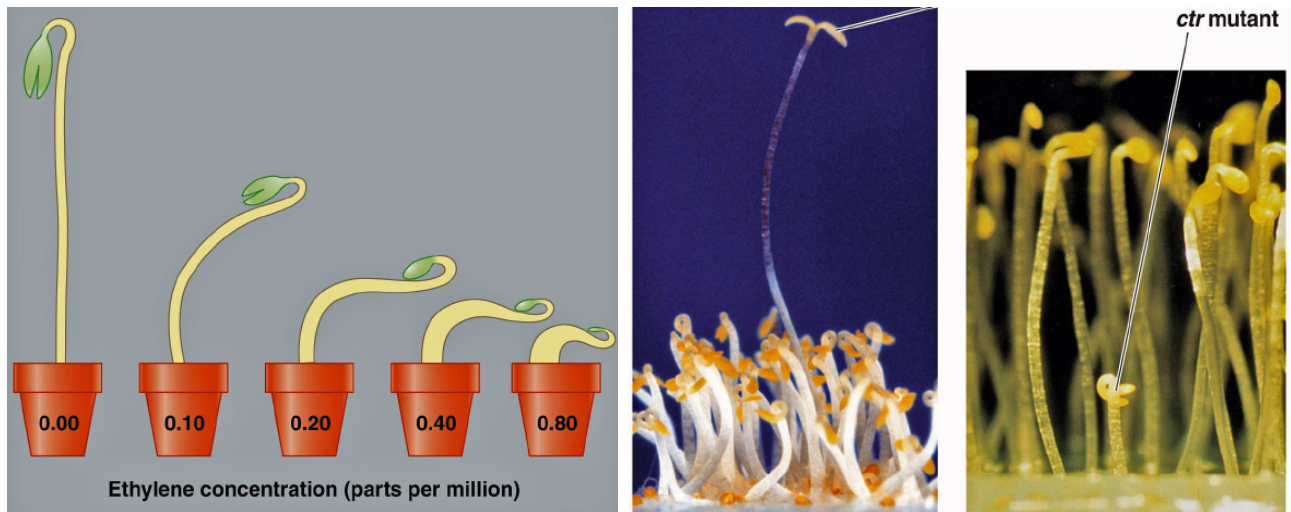
- Ethylene promotes female flower production in some members of the Cucurbitaceae, whereas high gibberellins may promote formation of male flowers.
- Ethylene is also responsible for the hypocotyl arch or apical hook common eudicot shoot germination.



- Ethylene functions to help germinating seeds overcome **mechanical stress**. A shoot tip that encounters an immovable object will grow around the object by changing its growth direction. This occurs through differential elongation of cell walls. When the shoot tip cannot penetrate the mechanical obstacle, ethylene is synthesized, which slows cell wall expansion.

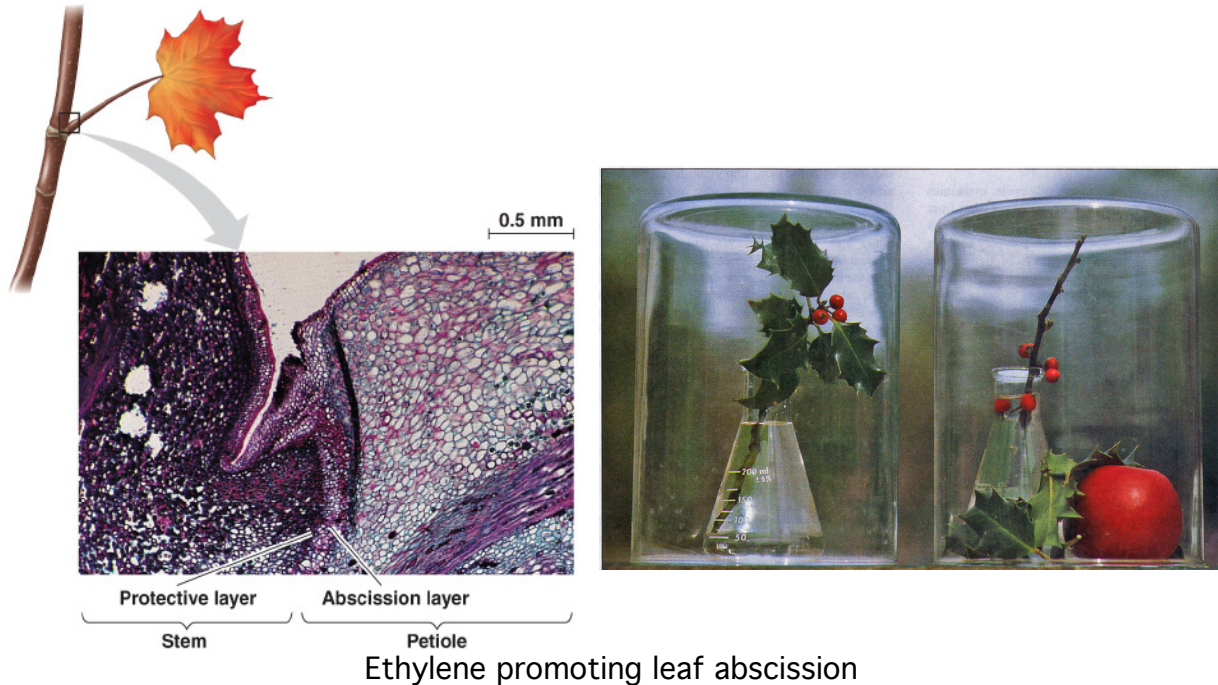
The walls thicken (more resistant to the pressure) and the stem curves to bypass the obstacle via horizontal growth. (Essentially the shoot is growing around the obstacle.) The stem tip pushes upward periodically to test for the obstacle. If the obstacle is encountered, ethylene initiates another cycle of stem thickening and horizontal growth. If no obstacle is encountered, the shoot resumes negative geotropic growth.

