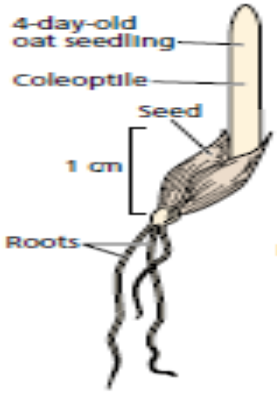
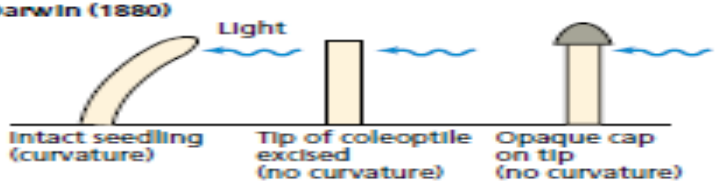
Auxin: The Growth Hormone

*(*Greek *auxein, meaning "to increase" or "to grow.")*

- It was the first growth hormone to be discovered in plants,
- It Required for viability (along with CK).
- Whereas the other plant hormones seem to act as on/off switches that regulate specific developmental processes, auxin and cytokinin appear to be required at some level more or less continuously.

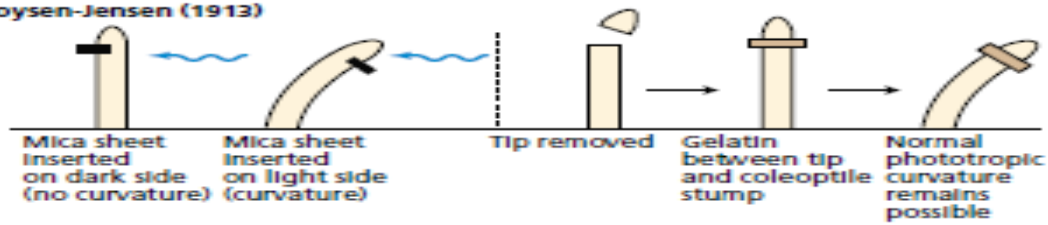


Darwin (1880)



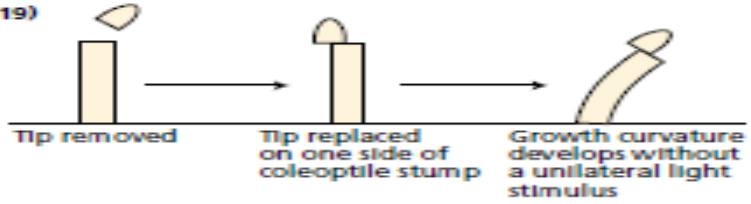
From experiments on coleoptile phototropism, Darwin concluded in 1880 that a growth stimulus is produced in the coleoptile tip and is transmitted to the growth zone.

Boysen-Jensen (1913)



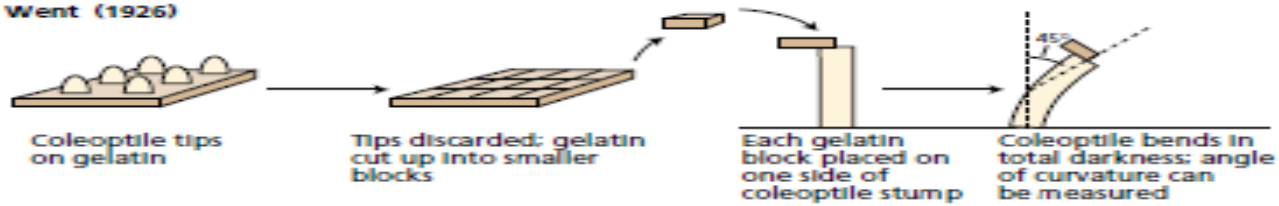
In 1913, P. Boysen-Jensen discovered that the growth stimulus passes through gelatin but not through water-impermeable barriers such as mica.

Paal (1919)

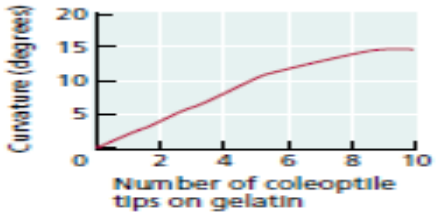


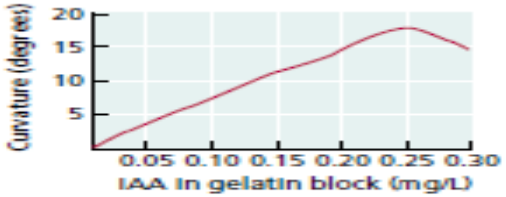
In 1919, A. Paal provided evidence that the growth-promoting stimulus produced in the tip was chemical in nature.

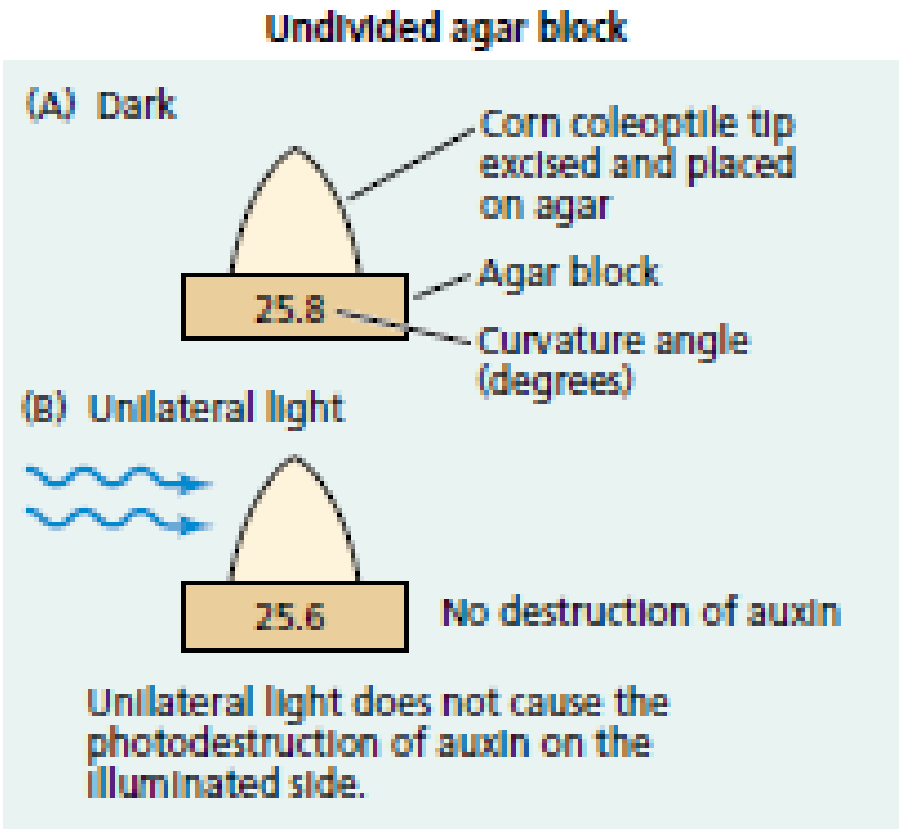
Went (1926)

