



Auxin: The First Discovered Plant Growth Hormone

Contents

- What is hormone ?
- Types of hormone
- Plant's hormone
- Introduction to Auxin
- Discovery of Auxin
- The Principle Auxin: Indole-3-Acetic acid
- IAA Synthesis
- Auxin Transport

Contents

- Chemiosmotic model of molar Auxin transport
- Auxin influx
- Auxin efflux
- Auxin signal transduction pathway
- Action of Auxin
- Cell elongation
- Plant tropisms
- Developmental effects of Auxin
- Summary
- References

WHAT IS HORMONE ?

- Hormones are chemical messengers that are produced in one cell and modulate cellular processes in another cell by interacting with specific proteins that function as receptors linked to cellular transduction pathway.

Types of hormones

Endocrine hormones

- Hormones that are transported to sites of action in tissues distant from their site of synthesis are referred to as endocrine hormones

Paracrine hormones

- Hormones that act on cells adjacent to the source of synthesis are referred to as paracrine hormones.

Plant hormones

- The plant development is regulated by six major hormones:
- Auxins
- Gibberellins
- Cytokinins
- Ethylene
- Abscisic acid
- Brassinosteroids

Plant hormones

- There are some signaling molecules (hormones) like jasmonic acid, salicylic acid and small polypeptides that play roles in resistance to pathogens and defence against herbivores.
- Strigolactone regulates the outgrowth of lateral buds.

Introduction to Auxin

- First growth hormone to be studied in plants
- Play important role in growth and development of plant
- Developmental processes like stem elongation, apical dominance, root initiation, fruit development, meristem development is controlled by auxin

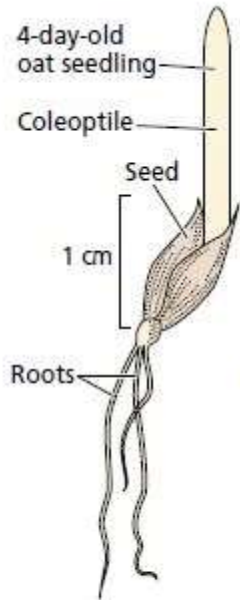
Introduction to Auxin

- Auxin and cytokinin are required for viability of the plant embryo
- Whereas other hormones act as regulators of discrete developmental processes

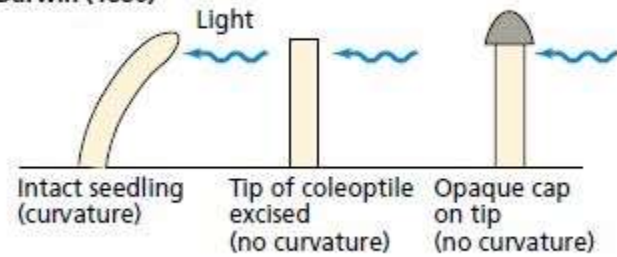
The Emergence of the Auxin concept

- Charles Darwin and his son Francis studied plant growth phenomena involving tropisms
- Their interest of study was the bending of plant towards light
- The phenomenon, which is caused by differential growth, is called phototropism
- Seedlings of canary grass (*Phalaris canariensis*)
- Young leaves are in protective organ called coleptile

- Coleoptiles are highly sensitive to light
- The power of movement in plants 1881
- 1926 Frits Went's studied growth promoting chemicals in tips of *Avena sativa*

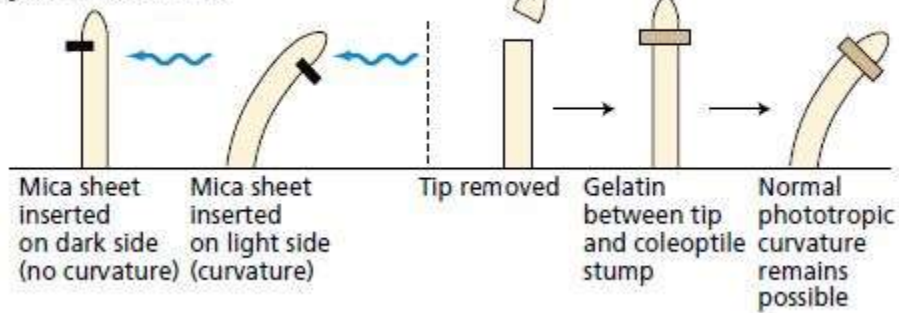


Darwin (1880)



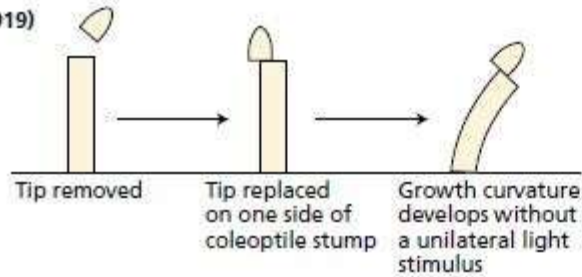
From experiments on coleoptile phototropism, Darwin concluded in 1880 that a growth stimulus is produced in the coleoptile tip and is transmitted to the growth zone.

Boysen-Jensen (1913)



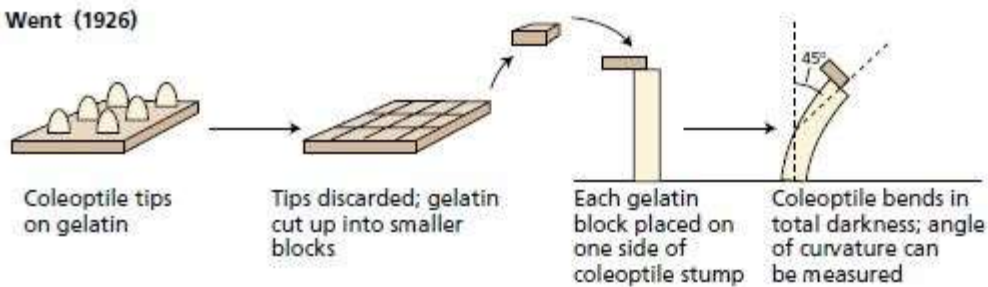
In 1913, P. Boysen-Jensen discovered that the growth stimulus passes through gelatin but not through water-impermeable barriers such as mica.

Paál (1919)



In 1919, A. Paál provided evidence that the growth-promoting stimulus produced in the tip was chemical in nature.

Went (1926)



In 1926, F. W. Went showed that the active growth-promoting substance can diffuse into a gelatin block. He also devised a coleoptile-bending assay for quantitative auxin analysis.

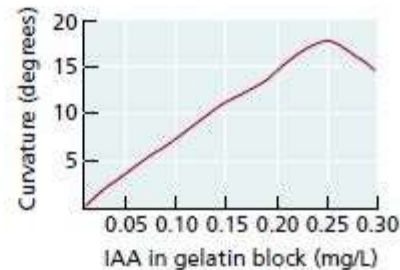
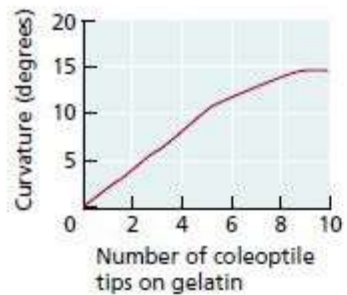
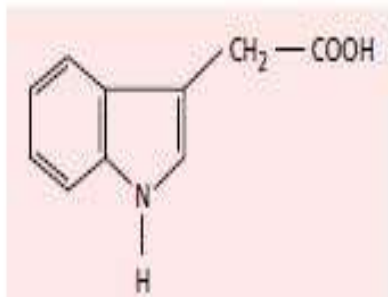


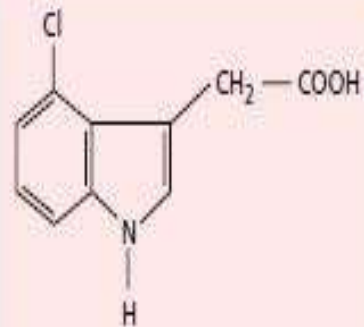
FIGURE 19.1 Summary of early experiments in auxin research.

The Principle Auxin: Indole-3-Acetic acid

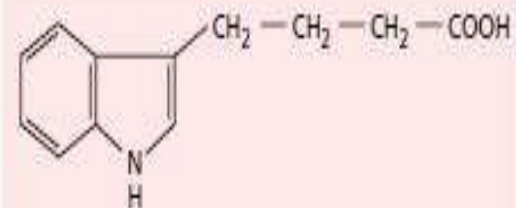
- In the mid 1930, it was determined that natural auxin is indole-3-acetic acid
- Various other auxins are found in plant
- Auxins are the compound with biological activities similar to those IAA
- Cell elongation in coleptile and stem section
- Cell division in callus cultures
- Formation of adventitious roots on detached leaves and stem



Indole-3-acetic acid
(IAA)



4-Chloroindole-3-acetic acid
(4-Cl-IAA)



Indole-3-butyric acid
(IBA)

FIGURE 19.3 Structure of three natural auxins. Indole-3-acetic acid (IAA) occurs in all plants, but other related compounds in plants have auxin activity. Peas, for example, contain 4-chloroindole-3-acetic acid. Mustards and corn contain indole-3-butyric acid (IBA).