This growth response is known as the **triple response** (decrease in elongation, stem thickening, and change in orientation of shoot growth to horizontal).



Mutant strains of *Arabidopsis* with an ethylene-insensitive (*ein*) gene do not exhibit reduced growth when exposed to ethylene. In a similar fashion, the *Arabidopsis* *eto* mutant over-produces ethylene and undergoes the triple response in the absence of obstacles. The *eto* mutant response can be reversed with ethylene synthesis inhibitors to achieve normal growth. A third mutant, the *ctr* mutant, over-produces ethylene resulting in unnecessary triple response but does not respond to ethylene inhibitors.

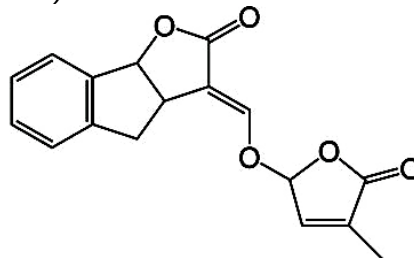
- Ethylene is responsible for initiating the programmed death (apoptosis) of sclerenchyma and xylem vessels and tracheids. Death involves intense cellular activity to degrade and salvage materials of the cytoplasm.
- Ethylene is the direct cause of leaf and fruit abscission, although ethylene synthesis is related to the decline in auxin production in the leaf. Ethylene promotes the degradation of the cell walls in the abscission zone cells. The declining levels of auxin in leaves signal the production of ethylene in the abscission zone cells as summer ends.



The auxin/ethylene interaction for abscission is also used commercially. Fruit growers may spray auxins on fruits to prevent the fruits from falling to the ground prior to harvest. Concentration is important; very high auxin level promotes ethylene production. High concentrations of CO_2 inhibit ethylene production so CO_2 can be used to prevent fruit ripening in grocer warehouses.

Strigolactones

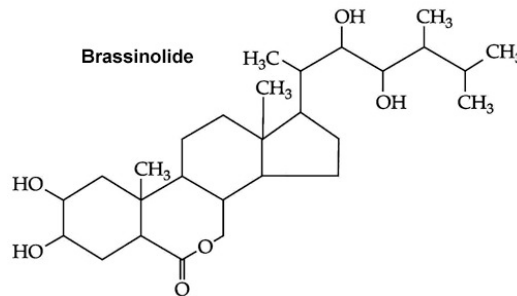
Strigolactones are chemical signal molecules that help promote seed germination, activate mycorrhizae associations (*discussed previously*) and may help to maintain apical dominance when activated by auxin. A parasitic plant host's roots may secrete strigolactones that attract a seedling parasitic to develop haustoria which penetrate the host. (This may be the parasite taking advantage of a signal intended to attract mycorrhizae.)