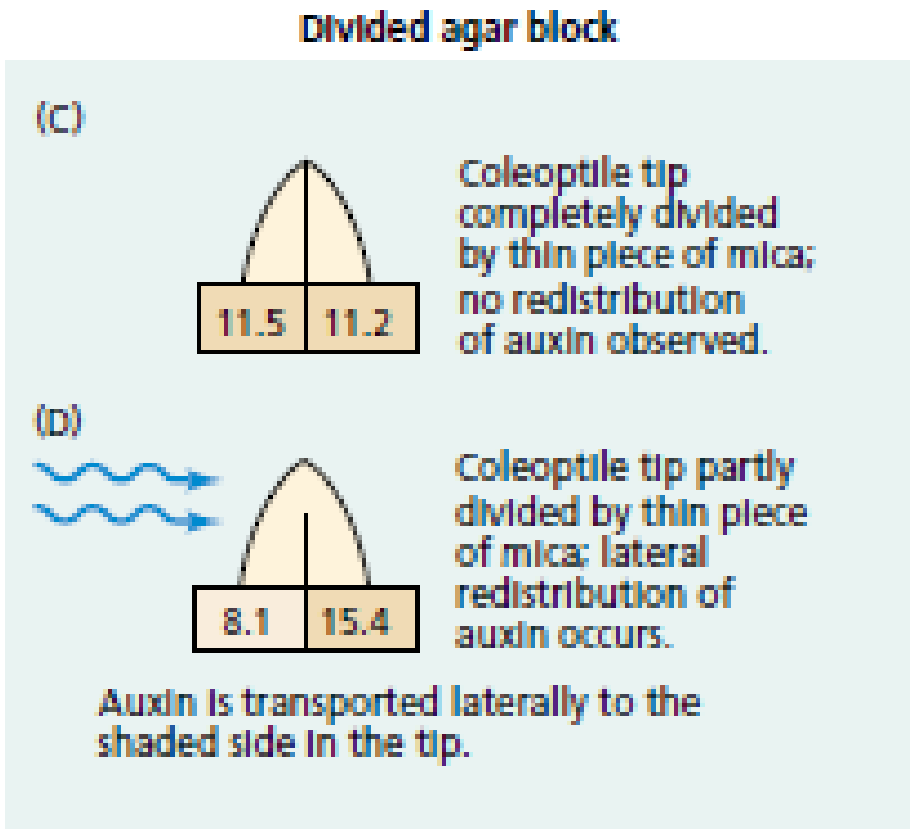


In 1926, F. W. Went showed that the active growth-promoting substance can diffuse into a gelatin block. He also devised a coleoptile-bending assay for quantitative auxin analysis.

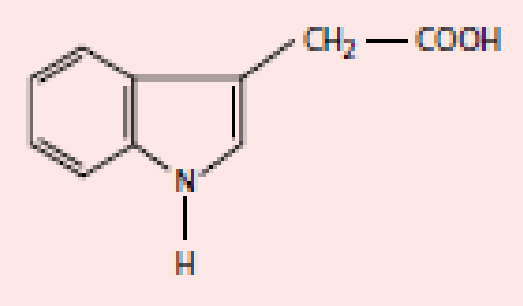




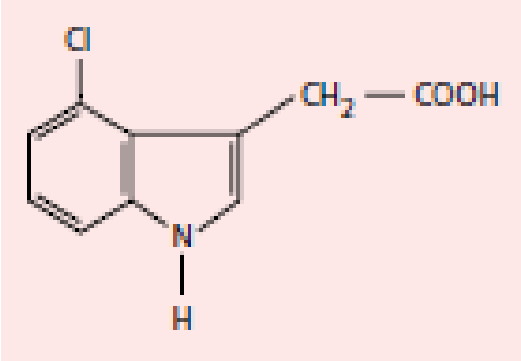

Undivided agar block
(A) Dark
Corn coleoptile tip excised and placed on agar
Agar block
25.8
Curvature angle (degrees)
(B) Unilateral light
25.6
No destruction of auxin
Unilateral light does not cause the photodestruction of auxin on the illuminated side.


Divided agar block
(C)
11.5
11.2
Coleoptile tip completely divided by thin piece of mica; no redistribution of auxin observed.
(D)
8.1
15.4
Coleoptile tip partly divided by thin piece of mica; lateral redistribution of auxin occurs.
Auxin is transported laterally to the shaded side in the tip.