IAA synthesis

- IAA biosynthesis is associated with rapidly dividing and growing tissues
- Primary site of auxin synthesis are shoot apical meristem and young leaves
- Root apical meristem also synthesize auxin
- Young fruits and seeds also have high level of auxin

- In Arabidopsis, auxin accumulate at tip of young leaves primodia
- In developed leaves move towards the margin
- Then shift at base of leaf and finally lamella center

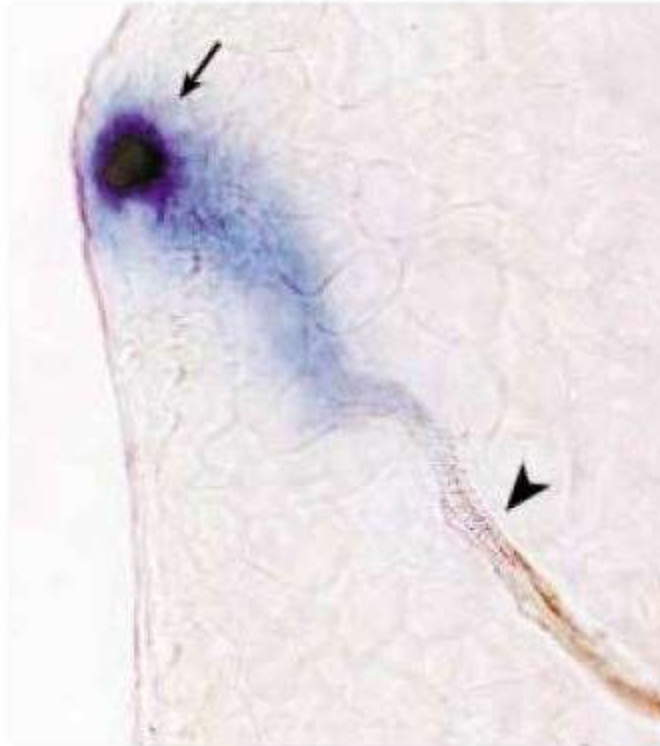


FIGURE 19.5 Detection of sites of auxin synthesis and transport in a young leaf primordium of *DR5 Arabidopsis* by means of a *GUS* reporter gene with an auxin-sensitive promoter. During the early stages of hydathode differentiation, a center of auxin synthesis is evident as a concentrated dark blue *GUS* stain (arrow) in the lobes of the serrated leaf margin. A gradient of diluted *GUS* activity extends from the margin toward a differentiating vascular strand (arrowhead), which functions as a sink for the auxin flow originating in the lobe. (Courtesy of R. Aloni and C. I. Ullrich.)

- GUS reporter gene is important analytical tool as its activity and location in the tissue can be visualized by treating the tissue with a substrate that produces a blue color when hydrolysed by the GUS enzyme
- When GUS gene fused to a DNA promoter sequence that respond to auxin, the distribution can be find out

- IAA is related to amino acid tryptophan and indol-3-glycerol phosphate-both are precursor for IAA synthesis

Auxin transport

- Transport polarly
- Main axes of shoots and roots have apex-base structural polarity
- This structural polarity is dependent on the polarity of auxin transport
- Went discovered that IAA moves mainly from the apical to basal end
- This type of unidirectional transport is termed polar transport

Polar transport require energy and is gravity independent

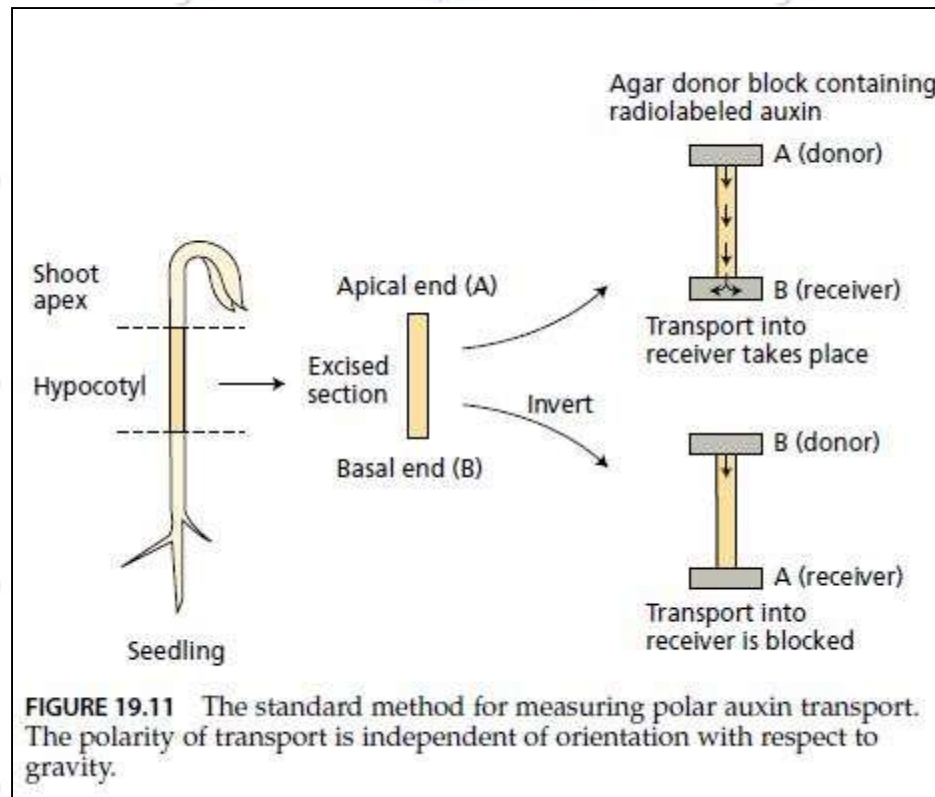




FIGURE 19.12 Roots grow from the basal ends of these bamboo sections, even when they are inverted. The roots form at the basal end because polar auxin transport in the shoot is independent of gravity. (Photo ©M. B. Wilkins.)

Polar transport require energy and is gravity independent

- Polar transport proceeds in a cell to cell fashion
- The auxin exits the cell through the plasma membrane, diffuses across the compound middle lamella and enters the next cell through its plasma membrane
- Export of auxin from cell is termed auxin efflux
- Entry of auxin into cell is called auxin uptake or influx
- The overall process requires metabolic energy

Polar transport require energy and is gravity independent

- The velocity of polar auxin transport can exceed 3mmh^{-1}
- faster than the rate of diffusion
- slower than phloem translocation rates
- Polar transport is also specific for active auxins, both natural and synthetic.
- Neither inactive auxin analogs nor auxin metabolites are transported polarly,
- polar transport involves specific protein carriers on the plasma membrane that can recognize the hormone and its active analogs.

