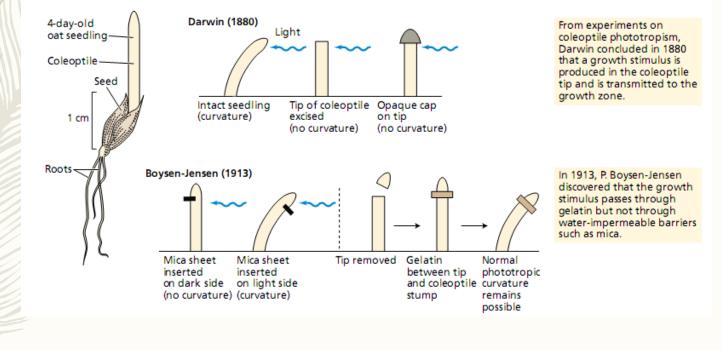
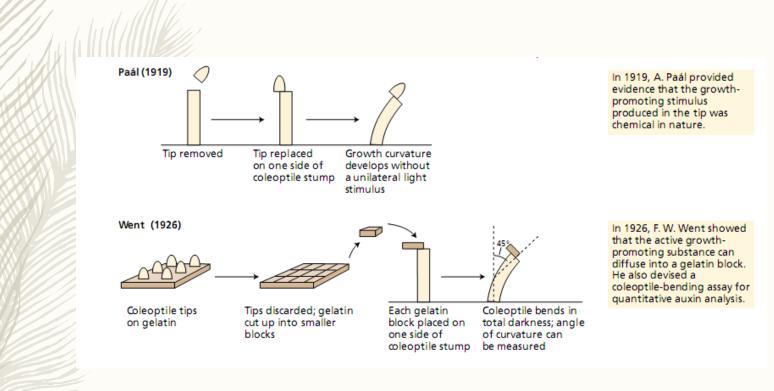
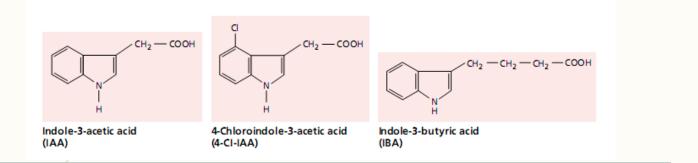
Auxins







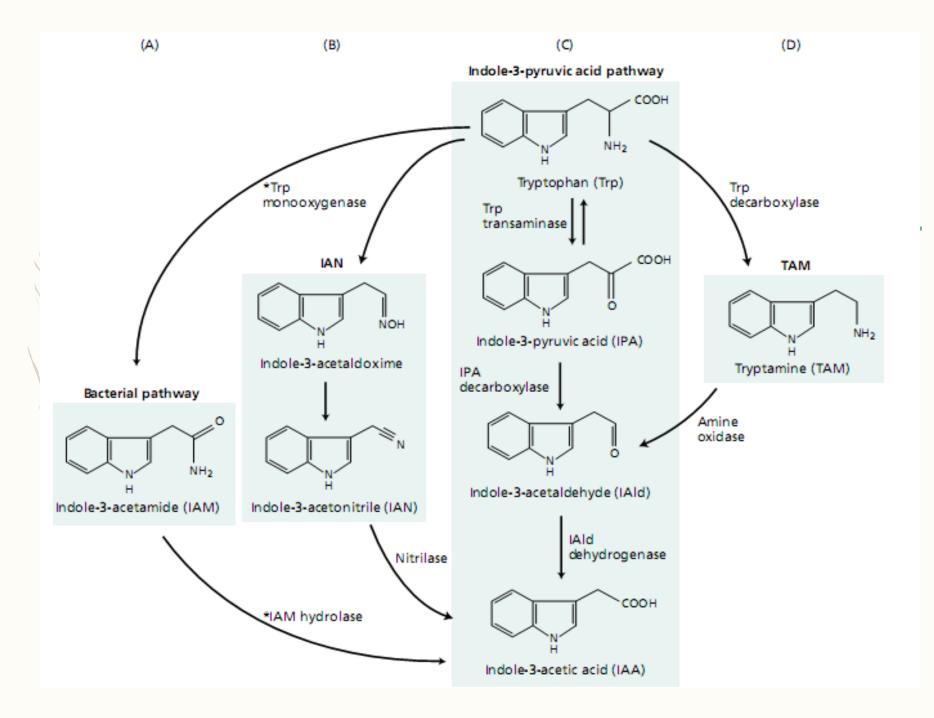
- 3 essential regions for auxins to bind to the auxin receptor
- 1) planar aromatic ring-acts as binding platform
- 2) a carboxylic acid acts as binding site
- 3) hydrophobic transition region-separates the 2 binding sites

Antiauxins

- Alpha-(p-chlorophenoxy)isobutyric acid
- No auxin activity, competes with IAA for specific receptors without triggering auxin response

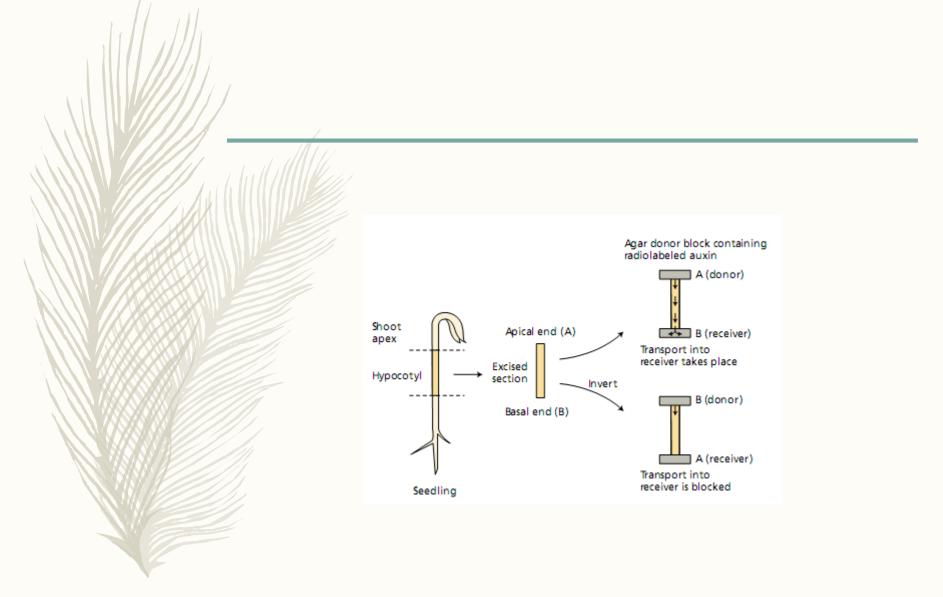
Biosynthesis

- In dividing tissues-shoot apical meristems, young leaves, developing fruits
- IPA Pathway-deamination followed by decarboxylation and then oxidation by specific dehydrogenase
- TAM pathway-deamination & decarboxylation is reversed
 & diferent enzymes are involved
- IAN pathway-cruciferaceae, gramineae & musaceae
- IAM pathway-indole-3-acetamide (IAM) as an intermediate--used by various pathogenic bacteria, such as *Pseudomonas savastanoi* and *Agrobacterium*