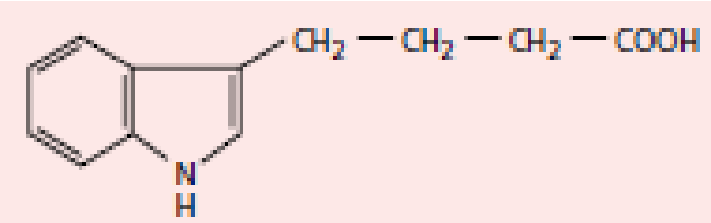
The Principal Auxin in Higher Plants Is Indole-3-Acetic Acid



Indole-3-acetic acid
(IAA)



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

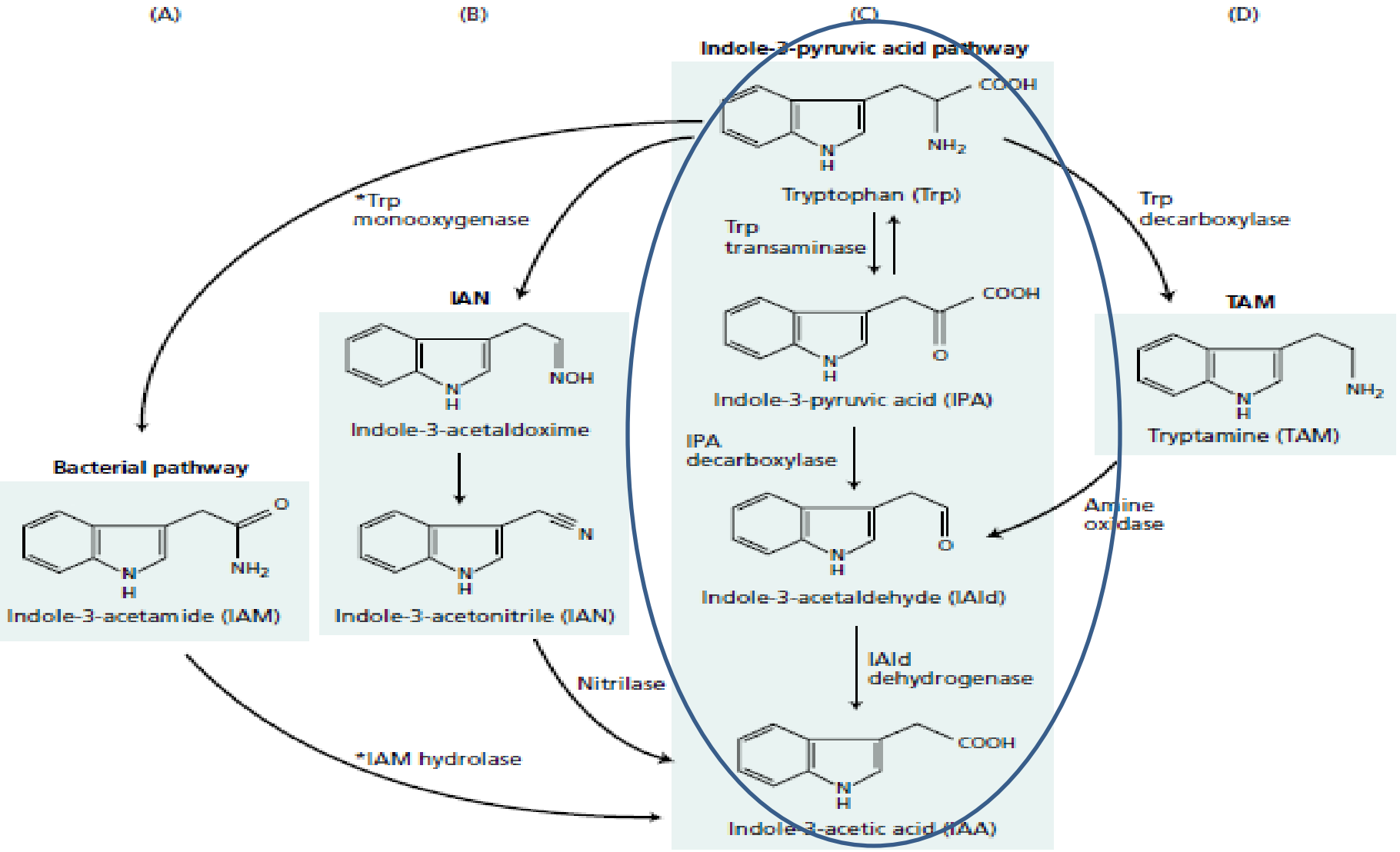


Indole-3-butyric acid
(IBA)

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).

Multiple Pathways Exist for the Biosynthesis of IAA

Brassicaceae (mustard family), Poaceae (grass family), and Musaceae (banana family).



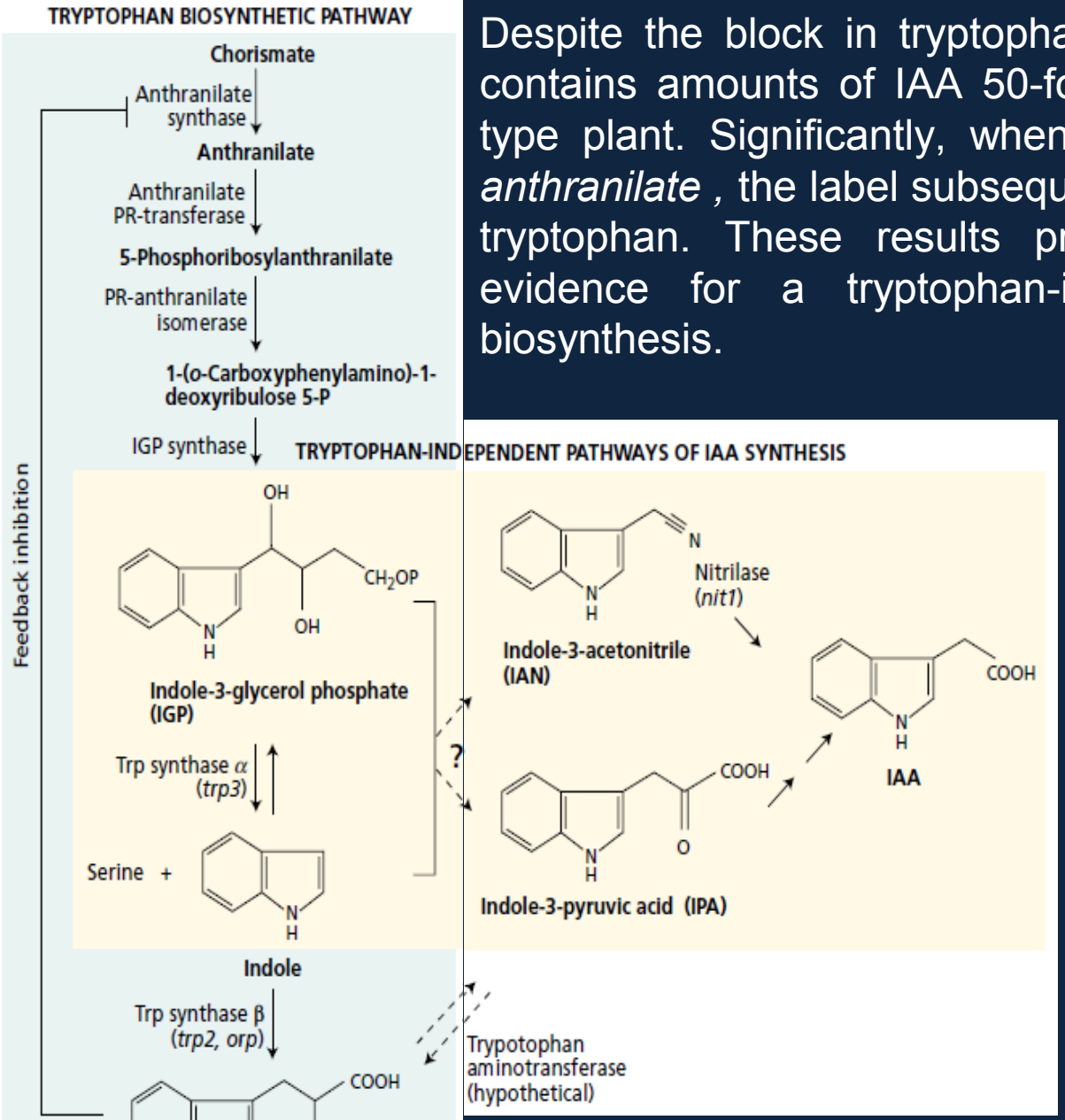
Tryptophan independent pathway;