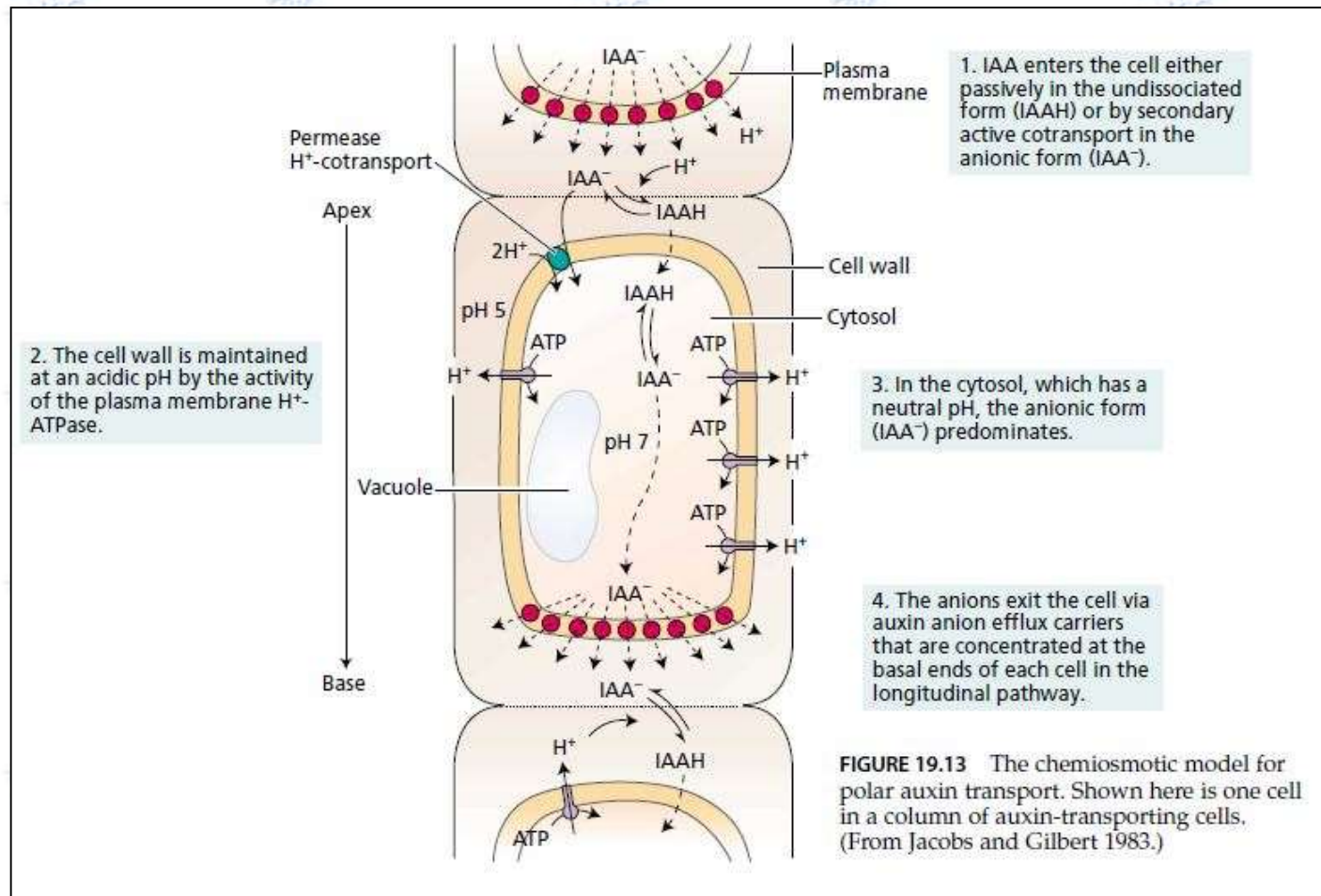
Polar transport require energy and is gravity independent

- The major site of basipetal polar auxin transport in stems and leaves is the vascular parenchyma tissue.
- Acropetal polar transport in the root is specifically associated with the xylem parenchyma of the stele

A Chemiosmotic Model Has Been Proposed to Explain Polar Transport

- The discovery in the late 1960s
- According to the chemiosmotic model for polar auxin transport,
- auxin uptake is driven by the proton motive force ($\Delta E + \Delta pH$) *across the plasma membrane*,
- while auxin efflux is driven by the membrane potential, ΔE .

A Chemiosmotic Model Has Been Proposed to Explain Polar Transport



1. IAA enters the cell either passively in the undissociated form (IAAH) or by secondary active cotransport in the anionic form (IAA⁻).

2. The cell wall is maintained at an acidic pH by the activity of the plasma membrane H⁺-ATPase.

3. In the cytosol, which has a neutral pH, the anionic form (IAA⁻) predominates.

4. The anions exit the cell via auxin anion efflux carriers that are concentrated at the basal ends of each cell in the longitudinal pathway.

FIGURE 19.13 The chemiosmotic model for polar auxin transport. Shown here is one cell in a column of auxin-transporting cells. (From Jacobs and Gilbert 1983.)

PIN and ABCB transporters regulate cellular auxin

- PIN proteins(named after the pin-shaped inflorescences formed by the *pin1* mutant of *Arabidopsis*; Figure 19.14A) are localized to the membrane of ER
- Regulate IAA metabolite formation by transporting IAA into the ER

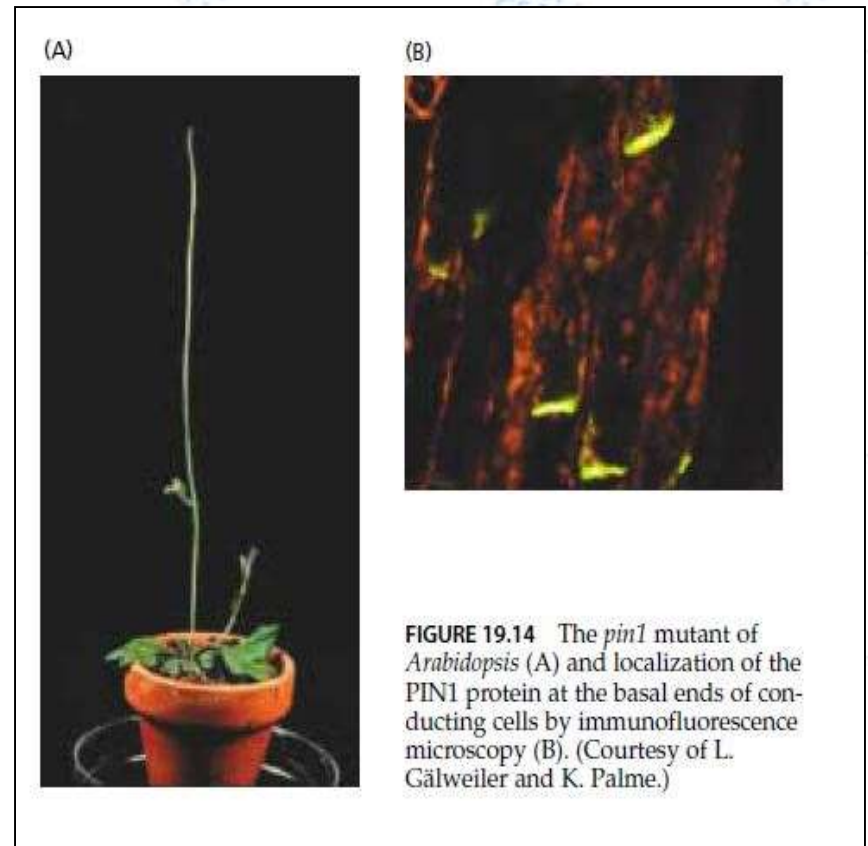


FIGURE 19.14 The *pin1* mutant of *Arabidopsis* (A) and localization of the PIN1 protein at the basal ends of conducting cells by immunofluorescence microscopy (B). (Courtesy of L. Gälweiler and K. Palme.)

PIN and ABCB transporters regulate cellular auxin

- ABCB4 is a PGP-type transporter
- Regulate auxin level into root hair cells
- At low auxin concentration, it appear to function in the cellular uptake

Inhibitors of Auxin Transport Block Auxin Efflux

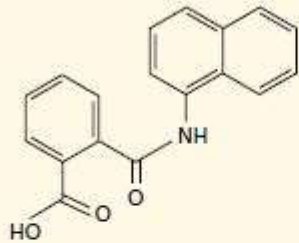
- Several compounds have been synthesized that can act as **auxin transport inhibitors (ATIs)**, including
 - **NPA (1-Nnaphthylphthalamic acid)**
 - TIBA (2,3,5-triiodobenzoic acid)
 - CPD(2- carboxyphenyl-3-phenylpropane-1,3-dione)
 - NOA (naphthoxyacetic acid)
 - Gravacin

Inhibitors of Auxin Transport Block Auxin Efflux

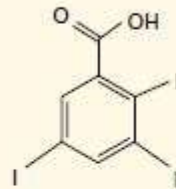
- NPA, TBA, CPD and gravacina are auxin efflux inhibitors
- NOA is an auxin influx inhibitor
- AEs can interfere with the trafficking of the plasma membrane proteins to which they bind, apparently by altering protein-protein interactions.

Inhibitors of Auxin Transport Block Auxin Efflux

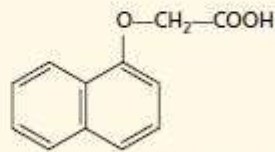
Auxin transport inhibitors not found in plants



NPA (1-N-naphthylphthalamic acid)

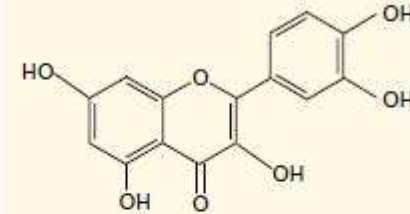


TIBA (2,3,5-triodobenzoic acid)

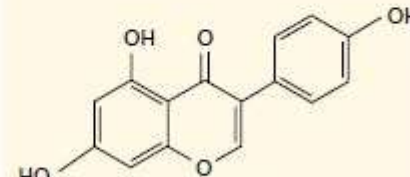


1-NOA (1-naphthoxyacetic acid)

Naturally occurring auxin transport inhibitors



Quercetin (flavonol)



Genistein

FIGURE 19.16 Structures of auxin transport inhibitors.