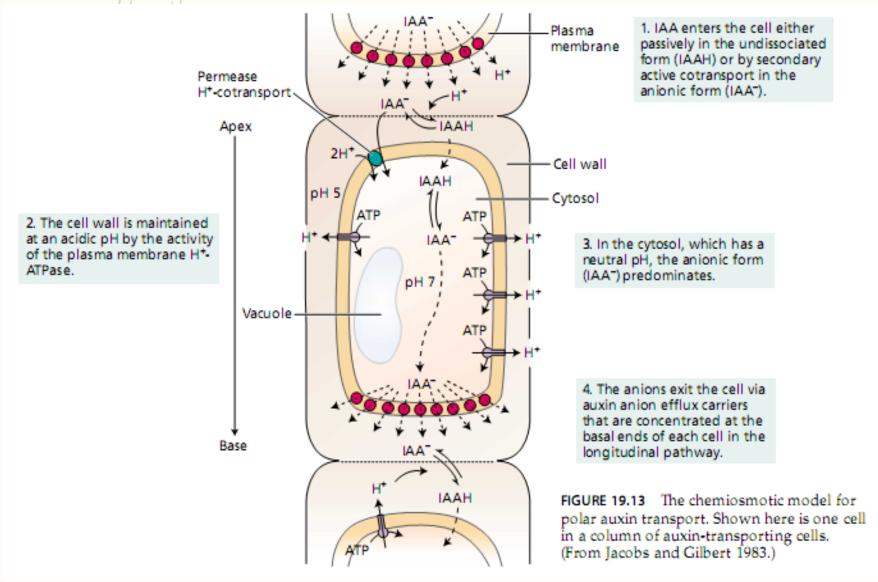


Polar transport

- Only plant growth hormone that is transported polarly. Polar transport contributes towards auxin gradient from shoot to root, which affect various developmental process, including stem elongation, apical dominance, wound healing and leaf senescence.
- Polar transport proceeds from cell to cell, rather than symplast
- High energy requiring process



A Chemiosmotic Model Has Been Proposed to Explain Polar Transport



- Auxin efflux & influx takes place
- The rate of polar transport is ~1cmh-1 (10 times faster than diffusion)
- Polar transport of auxins require protein carriers that can recognize the hormone (Inactive auxin analogs and auxin metabolites are not transported polarly)
- In roots acropetal transport, small amount of basipetal auxin transport also noticed.

Inhibits Au transport

- Blocks Au efflux
- NPA(1-N-naphthylphthalamic acid)
- TIBA(2,3,5-triiodobenzoic acid)
- Cause stunting, inhibition of root growth, loss of gravitropic & phototropic responses
- TIBA inhibits polar transport directly by binding to the Au binding site on the efflux carrier. In contrast phototropins bind to an associated protein that regulates the efflux carrier

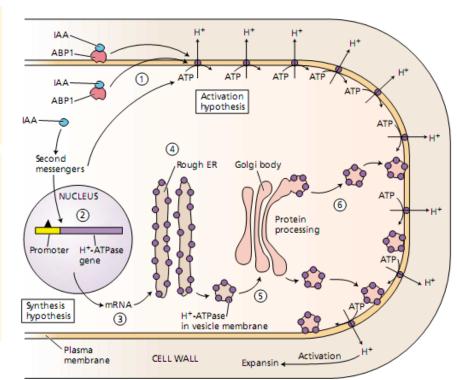
Auxin is transported nonpolarly via phloem

- Moves at greater speed and is passive
- Plant may be prefering phloem for long distance transport, for controlling process such as cambium division and branch root formation.
- Polar and phloem transport are not independent. Au can be transferred from the non polar phloem pathway to the polar transport pathway

PHYSIOLOGICAL EFFECTS OF AUXIN: CELL ELONGATION

Activation hypothesis: Auxin binds to an auxinbinding protein (ABP1) located either on the cell surface or in the cytosol. ABP1-IAA then interacts directly with plasma membrane H⁺-ATPase to stimulate proton pumping (step 1). Second messengers, such as calcium or intracellular pH, ould also be involved.

Synthesis hypothesis: IAA-induced second messengers activate the expression of genes (step 2) that encode the plasma membrane H*-ATPase (step 3). The protein is synthesized on the rough endoplasmic reticulum (step 4) and targeted via the secretory pathway to the plasma membrane (steps 5 and 6). The increase in proton extrusion results from an increase in the number of proton pumps on the membrane.



- Au promotes growth in stems & coleoptiles but inhibit growth in roots
- More IAA inhibits, opt is 10⁻⁶ to 10⁻⁵M
- Outer tissues of dicots are targets of Au action-expt.
 By splitting stem sections from growing portion of dicot stem & incubating in buffer
- -Au increases the extensibility of the cell wall
- Growth rate=m(P-Y)
- m=irreversible extensibility
- P=Turgor pr.
- -Y=yield threshold

- Au induced H+ extrusion acidifies the cell wall, increasing its extensibility: Acid growth hypothesis
- Specific proteins mediate acid induced loosening of cell wall
- Hydrolytic enz thought to get activated at low pH
 Expansins cause cell wall loosening in response to acid pH
- Au maintains the capacity for acid induced loosening of cell wall by
- 1)uptake/generation of osmotic solutes
- 2) activates enz involved in cell wall polysaccharide biosynthesis