Although a tryptophan-independent pathway of IAA biosynthesis had long been suspected because of the low levels of conversion of radiolabeled tryptophan to IAA.

The orange pericarp (*orp) mutant of maize is* missing both subunits of tryptophan synthase. As a result, the pericarps surrounding each kernel accumulate glycosides of anthranilic acid and indole. The orange color is due to excess indole.

Despite the block in tryptophan biosynthesis, the *orp* mutant contains amounts of IAA 50-fold higher than those of a wild-type plant.

Despite the block in tryptophan biosynthesis, the *orp* mutant contains amounts of IAA 50-fold higher than those of a wild-type plant. Significantly, when *orp seedlings were fed [15N] anthranilate ,* the label subsequently appeared in IAA, but not in tryptophan. These results provided the best experimental evidence for a tryptophan-independent pathway of IAA biosynthesis.

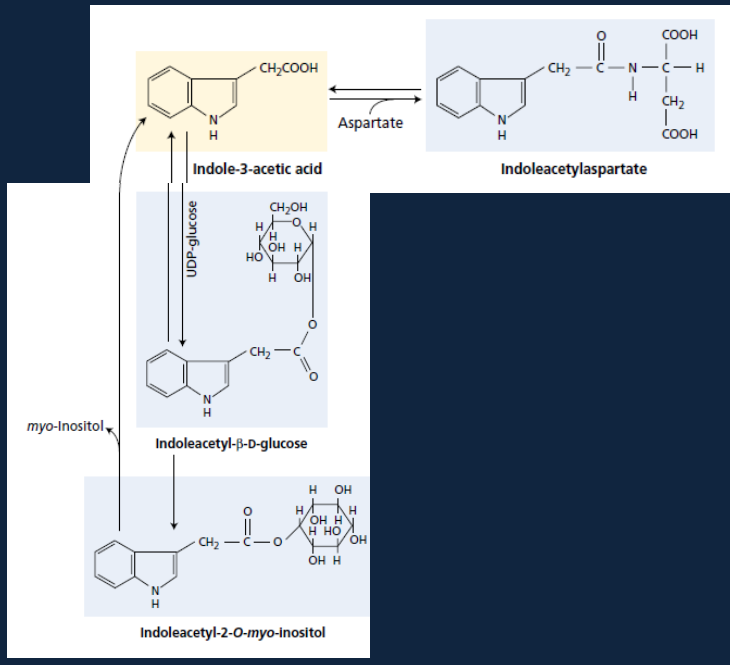


Most IAA in the Plant Is in a Covalently Bound Form

• Low-molecular-weight conjugated auxins include esters of IAA with glucose or *myo-inositol and amide* conjugates such as **IAA-*N*-aspartate .**

• High-molecular-weight IAA conjugates include **IAA glucan** (7–50 glucose units per IAA) and **IAA-glycoproteins** found in cereal

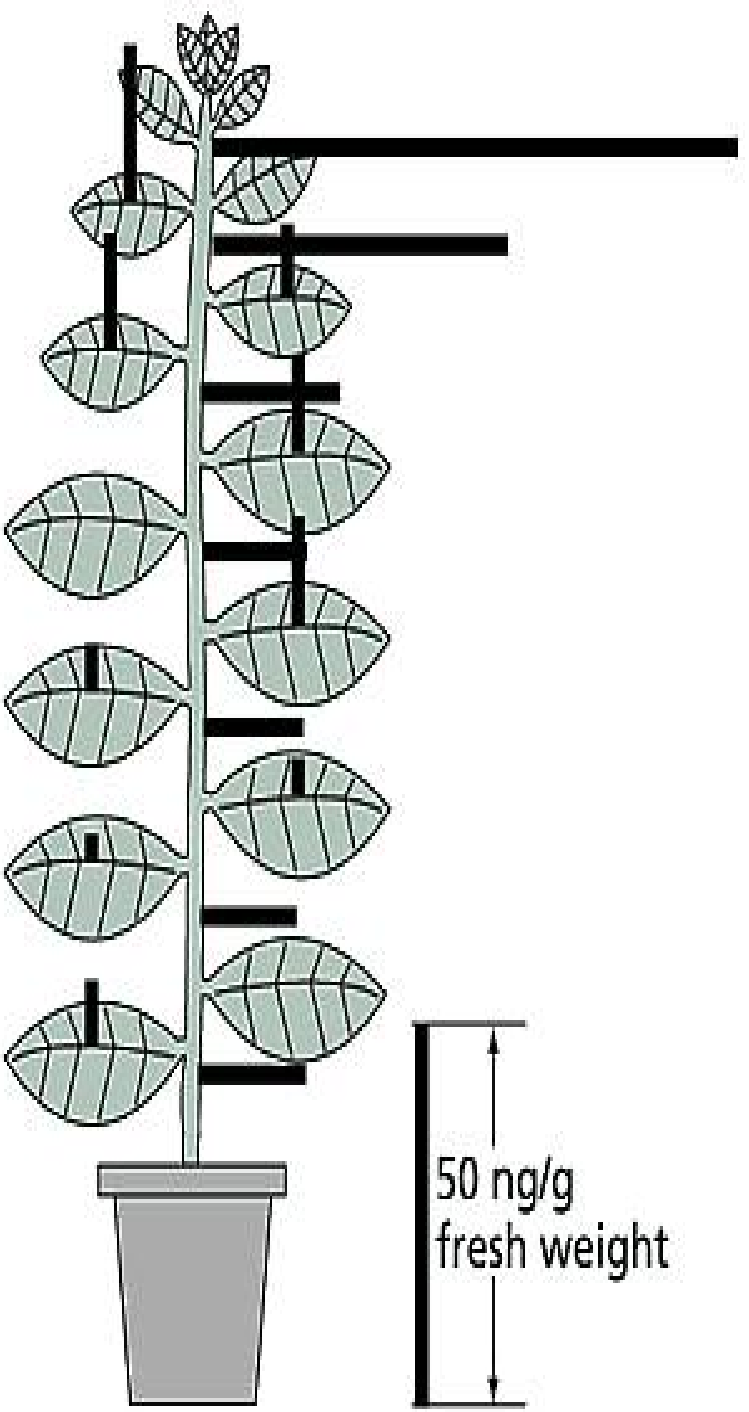
Regulation of the levels of free auxin.