Role of protein trafficking

- The plasma membrane localization of auxin transport proteins involves the movement of newly synthesized proteins through the endomembrane secretory system
- the PIN1 protein is localized at the basal ends (top) of root cortical parenchyma cells
- Treatment of *Arabidopsis seedlings with brefeldin A* (BFA), which causes Golgi vesicles and other endosomal compartments to aggregate near the nucleus, causes PIN to accumulate in these abnormal intracellular compartments(see Figure 19.17B).

Role of protein trafficking

- When the BFA is washed out with buffer, the normal localization on the plasma membrane at the base of the cell is restored (see Figure 19.17C).
- When cytochalasin D, an inhibitor of actin polymerization, is included in the buffer washout solution, normal relocation of PIN to the plasma membrane is prevented (see Figure 19.17D).

Role of protein trafficking

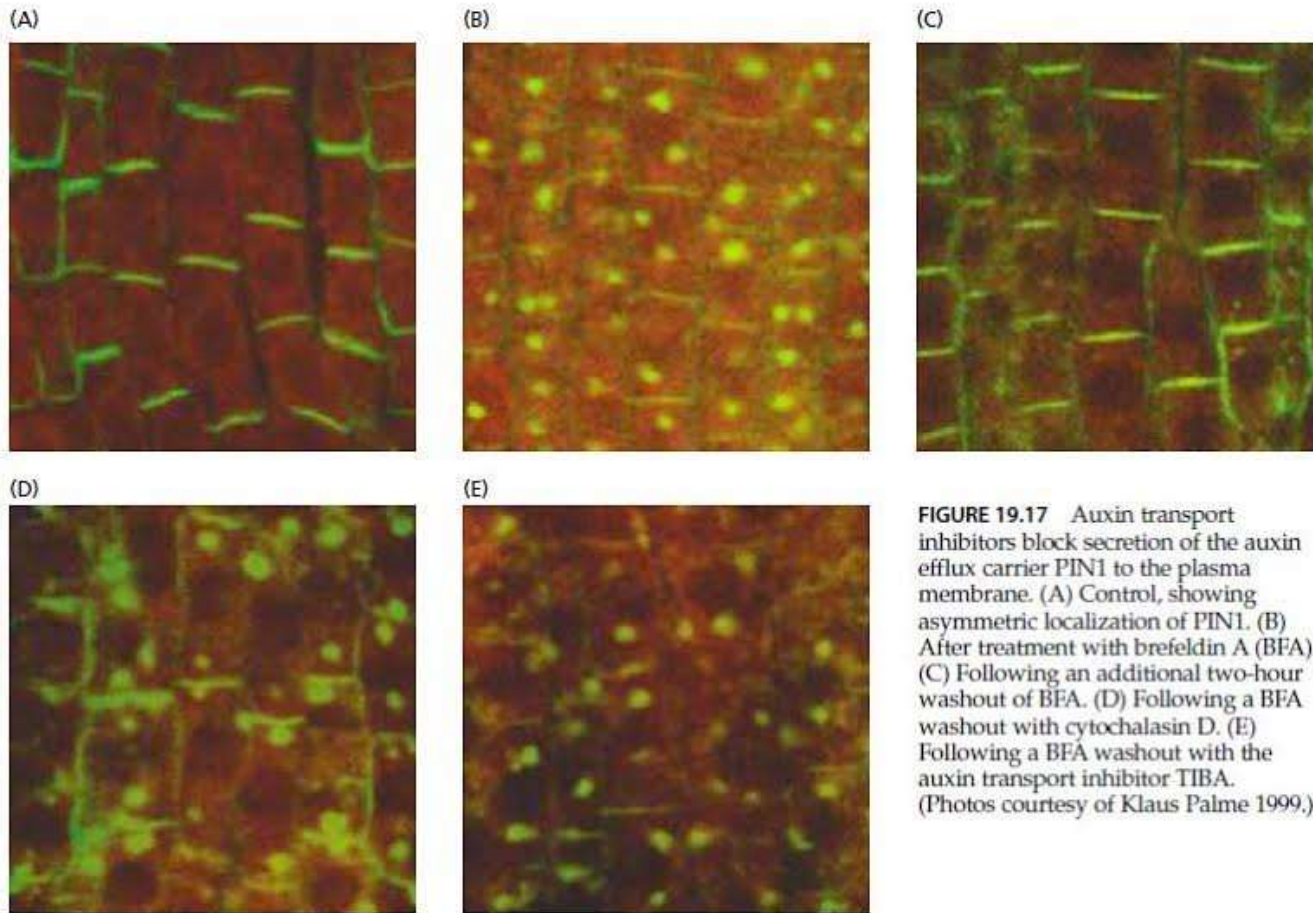


FIGURE 19.17 Auxin transport inhibitors block secretion of the auxin efflux carrier PIN1 to the plasma membrane. (A) Control, showing asymmetric localization of PIN1. (B) After treatment with brefeldin A (BFA). (C) Following an additional two-hour washout of BFA. (D) Following a BFA washout with cytochalasin D. (E) Following a BFA washout with the auxin transport inhibitor TIBA. (Photos courtesy of Klaus Palme 1999.)

Role of protein trafficking

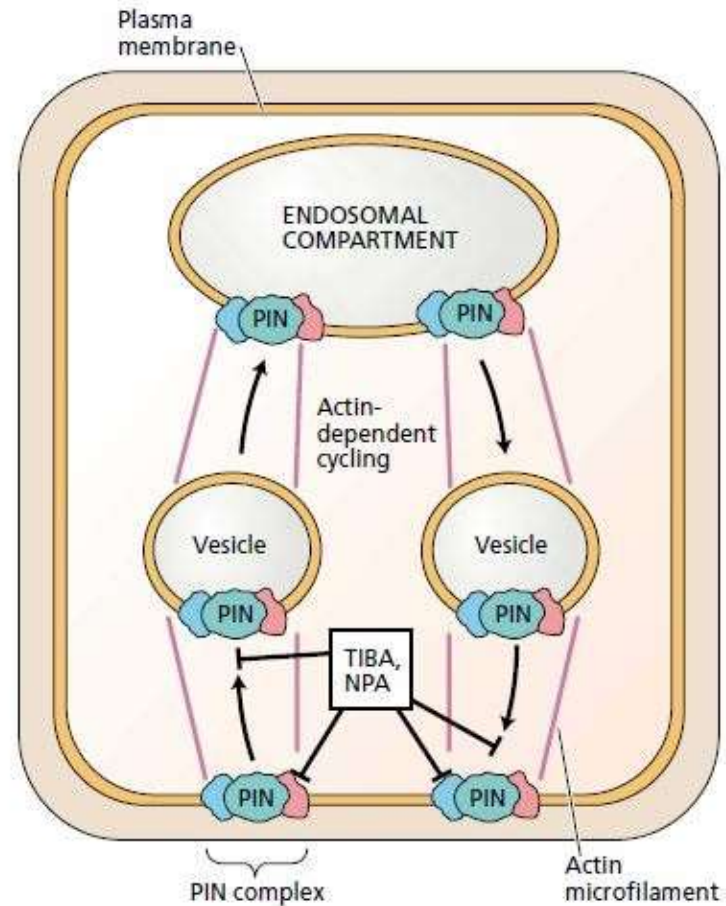
- These results indicate that PIN is rapidly cycled between the plasma membrane at the base of the cell and an unidentified endosomal compartment by an actin-dependent mechanism
- High concentration of AEs like TIBA and NPA added to the washout buffer, they prevented the normal relocalization of PIN on the plasma membrane

Role of protein trafficking

- TIBA and NPA altered the endocytotic cycling of the plasma membrane H-ATPase and other proteins.
- TIBA and NPA must also be able to directly inhibit the transport activity of PIN complexes on the plasma membrane—by binding either to PIN (as TIBA does) or to one or more regulatory proteins (as NPA does)

Role of protein trafficking

FIGURE 19.18 Actin-dependent PIN cycling between the plasma membrane and an endosomal compartment. Auxin transport inhibitors TIBA and NPA both interfere with relocalization of PIN1 proteins to basal plasma membranes after BFA washout (see Figure 19.17). This suggests that both of these auxin transport inhibitors interfere with PIN1 cycling.