PHYSIOLOGICAL EFFECTS OF AUXIN: PHOTOTROPISM AND GRAVITROPISM

Phototropism may be mediated by the lateral distribution of Au

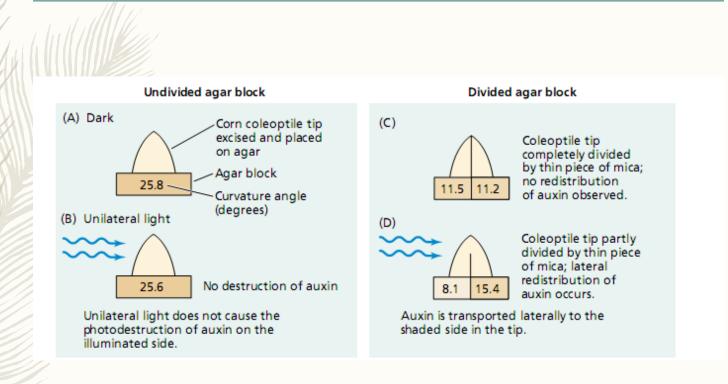
According to the Cholodny–Went model of phototropism, the tips of grass coleoptiles have three specialized functions:

- 1. The production of auxin
- 2. The perception of a unilateral light stimulus
- The lateral transport of IAA in response to the phototropic stimulus

Thus, in response to a directional light stimulus, the auxin produced at the tip, instead of being transported basipetally, is transported laterally toward the shaded side.

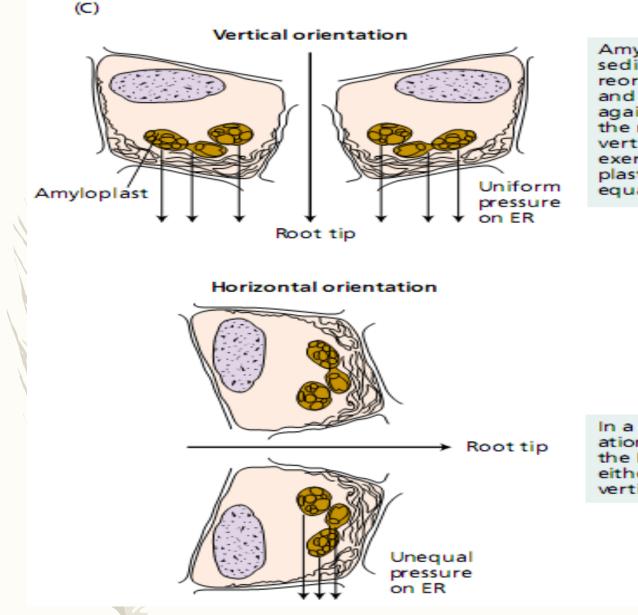
- phototropins 1 and 2, are the two flavoproteins, photoreceptors for the blue-light signaling pathway
- Phototropin 1 displays a lateral gradient in phosphorylation during exposure to low-fluence unilateral blue light.
- According to the current hypothesis, the gradient in phosphorylation induces the movement of Auxin to the shaded side of the coleoptile
- Once the auxin reaches the shaded side of the tip, it is transported basipetally to the elongation zone, where it stimulates cell elongation.
 - The acceleration of growth on the shaded side and the slowing of growth on the illuminated side (differential growth) give rise to the curvature toward light.

Cholodny Went model using the agar block/coleoptile curvature bioassay



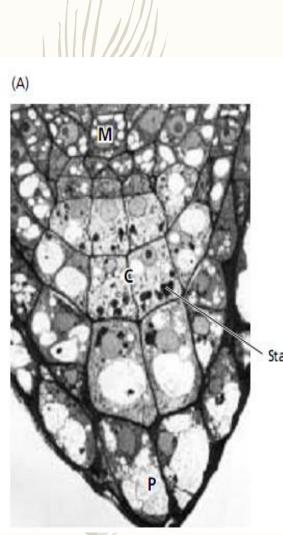
Gravitropism Also Involves Lateral Redistribution of Auxin

- Statoliths Serve as Gravity Sensors in Shoots and Roots
- Amyloplasts that function as gravity sensors are called statoliths, and the specialized gravity-sensing cells in which they occur are called statocytes.
- In shoots and coleoptiles, gravity is perceived in the starch sheath, a layer of cells that surrounds the vascular tissues of the shoot. The starch sheath is continuous with the endodermis of the root, but unlike the endodermis it contains amyloplasts.

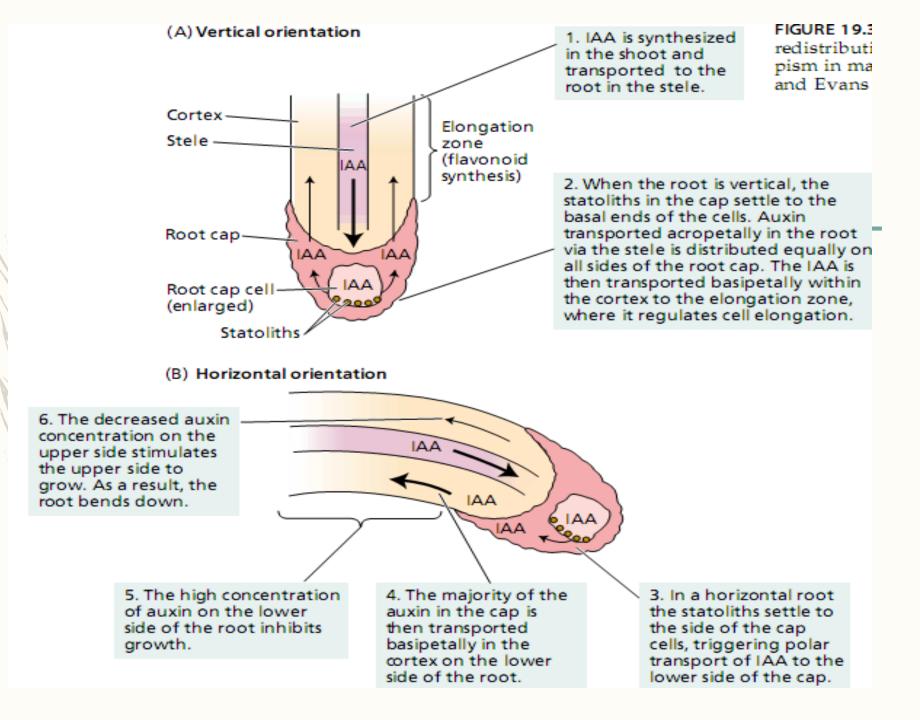


Amyloplasts tend to sediment in response to reorientation of the cell and to remain resting against the ER. When the root is oriented vertically, the pressure exerted by the amyloplasts on the ER is equally distributed.