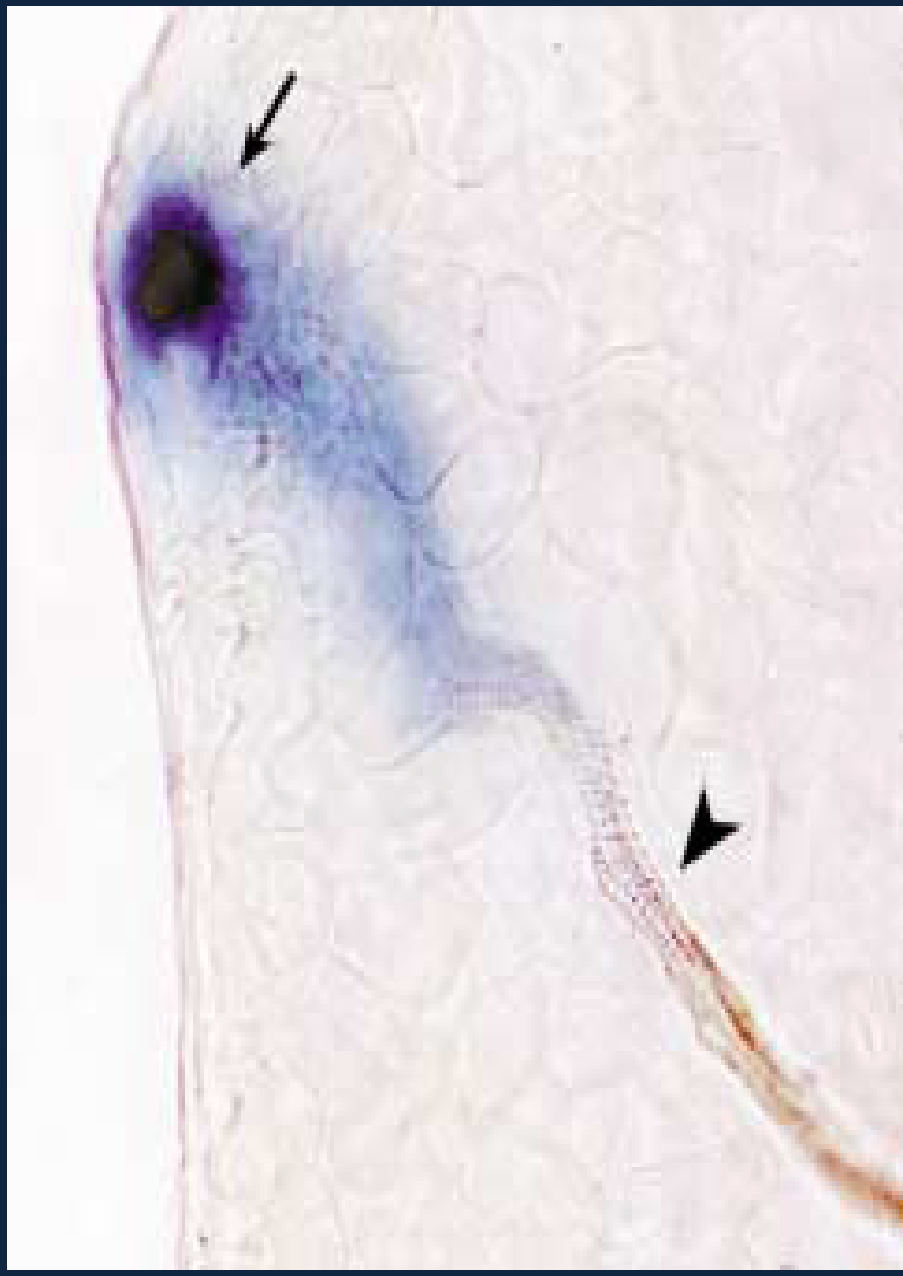
Environmental stimuli such as light and gravity have been shown to influence both the rate of auxin conjugation (removal of free auxin) and the rate of release of free auxin (hydrolysis of conjugated auxin).
The formation of conjugated auxins may serve other functions as well, including storage and protection against oxidative degradation.

IAA Is Synthesized in Meristems,Young Leaves, and Developing Fruits and Seeds

IAA biosynthesis is associated with **rapidly dividing and rapidly growing tissues, especially in shoots**. Although virtually all plant tissues appear to be capable of producing low levels of IAA, **shoot apical meristems, young leaves, and developing fruits and seeds** are the primary sites of IAA synthesis. In very young leaf primordia of *Arabidopsis, auxin is* synthesized at the tip. During leaf development there is a gradual shift in the site of auxin production basipetally along the margins, and later, in the central region of the lamina. The basipetal shift in auxin production correlates closely with, and is probably causally related to, the basipetal maturation sequence of leaf development and vascular differentiation.