AUXIN SIGNAL TRANSDUCTION PATHWAYS

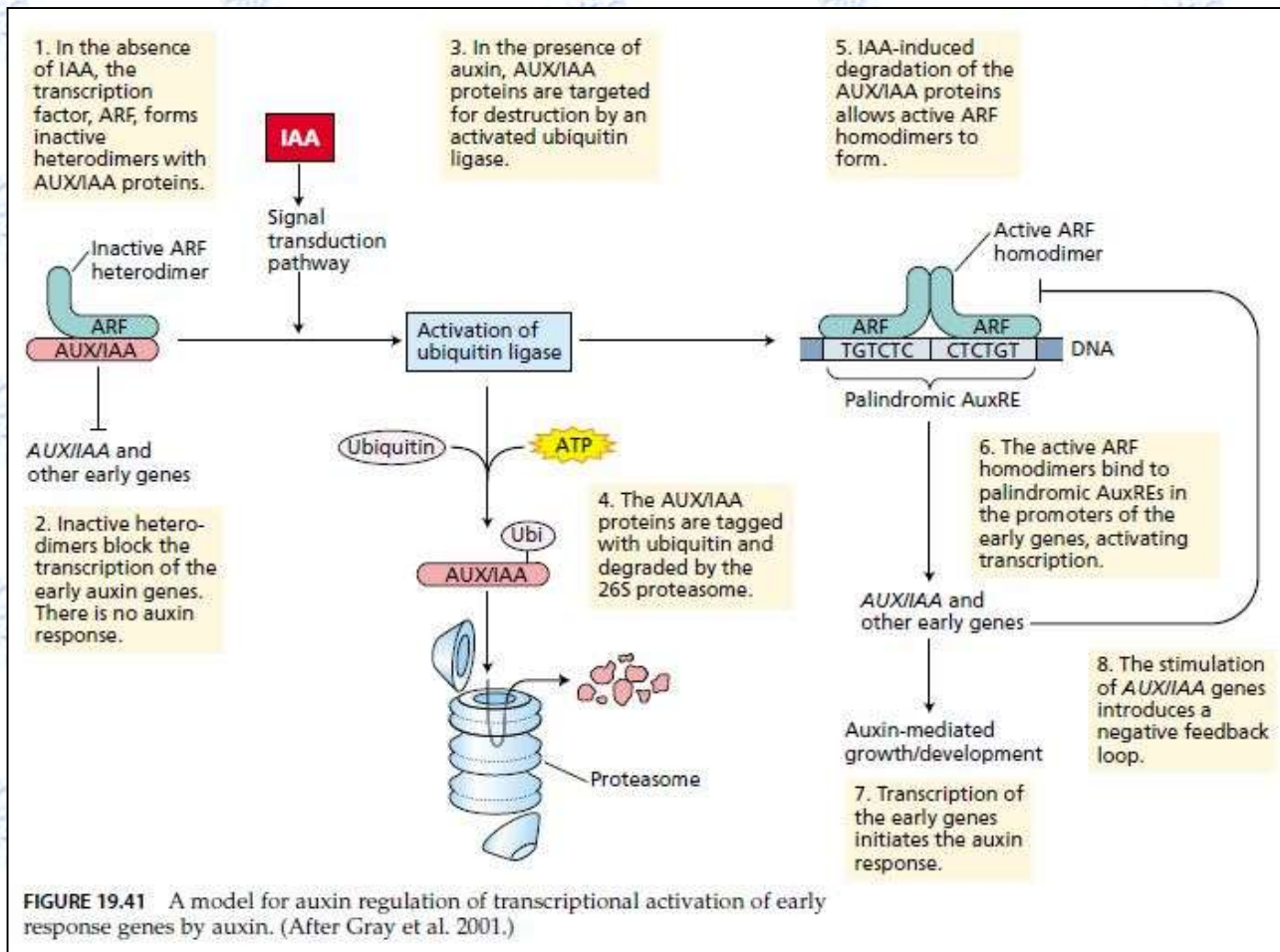
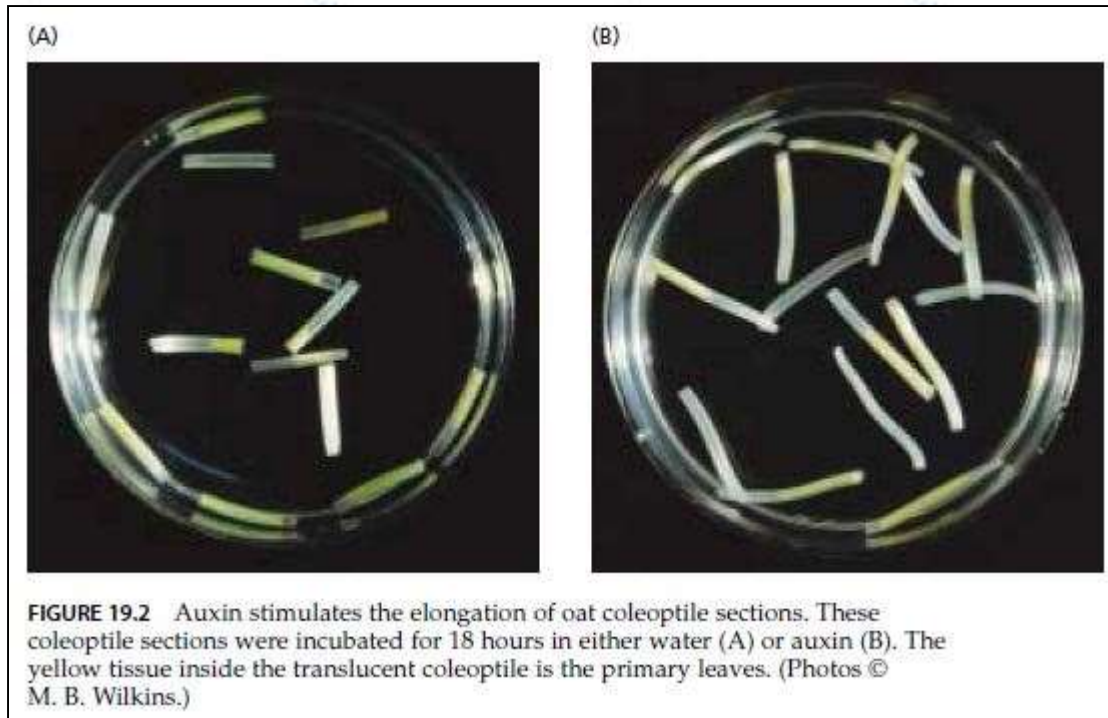


FIGURE 19.41 A model for auxin regulation of transcriptional activation of early response genes by auxin. (After Gray et al. 2001.)

CELL ELONGATION

- Auxin was discovered as the hormone involved in the bending of coleoptiles toward light
- Auxins Promote Growth in Stems and Coleoptiles, While Inhibiting Growth in Roots
- Auxin transport basipetally from shoot apex to the tissue below
- Auxin causes continued elongation of these cells

CELL ELONGATION



Action of auxin: Plant tropism

- 3 main guidance systems control the orientation of the plant axis
- Phototropism
- Gravitropism
- Thigmotropism

Phototropism is mediated by the lateral redistribution of auxin

- Charles and Francis Darwin provided the first clue concerning the mechanism of phototropism.
- When a shoot is growing vertically, auxin is transported polarly
- Auxin can also be transported vertically
- Cholodny- went model of phototropism
- Perception of a unilateral light stimulus
- A decrease in basipetal IAA transport and diversion to lateral transport in response to the phototropic stimulus

Phototropism is mediated by the lateral redistribution of auxin

- Precise sites of auxin production, light perception and lateral transport have been difficult to define.
- In maize coleoptiles auxin accumulation zone is upper 1-2mm of tip, the zones of photosensing and lateral transport are within the upper 5mm of the tip.
- Similar zones are observed in true shoots of all monocots and dicots.
- Two flavoproteins, Phototropin 1 and 2 are photoreceptors for the blue light signaling pathway.
- The action spectrum for blue light activation of kinase activity matches the action spectrum for phototropism.

Phototropism is mediated by the lateral redistribution of auxin

- Phototropin 1 displays a lateral gradient in phosphorylation.
- Phototropin phosphorylation results in dissociation of plasma membrane proteins
- In coleoptiles, phototropin phosphorylation gradient induce the lateral movement of auxin.