In a horizontal orientation the pressure on the ER is unequal on either side of the vertical axis of the root.



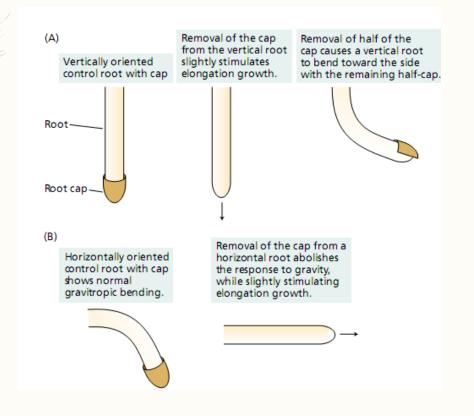
Statolith



Gravity perception without statoliths? An alternative

mechanism of gravity perception that does not involve statoliths has been proposed for the giantcelled freshwater Chara.

- Auxin Is Redistribution Laterally in the Root Cap
- In addition to functioning to protect the apical meristem as the tip penetrates the soil, the root cap is the site of gravity perception.
- a chemical messenger is presumed to be involved in communication between the cap and the elongation zone.
- cap produces a root growth inhibitor (IAA)



Other physiological effects of Auxin

- Regulates apical dominance
- Promotes formation of lateral roota and adv. Roots
- Delays onset of leaf abscission
- Regulates floral bud development
- Promotes fruit development
- Induces vascular differentiation

Synthetic Au Applications

- Rooting in cuttings
- Flowering in pineapple
- Prevention of leaf and fruit devlopment
- Induction of parthenocarpy
- 2.4-D & dicamba-commonly used

Molecular mechanism of Auxin action

- Au binds to the receptors at 3 locations 1)ER
 (ABP1-22kDa, dimer) 2) plasma membrane 3)
 tonoplast
- Au binding to ABP1 increases membrane voltage
 - --Ab, blocking H+ATPase block hyperpolarization response of Au
 - --some ABP1 Ab mimics the effect of Au by bringing about hyper polarization of tobacco mesophyll protoplasts, stimulation of H+ extrusion etc.

Signaling intermediates in Au action

- In cell cycle, Au stimulates synthesis of cyclin dependent protein kinase (CDK)
- CDK's together with their regulatory subunits, the cyclins regulate the transitions from G1 to S and from G2 to mitosis during cell cycle.
- MAPkinases that play a role in signal transduction by phosphorylating proteins in a cascade that ultimately activates transcription factors have also been implicated in auxin responses. (when tobacco cells are deprived of auxin, they arrest at the end of the G1or the G2 phase and cease dividing; if auxin is added back into the culture medium, the cell cycle resumes

Contd.....

- Ca+ (2,4-D, free Ca+ rose from 280-380nm)
- Auxin decreases cytosolic pH by 0.2 units, which inturn can promote the activity of H+-ATPase, which is active under low pH
- Auxin stimulates phospholipase A2(PLA2)

---fatty acids released from membrane phospholipids by PLA2 serve as substrate for lipoxygenase—the other product of the reaction lysophosphatidylcholine, can activate protein kinase (PLA2 pathway in Auxin action)

Auxin alters gene expression

Rapid changes in the levis of translatable mRNA Au induced genes fall into 2 classes

- --primary responsive genes (on binding of Au to receptor there is the activation of select group of preexisting transcription factors
- --pri responsive genes have 3 main functions
- 1) encodes protein that regulate transcription of sec response genes
- 2) involved in cell-cell signalling
- 3) involved in stress adaptation
- --5 major class of early Au responsive genes
- --Aux/IAA gene family, SAUR(small Au upregulated RNA's), GH3, ACC synthase, Glutathione-S-transferase (GST's)(a class of proteins stimulated by stress conditions)

Cytokinins

Discovery

1913-G.Haberlandt-austria-Vas. Tissue contains water soluble substance, stimulating the cell division in potato

Many substances tested to initiate and sustain cell poliferation - yeast extract, tomato juice, coconut milk (zeatin)

Kinetin – 1st cytokinin discovered, discovered as a DNA breakdown product

1940-50 - Skoog (Univ. Wisconsin)

Adenine (Aminopurine) - promotive effect

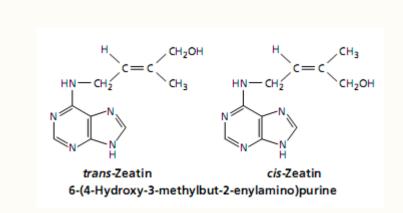
Autoclaved sperm DNA - promoting effect

Skoog & Miller – fractionating heat treated DNA – kinetin – adenine derivative (6furfurylaminopurine)

Zeatin - abudant natural cytokinin, from extracts of immature endosperm of zeamays-(Miller & Letham)

Cis & trans forms (regulated by isomerases)

All cytokinin compounds - N6 substituted aminopurines- ex. BAP, diphenyl urea

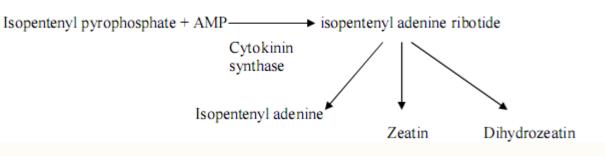


Bioassay

- 1) cell proliferation bioassay using tobacco pith tissue
- 2) cotyledonary expansion - raddish

Cytokinin occur in both free and bound forms Free- zeatin, dihydrozeatin & isopentenyladenine Bound - plant tRNA's contain zeatin as hyper modified base

Biosynthesis



Transport

Root to shoot via xylem along with water and minerals major site of synthesis - Root & shoot meristem, Young maize embryos, young developing leaves.

Transporeted as zeatin ribosides, once they reach leaf they are freed A signal from the shoot can regulate the cytokinin transport from root.