Strigolactone Structure

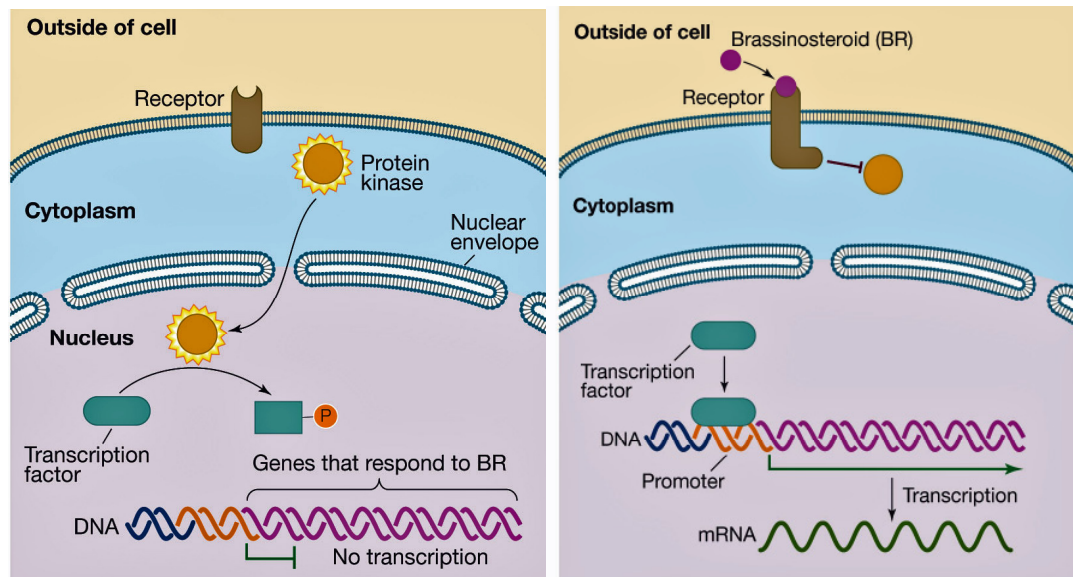
Brassinolides (Brassinosteroids)

Brassinolides is a plant steroid discovered in pollen of members of the mustard family, and studied in *Arabidopsis*. Chemically the brassinosteroids are similar to animal steroid hormones, and have similar genes that code for the steroid synthesis pathways.



The brassinosteroids functions overlap those of other plant hormones, making them challenging to study and their effects may be additive.

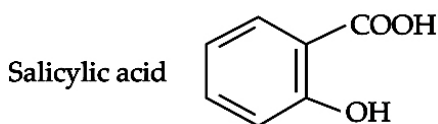
- Brassinosteroids activate signal transduction pathways that promote cell elongation and cell division. Without the brassinosteroid, a kinase inhibits a transcription factor. The brassinosteroid signal reception deactivates the kinase, promoting gene transcription.



- Brassinosteroids also promote differentiation of xylem tissue, and perhaps other tissues, too.
- Brassinosteroids can affect leaf abscission.
- Brassinosteroids promote pollen tube growth.
- Brassinosteroids promote seed germination
- Absence of brassinosteroids results in dwarf plants.

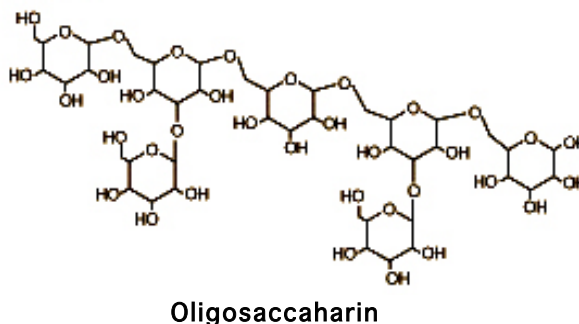
Salicylic Acid

Salicylic acid is known to activate defense genes for resistance against pathogen invaders, known as the **hypersensitive response**. (*See plant defense responses discussion.*) Salicylic acid, a phenolic extract from willow bark, was long used as an analgesic. It is now prepared commercially and is the active ingredient of aspirin.



Oligosaccharins (Oligosaccharides)

Oligosaccharins are short chain sugars in cell walls that may have a role in defense against pathogens. When a pathogen “chomps” on the oligosaccharide, the oligosaccharide activates a signal transduction pathway leading to plant defense responses (*discussed with plant defenses*). They may also help regulate growth, differentiation and flower development by activating signal transduction pathways.

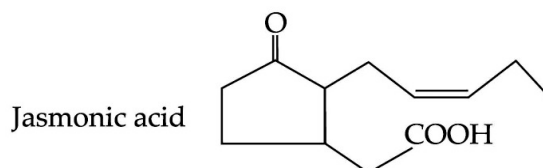


Systemin

Systemin is a small peptide found in wound tissue. It may stimulate defense activities as the signal molecule (ligand) that activates the signal transduction pathways that include the jasmonates.

Jasmonates

Jasmonates are a group of fatty acid derivatives. They appear to have a role in seed germination, root growth, and the storage of protein (especially in seeds).



Jasmonates synthesized in response to signal molecules produced in wound areas are involved in the signal transduction pathways that result in secondary metabolites (protease inhibitors) that poison the predator, or synthesis of volatile molecules that attract the predator’s predators. (*We will discuss plants and their defenses at the end of this unit.*)