Phototropism is mediated by the lateral redistribution of auxin

- Agar block\ coleoptiles curvature bioassay supported the colodny-went models prediction.
- Acidification of the apoplast plays a role in phototropic growth.
- Phototropic auxin movement involves inhibition of ABCB 19, destabilization of PIN1 and inhibition\ or relocalization of laterally localized PIN3 proteins

Phototropism is mediated by the lateral redistribution of auxin

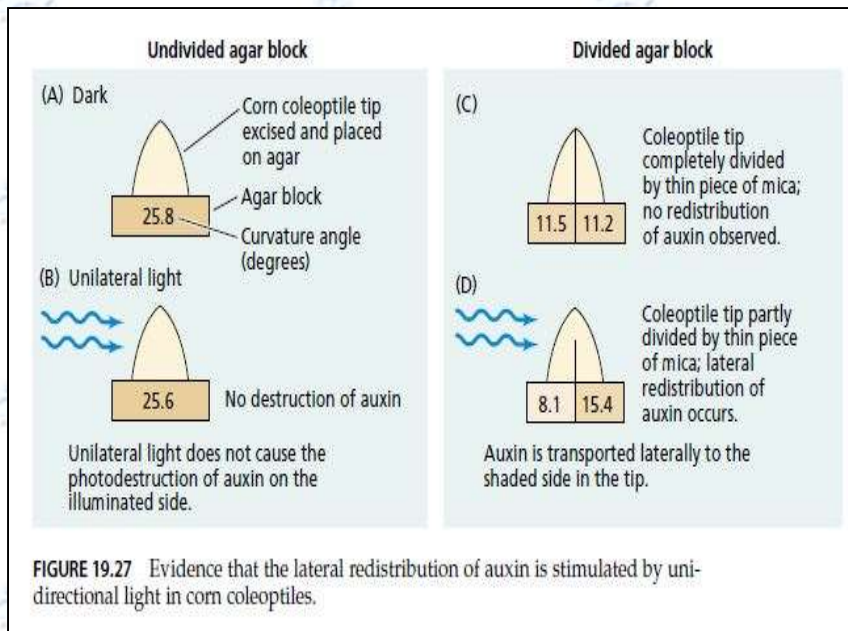


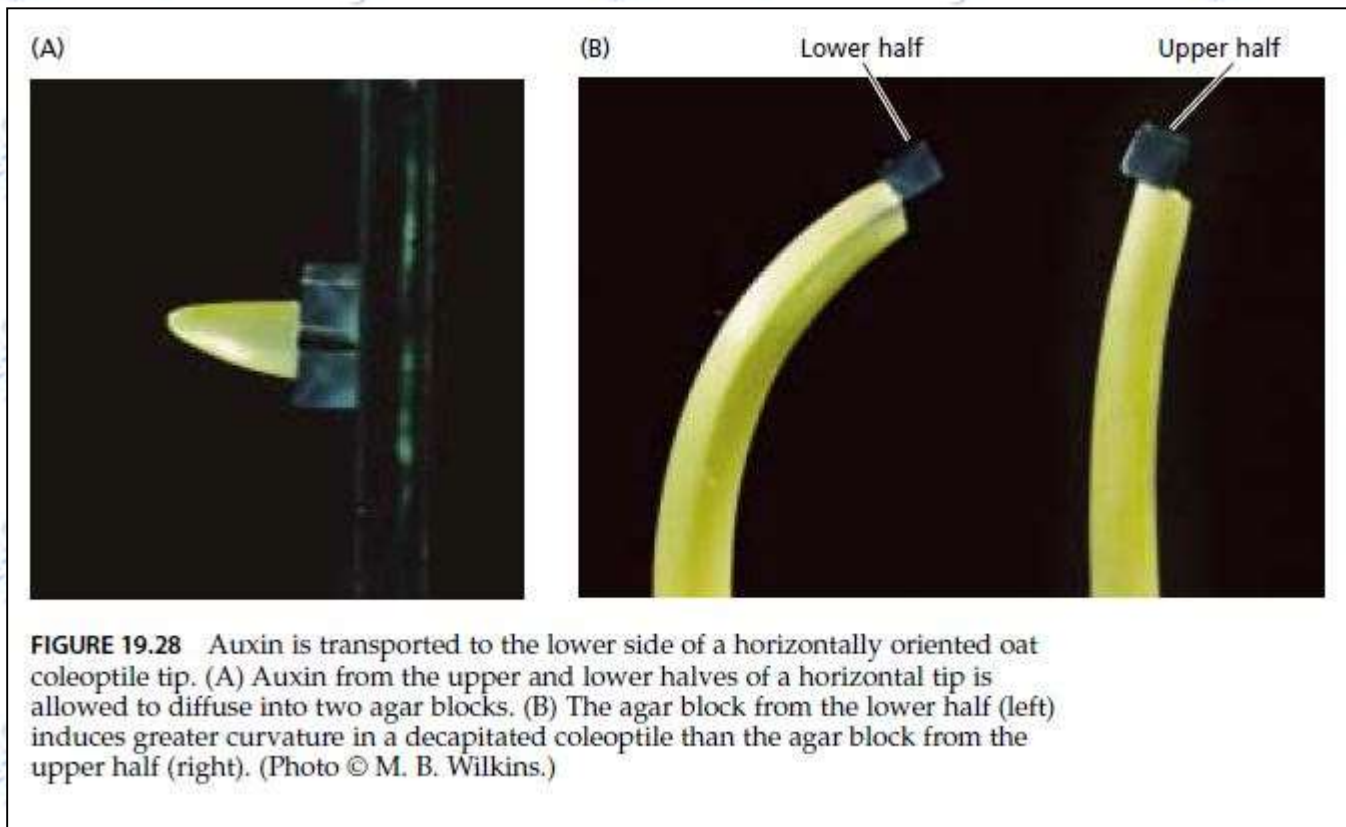
FIGURE 19.29 Lateral auxin gradients are formed in *Arabidopsis* hypocotyls during the differential growth responses to light (A) and gravity (B). The plants were transformed with the *DR5::GUS* reporter gene. Auxin accumulation on the shaded (A) or lower (B) side of the hypocotyls is indicated by the blue staining shown in the insets. (Photos courtesy of Klaus Palme.)



Gravitropism involves lateral redistribution of auxin

- When dark grown *Avena* seedlings are oriented horizontally, the coleoptiles bend upwards in response to gravity.
- Tissues below the tip are able to respond to gravity as well.
- Lateral distribution of auxin is more difficult to demonstrate in shoot apical meristem than in coleoptiles.

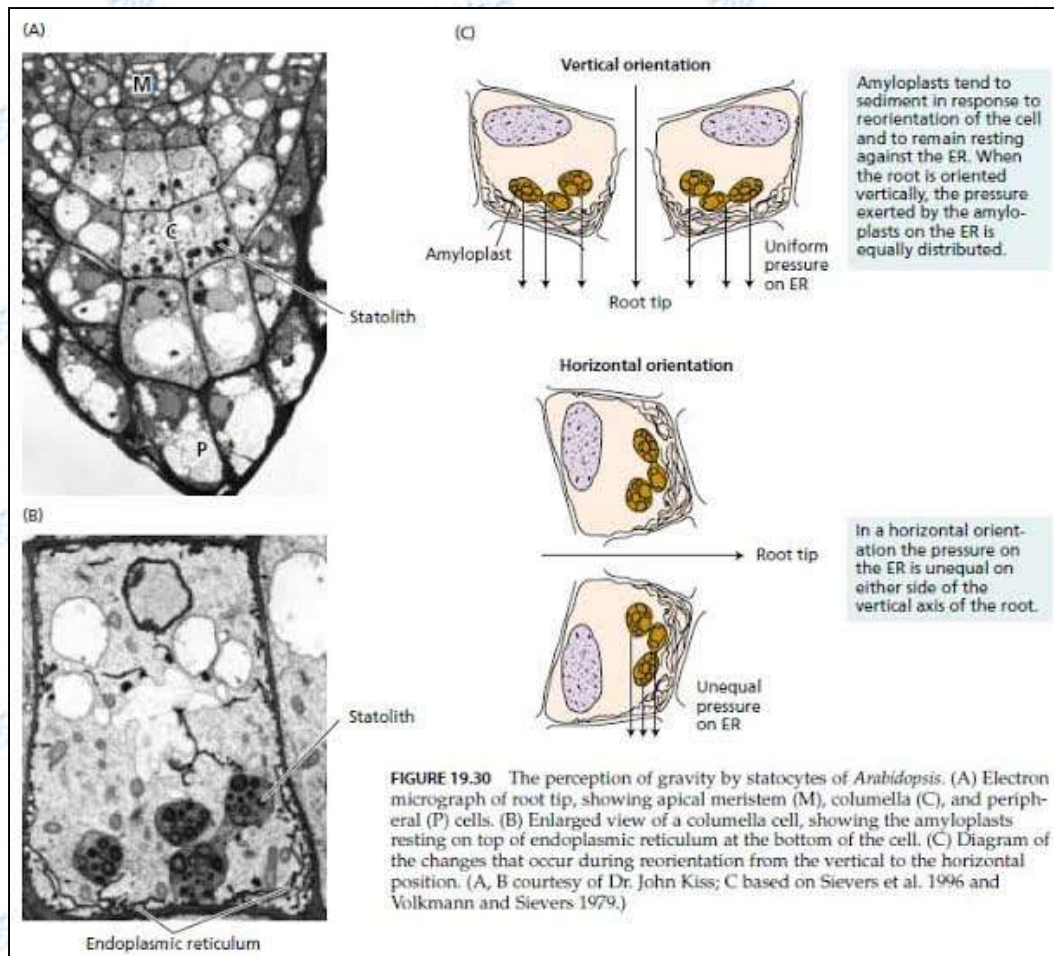
Gravitropism involves lateral redistribution of auxin