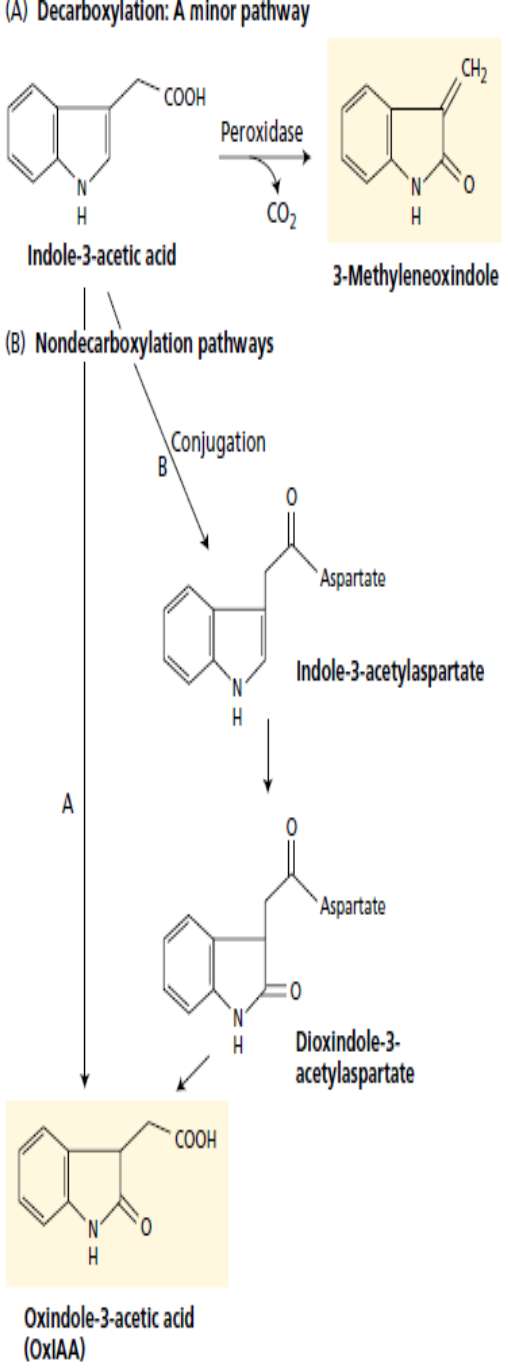
As shown in figure, during early stages of hydathode differentiation a center of high auxin synthesis is evident as a concentrated dark blue GUS stain (arrow) in the lobes of serrated leaves of *Arabidopsis (Aloni et al. 2002).* A diffuse trail of GUS activity leads down to differentiating vessel elements in a developing vascular strand. This remarkable micrograph captures the process of auxin-regulated vascular differentiation in the very act.



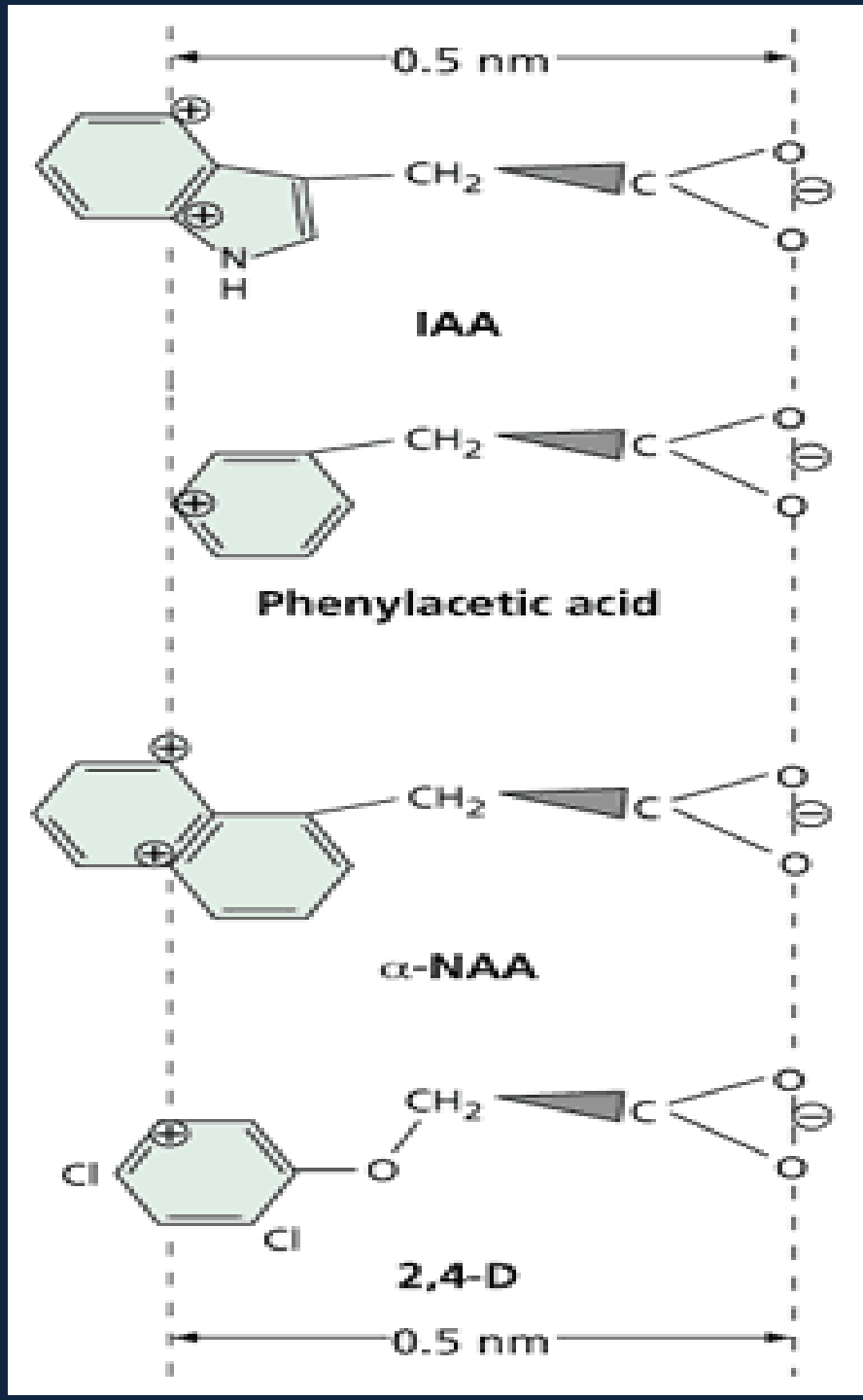
Two Subcellular Pools of IAA Exist: The Cytosol and the Chloroplasts