Cytokinins are rapidly metabolized by plant tissues Glucosidases Cytokin bases nucleotides/glucosides Active form (Storage forms, metabolically inactive) (germination initiated) -in dormant seed

Zeatin, Zeatin riboside Cytokinin oxidase denine or its derivatives (inactivates hormones, thus regulates it)

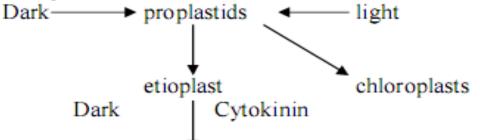


Biological roles

- 1) shoot apical meristems of cytokinin overproducing plants produce more leaves
- 3) >Chl
- 4) Adv. Shoots from leaf veins and petioles
- 5) Leaf senescence retarded
- 6) Apical dominace reduced
- 7) Cytokinin overproducing plants stunted & shortened internodes
- Rooting of stem cuttings reduced
- 9) Aux/cytokinin ratio regulates morphogenesis in cultured tissues
- 10) Modify apical dominance and promote lateral bud growth
- 11) Aux/cytokinin regulates plany cell cycle

CDK's (cyclin-the regulatroy subunit is regulated by cytokinin)

- 12) delays leaf senescence (controlled by root derived cytokinins to leaves)
- promotes nutrient mobilization (metabolism of traeted area stimulated, so nutrients move to that side – creates a new source-sink relationship)
- 14) promotes chloroplast maturation



Extensive grana, chl & Ps enz.

 promotes cell expansion in leaves and cotyledons (increase in mechanical extensibility of cell walls, not accompanied by H+ extrusion
 inhibits cell elongation in stems and roots Tobacco plants overexpressing the gene for cytokinin oxidase. Shoot growth is strongly

inhibited in the transgenics



Transgenics overproducing cytokinin oxidase

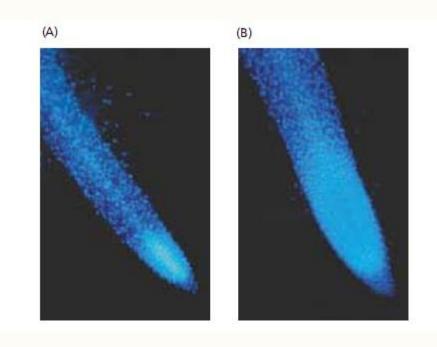


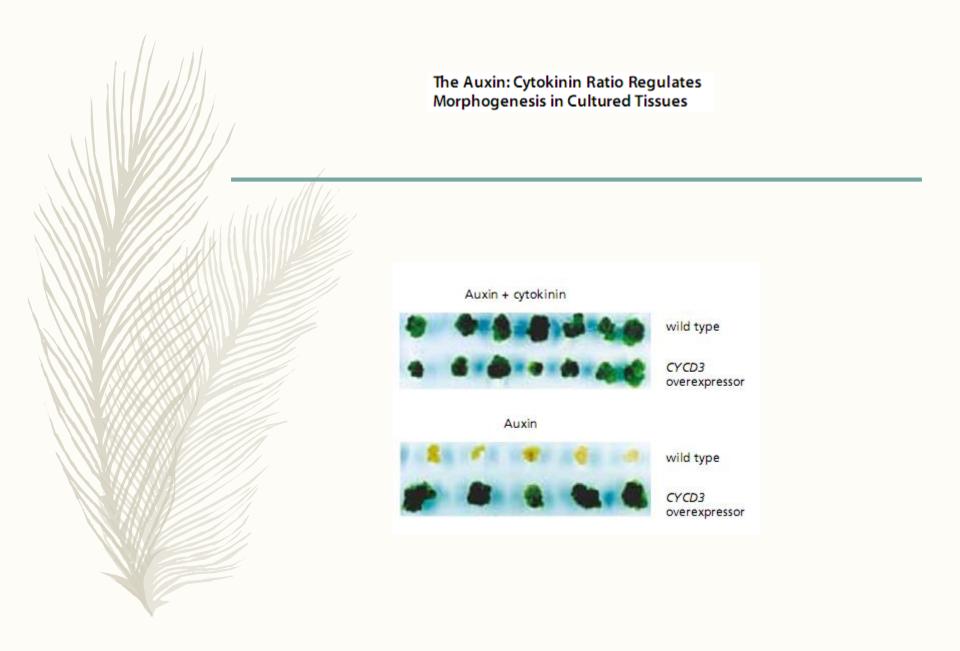
wild

Cytokinin suppresses the growth of roots.



Cytokinin suppresses the size and cell division activity of roots. (A) Wild type. (B) AtCKX1.





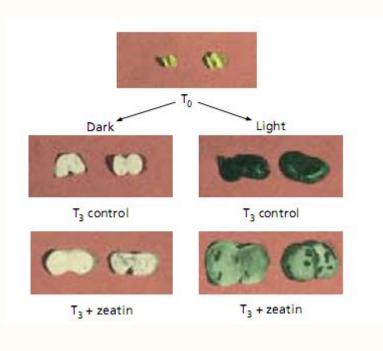
Leaf senescence is retarded in a transgenic tobacco plant containing a cytokinin biosynthesis gene, ipt.





ipt Age-matched control: een advanced senescence, tic no photosynthesis

The effect of cytokinin on the expansion of radish cotyledons.



Mode of Action

Cytokinin receptor

1) ZBP-67 Kda - high affinity for zeatin

2) CKI 1 (Cytokinin independent 1) - 125 Kda (similar to ETR 1)

Hormone binding _____induce histidine kinase activity _____ autophosphorylation of histidine

Cytokinin signal transduction involves Ca 2+ as a secondary messenger (Ca 2+ and calmodulin regulated protein kinases)

Increase mRNA level- soyabean 20 different mRNA's increase 2-20 fold within 4 h (of pri. responsive genes)

Construction of cDNA libraries from cytokinin treated and starved tissues Nitrate reductase induced by cytokinin (partially substitutes for light)