Dense plastids serve as gravity sensors

- Gravity does not form gradient.
- The only way of sensing gravity is through the motion of a sedimenting body.
- In plants intracellular gravity sensors are large, dense amyloplast.
- Statoliths –amyloplasts that function as gravity sensors.
- Statocytes-specialized gravity sensing cells in which statoliths are present.

Dense plastids serve as gravity sensors



Gravity perception in shoots and coleoptiles

- In shoots and coleoptiles, gravity is perceived in the starch sheath.
- Starch sheath is continuous with the root endodermis and contains amyloplasts.
- In scarecrow (scr) mutant of Arabidopsis, the cell layer remains undifferentiated.
- On the basis of phenotypes of these two mutants following conclusion is drawn.

Gravity perception in shoots and coleoptiles

- The starch sheath is required for gravitropism in shoots. The starch sheath contains ABCB19 and PIN3, which function coordinately in the bundle sheath to restrict auxin streams to the vascular cylinder. Selective regulation of the downward auxin transport stream conducted by PIN1 inside the vascular cylinder and selective restriction of lateral auxin movement into starch sheath cells appears to play a fundamental role in tropic bending.

Gravity perception in shoots and coleoptiles

- The root endodermis, which does not contain statoliths, is not required for gravitropism in roots, tropic bending in roots involves rerouting of auxin in a basipetal direction at the root tip rather than direct lateral movement from the central cylinder

Gravity sensing may involve pH and calcium ions as second messengers

- **Gravistimulation** – when n gravity sensing mechanisms detect that the root or shoot axis is out of alignment with the gravity vector, signal transduction mechanisms transmit this information to initiate corrective differential growth.
- Localized changes in Ph and Ca^{2+} gradients are part of of that signaling.
- Changes in intracellular pH can be detected early in root columella cells responding to gravity.

Gravity sensing may involve pH and calcium ions as second messengers

- Rapid changes in Ph were observed in Arabidopsis roots, after roots were roated to a horizontal position.
- Activation of the plasma membrane H⁺-ATPase is one of the initial event that mediates root gravity perception.
- Ca²⁺ release from storage pools might be involved in root gravitropic signal transduction.
- Treatment of maize roots with EGTA, prevents ca²⁺ uptake by cells and inhibits root gravitropism.
- Localized changes in internal ca²⁺ pools have been observed in root thigmotropism responses.

Gravity sensing may involve pH and calcium ions as second messengers



FIGURE 19.35 A corn root bending toward an agar block containing calcium placed on the cap. (Courtesy of Michael L. Evans.)

Auxin is redistributed laterally in the root cap

- Root cap is the site of gravity perception.
- Graviresponsive signaling events initiated in the root cap must induce production of a chemical messengers.
- Microsurgery experiments showed that the cap supplies a root growth inhibitor to the lower side of the root.
- IAA is more inhibitory to root growth than ABA.
- IAA is delivered to the root apex by the acropetal PIN1\ABCB19 directed stream.
- IAA synthesized in the root meristem is excluded from root cap apical cells by the combined activity of PIN3,PIN4 and ABCB1, while AUX1- mediated auxin uptake in lateral root cap cells drives a basipetal auxin stream out of the root apex.

Auxin is redistributed laterally in the root cap

- PIN2 conducts auxin away from the lateral root cap to the elongation zone.
- An auxin reflux loop is thought to redirect auxin back into the acropetal stellar transport stream.
- According to the current model for gravitropism, basipetal auxin transport in a vertically oriented root is equal on all sides.
- The downward movement of auxin across a horizontal root cap has been confirmed using a reporter gene construct, DR5:GFP.
- PIN3 is thought to participate in the redirection of auxin in roots.

Auxin is redistributed laterally in the root cap

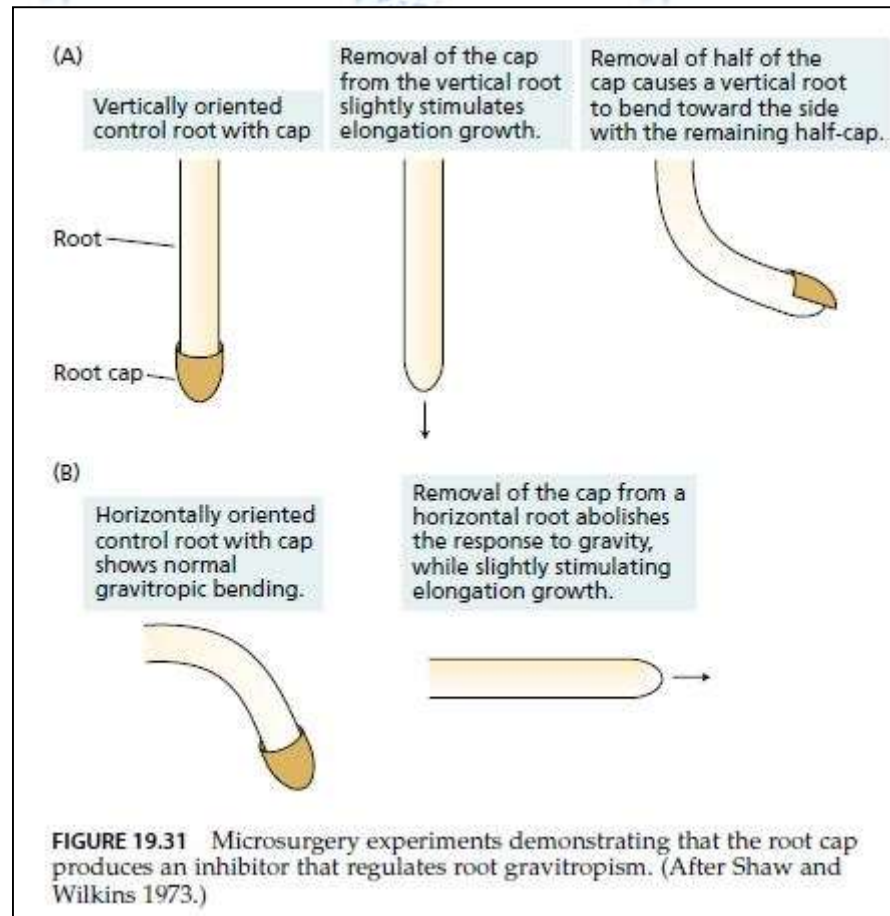


FIGURE 19.31 Microsurgery experiments demonstrating that the root cap produces an inhibitor that regulates root gravitropism. (After Shaw and Wilkins 1973.)

Developmental effects of auxins

- Influences every stage of plants life cycle from germination to senescence
- Polar auxin streams directed by PIN proteins which helps in plant organogenesis
- Tissue elongation and maturity
- Loss of PIN proteins can cause severe embryonic defect
- Auxin may act conjugation with other hormones, Ca and reactive oxide for plant development

Auxin Regulates Apical Dominance

- Growing apical bud inhibits the lateral bud growth
- In 1920, Skog and Thimann performed an experiment on bean and result showed that outgrowth of auxillary bud is inhibited by auxin that is transported basipetally
- The auxin content of bud increases by decapitation of shoot apex
- Auxin control auxillary bud growth by acting in xylem and interfascicular sclerechyma of shoot

Auxin Regulates Apical Dominance

- Strigolactones as signal that interact with auxin in regulating apical dominance
- Strigolactone is a group of terpenoids that produced in both shoots and roots and is transported through xylem

Auxin Regulates Apical Dominance

FIGURE 19.36 Auxin suppresses the growth of axillary buds in bean (*Phaseolus vulgaris*) plants. (A) The axillary buds are suppressed in the intact plant because of apical dominance. (B) Removal of the terminal bud releases the axillary buds from apical dominance (arrows). (C) Applying IAA in lanolin paste (contained in the gelatin capsule) to the cut surface prevents the outgrowth of the axillary buds. (Photos ©M. B. Wilkins.)