The distribution of IAA in the cell appears to be regulated largely by pH. Because IAA^- does not cross membranes unaided, whereas IAAH readily diffuses across membranes

About one-third of the IAA is found in the **chloroplast**, and the remainder is located in the cytosol. IAA conjugates are located exclusively in the cytosol. IAA in the cytosol is metabolized either by conjugation or by nondecarboxylative catabolism

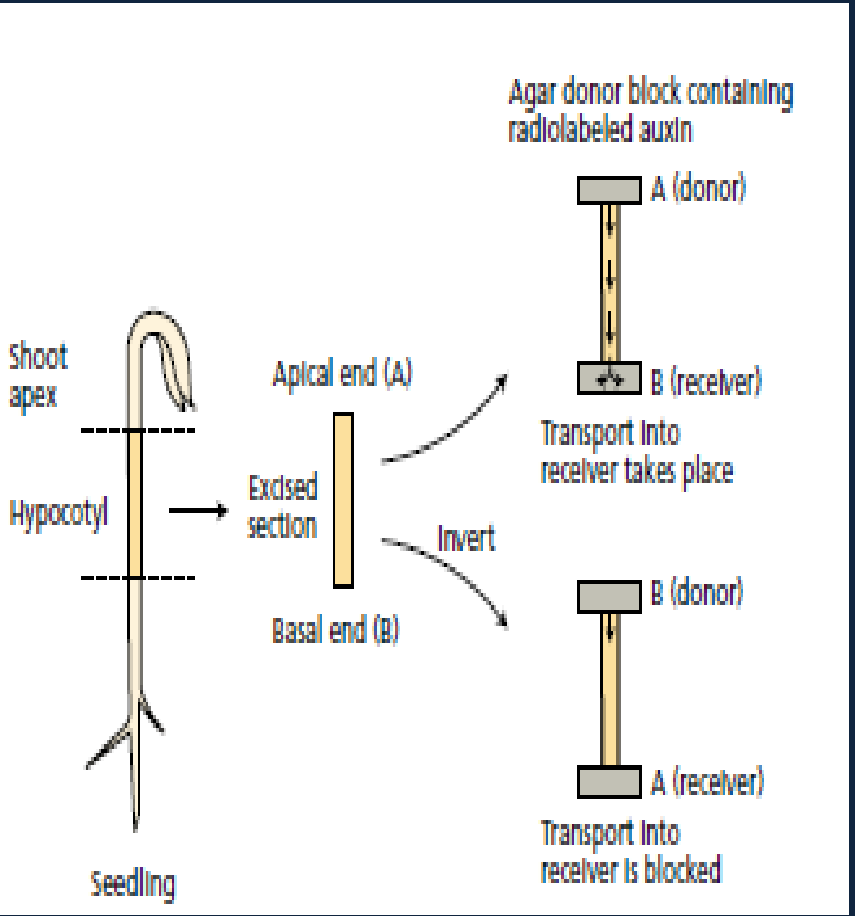


Diverse group of chemical shows auxin like activity ?



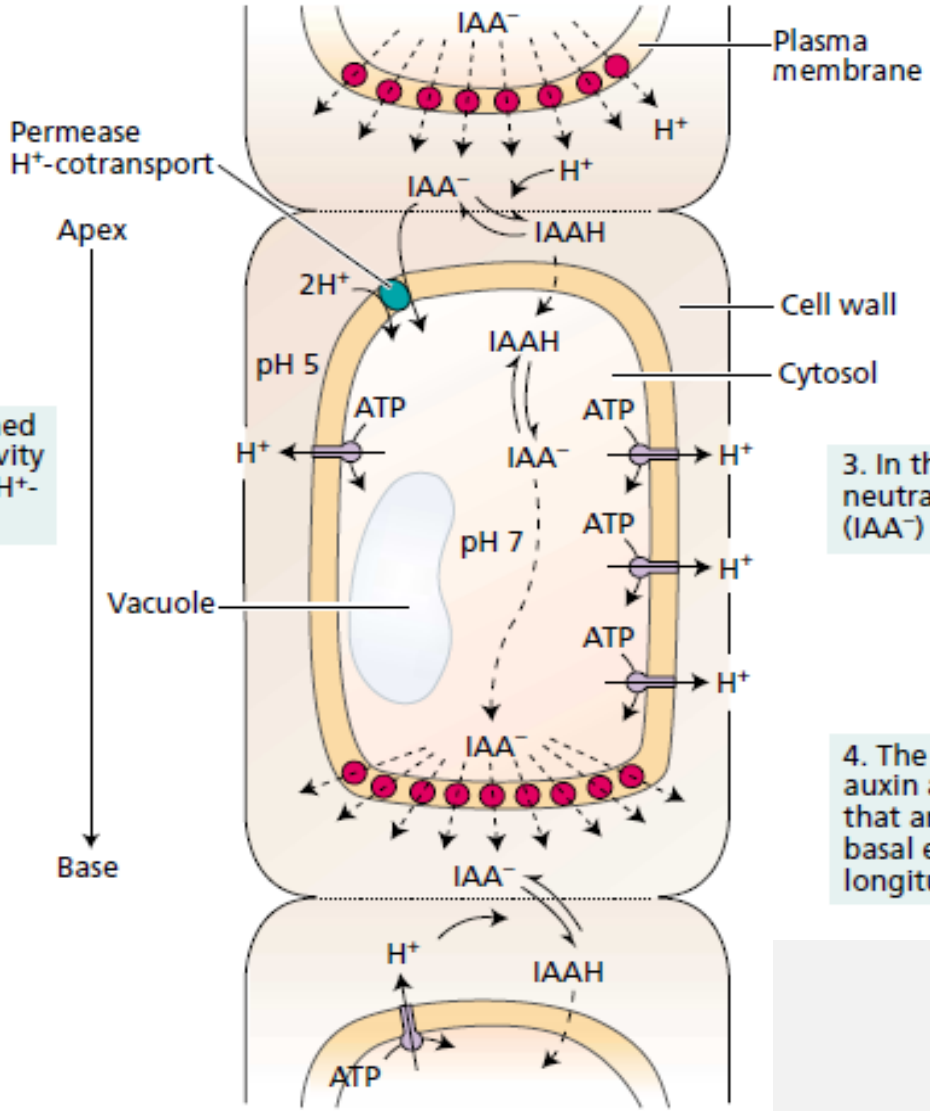
AUXIN TRANSPORT

Apex-base polarity → Polarity of auxin transport



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.