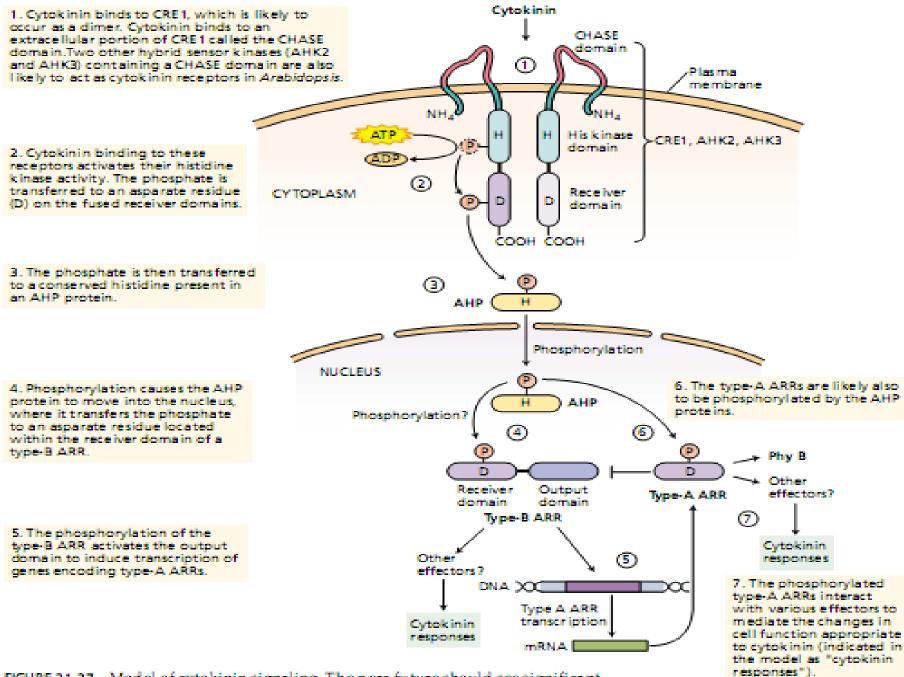
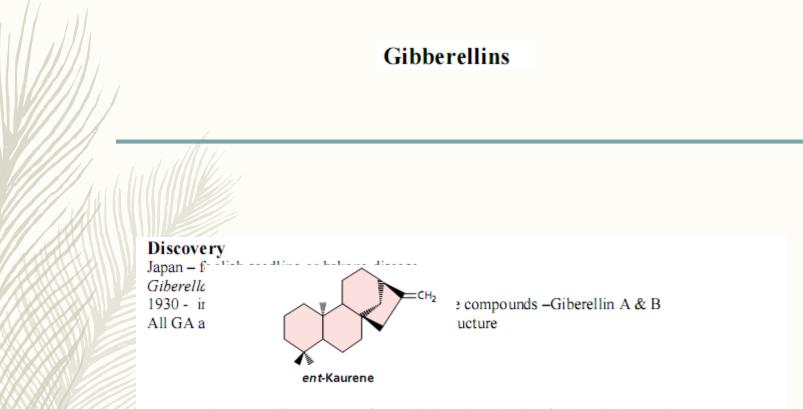
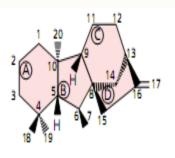


FIGURE 21.27 Model of cytokinin signaling. The near future should see significant refinement of this model, the tools are now in hand to analyze the interactions among these elements.



1958 – Jake Mac Millan – GA A₁ from a higher plant (seeds of *Phaseolus*) – seeds are better source

Numbered as GA_x in the order of their discovery (implies no close chemical similarity or metabolic reactions



t-giberellane structure

e C-19

ent-Gibberellane structure

Biosynthesis

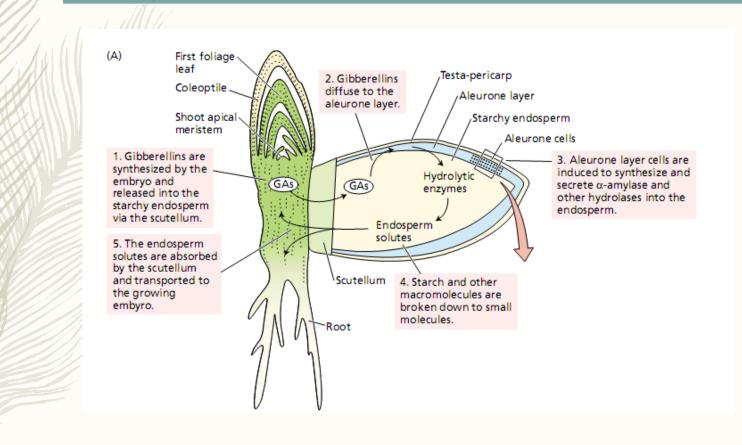
- 2 Terpenoid pathway
- 1) Mevalonic acid dependent pathway (precursor-mevalonic acid, in cytosol)
- Mevalonic acid independent pathway (precursor-GLAL-3-P & pyruvate, in chloroplasts)

GA are made up of 4 isoprene units – isoprene units are added succesively to produce geranyl pyrophosphate (C10), farnesyl pyrophosphate (C 15) and geranylgeranyl pyrophosphate (C 20)

Stage 1: Cyclization reactions GGPP_ ent – kaurene 2 enzs. Are localized in the proplastids of meristematic shoots AMO-1618, cycocel & Phosphon D are the specific inhibitors of this stage Stage 2: A methyl group oxidized to carboxylic group. Also one of the ring (B ring) contracts from a 6C to 5C ring – GA₁₂ aldehyde (1st GA formed and precursor to all other GA's) Enzs. Localized in ER Paclobutrazol inhibits this stage Stage 3: All steps catalyzed by enz. dioxygenase located in the cytosol 2 basic chemical changes hydroxylation at C-13 or C-3 or both A successive oxidation at C-20 (CH₂→CH₂OH→CHO), followed by a C loss of C-20

Bioassays

- Lettuce hypocotyl elongation bioassay
- 2) Dwarfrice leaf sheath bioassay
- 3) Barley aleurone layer alpha amylase bioassay



GA intermediates can be transported

High in developing seeds - decreases in mature seeds - mature seeds contain GA₁₂ aldehyde (the immediate GA precursor) - GA synthesis at young, growing buds, leaves & upper internodes – transported via phloem.