(A)



(B)



(C)



Auxin transport regulates floral bud and phyllotaxy

- In absence of PIN1, auxin movement to meristem and phyllotaxy is disturbed
- If lanolin paste is applying on side of apical meristem then leaf perinmodia can be induced
- Auxin with combine action of AUX1, PIN1 & ABCB19 can predict phyllotactic pattern

Auxin Promotes the Formation of Lateral and Adventitious Roots

- primary root is inhibited
- by auxin concentrations greater than 10^{-8} M
- High concentration initiate lateral and adventitious roots
- Cell division in pericycle is initiated by IAA transport acropetally
- PIN2, AUX1, ABCB19 & ABCB1 mediate uptake auxin into root
- LAX3 promote cell expansion & cell wall modification by uptake auxin into cortical & epidermal cell of roots

Auxin Promotes the Formation of Lateral and Adventitious Roots

- Cell division and growth is maintained by root and shoot derived auxin

Auxin induce vascular differentiation

- Vascular differentiation involves interaction of PIN1, ethylene and other hormones
- When apical bud is grafted onto a clamp of undifferentiated cells forms
- Auxins controls the regeneration of vascular tissues

Auxin induce vascular differentiation

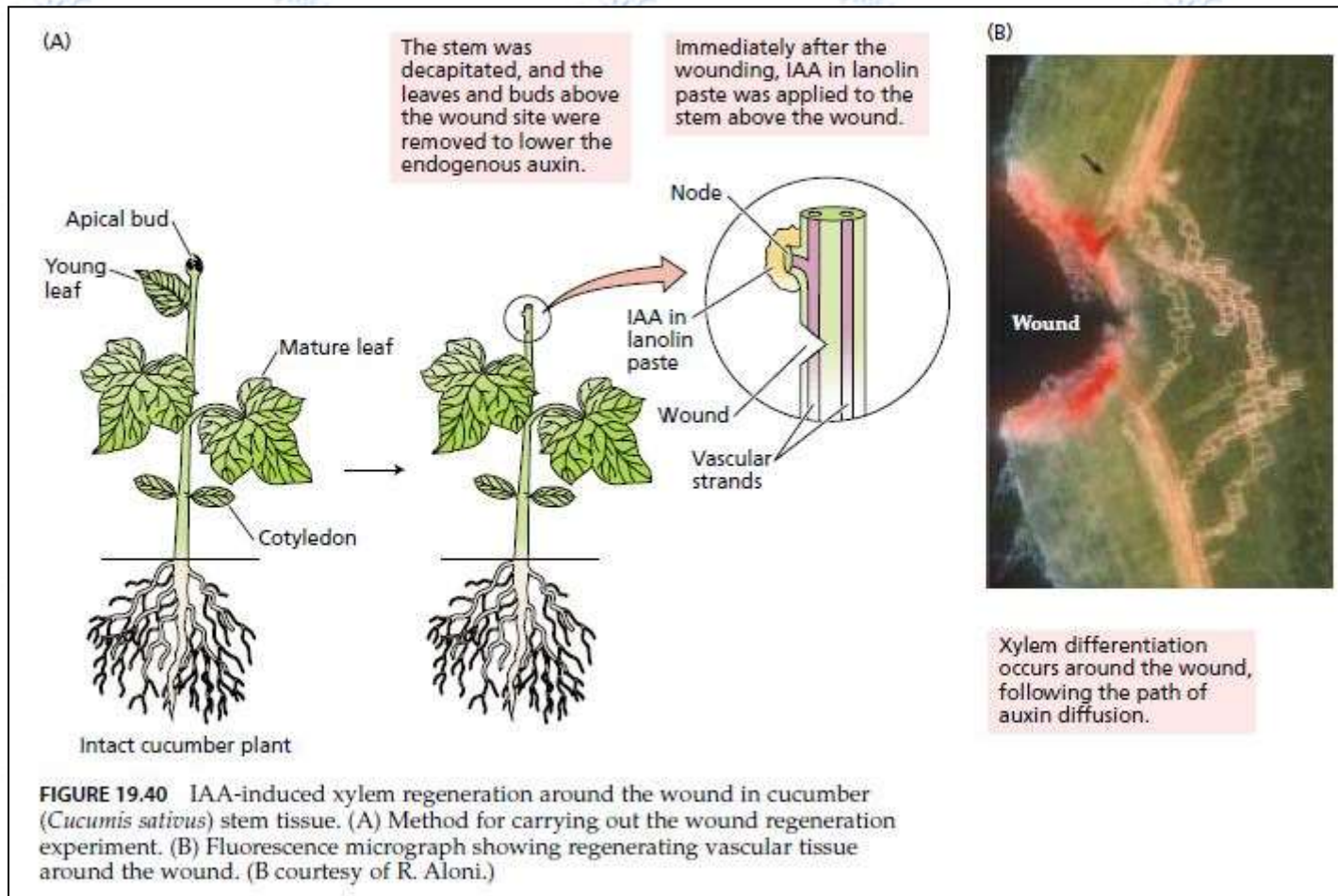


FIGURE 19.40 IAA-induced xylem regeneration around the wound in cucumber (*Cucumis sativus*) stem tissue. (A) Method for carrying out the wound regeneration experiment. (B) Fluorescence micrograph showing regenerating vascular tissue around the wound. (B courtesy of R. Aloni.)

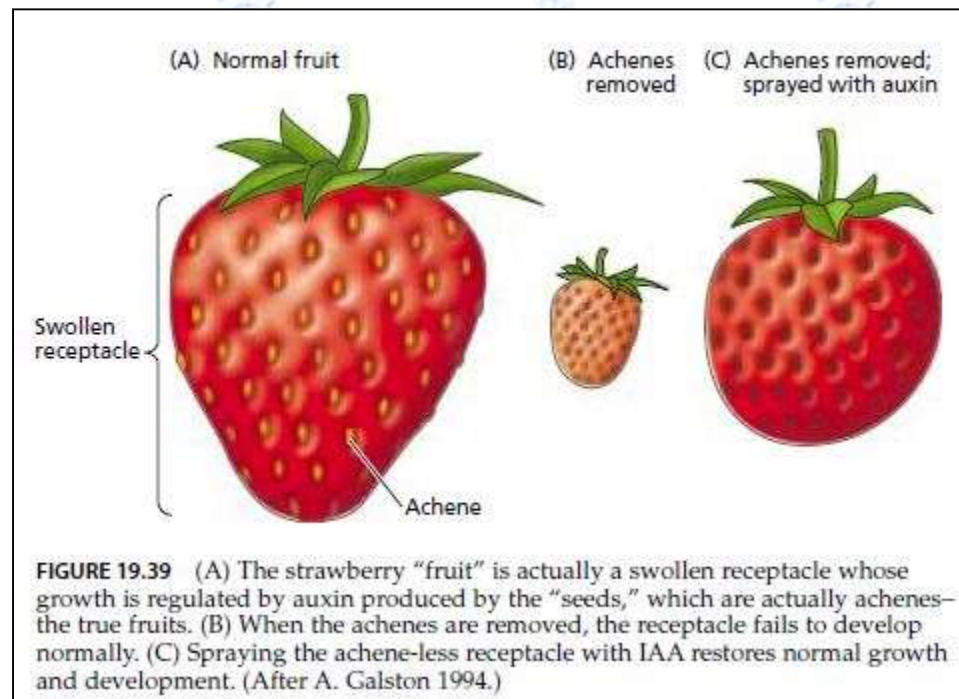
Auxins delays onset of leaf abscission

- Auxins level high in young leaves and low in senescing leaves when abscission started
- Auxin is transported from leaf blade normally prevent abscissions

Auxins promotes fruits development

- Regulation of fruit development
- After fruit set, fruit growth depends on auxins

Auxins promotes fruits development



Synthetic auxins have a variety of commercial uses

- Prevention of fruit & leaf drop, induction of parthenocarpic fruit development, thinning of fruit & rooting of cutting for plant propagation
- If excised leaf is dipped in auxins then rooting enhanced
- Parthenocarpic fruit can be induced by treatment of unpollinated flowers with auxins

Synthetic auxins have a variety of commercial uses

- 2,4-D and dicamba are synthetic auxins which used in herbicides
- 2,4-D also used as weed control while monocots are able to inactivate synthetic auxins rapidly than dicots



Conclusion