Initial steps of GA biosynthesis may occur in one tissue and metabolism to active GA in another

Ex. Intermediates of GA are transported from meristematic shoot tissues to green leaves

GA biosynthesis regulated

- 1) Photoperiod
 - LDP transfer from Sd to Ld shows alteration in GA metabolism
 - In LDp kept at SD, level of 13-hydroxylated GA relatively low, on transfer to LD, the levels of 13-hydroxylated GA pathway increases
- 2) Temperature: Vernalization
- 3) Feed Back control
- 4) By conjugation with sugars: GA glycosides

Effects of GA on growth and development

- stimulates stem growth in dwarf and rosette plants (target of GA action in the intercalary meristem)
- 2) regulates transition from juvenile to adult phase
- 3) influence floral initiation and sex determination
- 4) promotes fruit set (ex. apple)
- 5) promotes seed germination

Commercial applications

Commercial applications

- fruit production; increase stalk length of grapes, citrus delays senescence
- 2) malting of barley
- 3) increasing sugar cane vields
- 5) in plant breeding induce cones in conifers
- 6) GA synthesis inhibitors in floral crops

Mechanism of GA action

1) Promoting stem growth

-Mitosis increases markedly in the subapical region of the meristem. Stimulation of internode elongation is partly due to increased cell division activity in the intercalary meristem. Regulates the cell cycle in intercalary meristem

-Increase cell wall extensibility: XET - xyloglucan endotransglycosylase (activity increases), facilitates the penetration of expansins into the cell wall

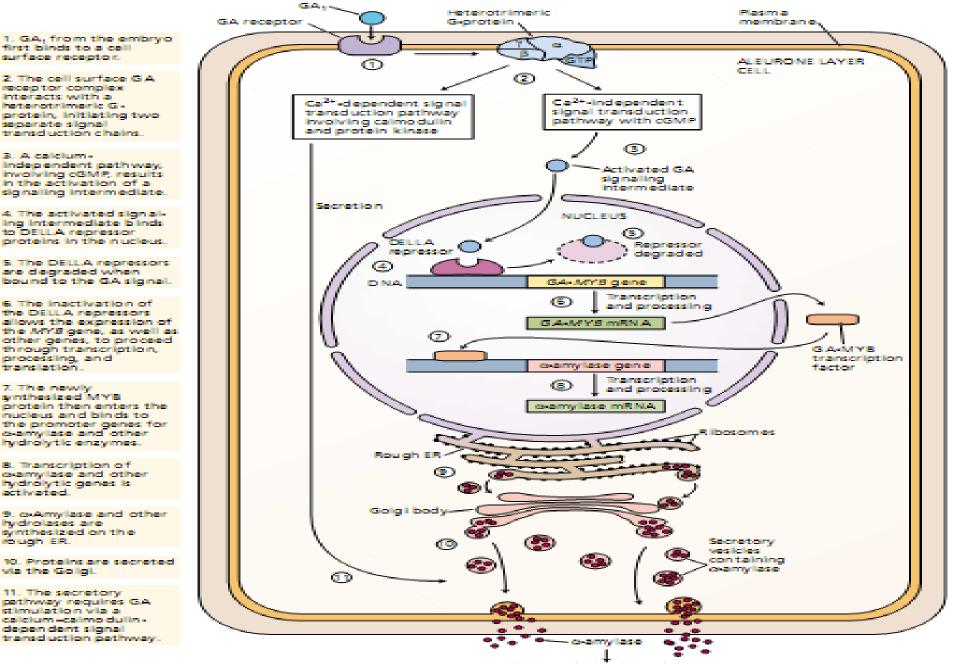
2) Mobilizing endosperm reserves

Mode of action

Receptors are localized on the membrane

GA – receptor – interaction with G protein - stimulates GDP/GTP exchange by heterotrimeric G proteins - induces the synthesis & secretion of alpha amylase gene.





Starch degradation in endosperm



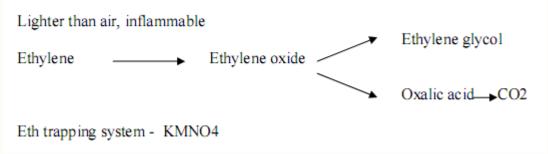
19th century – coal gas – illumination, street lamps
 Dimitry (Graduate student at St. Petersburg) – 1901 - triple response in pea seedlings (reduced stem elongation, increased lateral growth and abnormal horizontal growth)
 1910 - Cousins – oranges – premature ripening of bananas

Biosynthetic regions and period

Meristematic and nodal regions are most active

during fruit ripening, leaf senescence, abscission, wounding, stresses

Properties



| Biosynth Methioni | | , | Eth | |
|----------------------|----------------|------------------|-----------------|---|
| AdoMet | (S-adenosy | 'l methionine)_ | ACC Synthase | ACC (1-aminocyclopropane-1- carboxylic acid) |
| | antis | era, expressed i | n e. coli, sho | l factors, labile (unstable), purified using owed similarity to aminotransferase l by multigene family) |
| | ACC oxidase | Eth | Fe2+/ascor | bate oxidase family) |

Conjugated forms

N-malonyl ACC GACC (1-(V-L-glutamyl-amino) cyclopropane – 1- carboxylic acid

ACC synthase gene---antisense DNA

Eth production

- Fruit ripening > ACC, ACC oxidase, ACC synthase
- 2) Stress induced wounding, chilling, flooding, drought > Acc synthase

Auxin induced - > ACC synthase

Inhibitors of Eth production

Adomet \longrightarrow ACC (inhibited by AVG (aminoethoxyvinylglycine), AOA (aminooxyacetic acid) ACC \longrightarrow Eth (inhibited by CO²⁺)

Eth action inhibited by Ag+ of AgNO₃, CO₂ (5-10%)

Trans-cyclooctene, competitive inhibitor to the ethylene for binding to the receptor

Developmental and physiological effects

- 1) promotes fruit ripening
- 2) leaf epinasty when ACC from root is transported to shoot
- 3) Induces lateral cell expansion triple response pea
- 4) Hooks of dark grown seedlings interaction between eth and phytochrome
- Breaks seed and Bud dormancy cereals, peanuts, sprouting in potato tubers and other bulbs
- 6) Promotes elongation of submerged aquatic sps. ranunculus, nymphodes, deepwater rice (in absence of O2 eth synthesis is diminished but loss of eth by diffusion is retarded. In deep water rice eth stimulates internode elongation by increasing sensitivity of cells of intercalary meristem to GA)
- 7) Induce root and root hairs (High concentration required 10 ul/l)
- Induce flowering in pineapple (synchronization of fruit set, flowering initiation in mango, promotion of female flowers in cucumber)
- Enhances leaf senescence rate (senescence regulated by balance of eth and cytokinin)
- 10) Eth in abscission zone is regulated by Auxin

Triple response of etiolated pea seedlings. seedlings show a radial swelling, inhibition of elongation of the epicotyl, and horizontal growth of the epicotyl (diagravitropism).



Epinasty, or downward bending of the tomato leaves. Epinasty results when the cells on the upper side of the petiole grow faster than those on

the bottom.

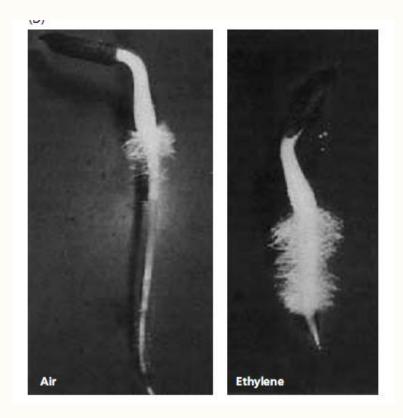


Inhibition of flower senescence by inhibition of ethylene action. Carnation flowers were held indeionized water for 14 days with (left) or without (right)silver thiosulfate (STS), a potent inhibitor of ethylene action.Blocking of ethylene results in a marked inhibition of floral

senescence.



Promotion of root hair formation by ethylene in lettuce seedlings.

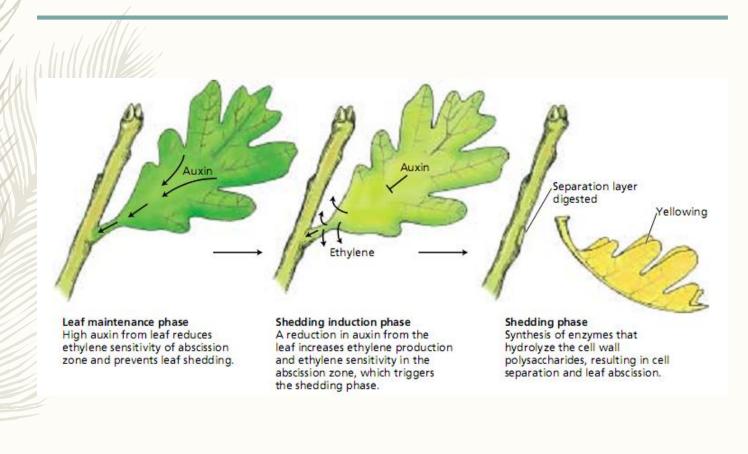


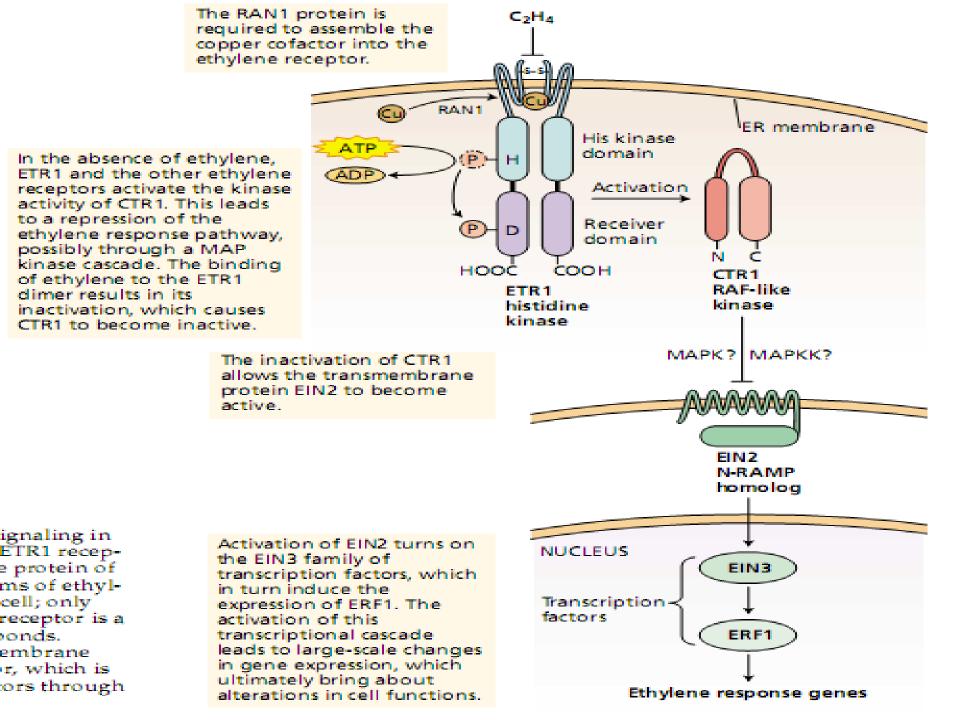
Effect of ethylene on abscission in birch (Betaul pendula). The plant on the left is the wild type; the plant on the right was transformed with a mutated version of the Arabidopsis ethylene

receptor, ETR1-1.



Schematic view of the roles of auxin and ethylene during leaf abscission.





Eth is difficult to apply in field as gas Eth releasing compound – ethephon – 2-chloroethyl phosphonic acid (trade name: Ethrel) – ACC and Auxins can trigger natural biosynthesis of ethylene Ethephon – hastens fruit ripening (apple and tomato), degreening of citrus, synchronize fruiting in pineapple, accelerates abscission of flowers and fruits, promotes female sex expression in cucumber

Commercial Uses

Ag+, Avg, transcyclooctene (anti eth)

Transgenic - antisense version of ACC synthase and ACC oxidase

Mode of Action

Eth - receptor-signal transduction pathway - protein

Increases m RNA levels – for cellulase, chitinase, B-1,3-glucanase, peroxidase, chalcone synthase (key enz. in flavanoid synthesis), a pathogenesis related protein, ripening related genes, synthesis related genes

Eth responsive elements (ERE's) -regulatory sequence – GCCGCC repeat motif -ERE binding proteins (EREBP's) (eth pri. response gene products)

Fruit ripening – regulated by eth dependent (lycopene & aroma biosynthesis, respiratory metabolism and ACC synthase & independent pathways (chlorophyllase, ACC oxidase, polygalactouranase)