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.

Auxin influx.

The first step in polar transport is auxin influx. According to the model, auxin can enter plant cells from any direction by either of two mechanisms:

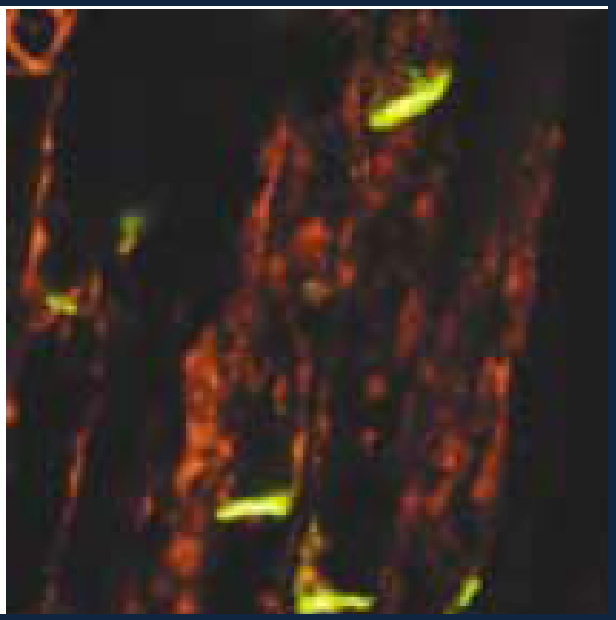
1. Passive diffusion of the protonated (IAAH) form
across the phospholipid bilayer
2. Secondary active transport of the dissociated (IAA–) form via a 2H+–IAA– symporter

Auxin efflux.

Once IAA enters the cytosol, which has a pH of approximately 7.2, nearly all of it will dissociate to the anionic form. Because the membrane is less permeable to IAA– than to IAAH, IAA– will tend to accumulate in the cytosol. However, much of the auxin that enters the cell escapes via an *auxin anion efflux carrier.*

A permease-type auxin uptake carrier, AUX1, related to bacterial amino acid carriers, has been identified in *Arabidopsis* roots.

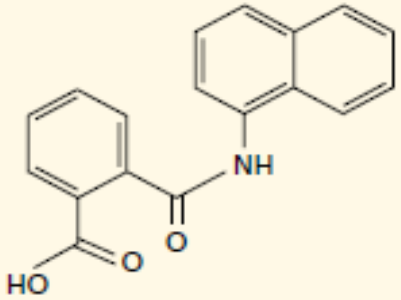
A family of putative auxin efflux carriers known as **PIN proteins (named after the pin-shaped** inflorescences formed by the *pin1 mutant* of *Arabidopsis; are localized* precisely as the model would predict—that is, at the basal ends of the conducting cells



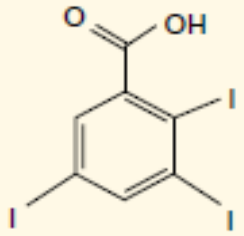
localization of the PIN1 protein at the basal ends of conducting cells by immunofluorescence microscopy

Inhibitors of Auxin Transport Block Auxin Efflux

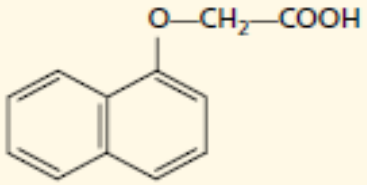
Auxin transport inhibitors not found in plants



NPA (1-N-naphthylphthalamic acid)

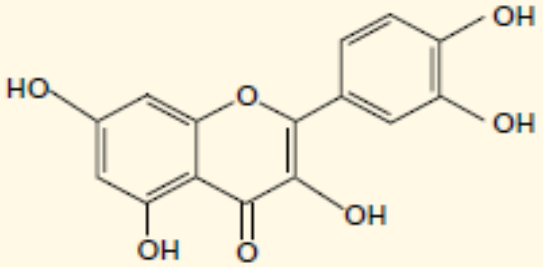


TIBA (2,3,5-triiodobenzoic acid)

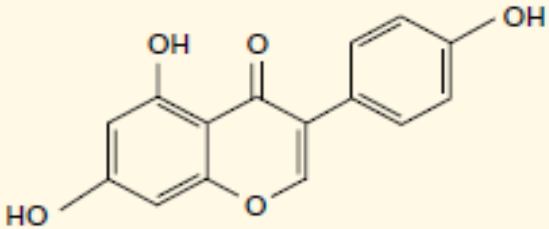


1-NOA (1-naphthoxyacetic acid)

Naturally occurring auxin transport inhibitors



Quercetin (flavonol)



Genistein

Auxin Is Also Transported Nonpolarly in the Phloem

Long-distance auxin transport in the phloem is important for controlling such processes as cambial cell divisions, callose accumulation or removal from sieve tube elements, and branch root formation.

Polar transport and phloem transport are not independentof each other.

It has been proposed that the asymmetrically oriented AUX1 permease promotes the acropetal movement of auxin from the phloem to the root apex.This type of polar auxin transport based on the asymmetric localization of AUX1 differs from the polar transport that occurs in the shoot and basal region of the root, which is based on the asymmetric distribution of the PIN complex.

AUX1 is also strongly expressed in a cluster of cells in the columella of the root cap, as well as in lateral root cap cells that overlay the cells of the distal elongation zone of the root. These cells form a minor, but physiologically important, basipetal pathway whereby auxin reaching the columella is redirected backward toward the outer tissues of the elongation zone

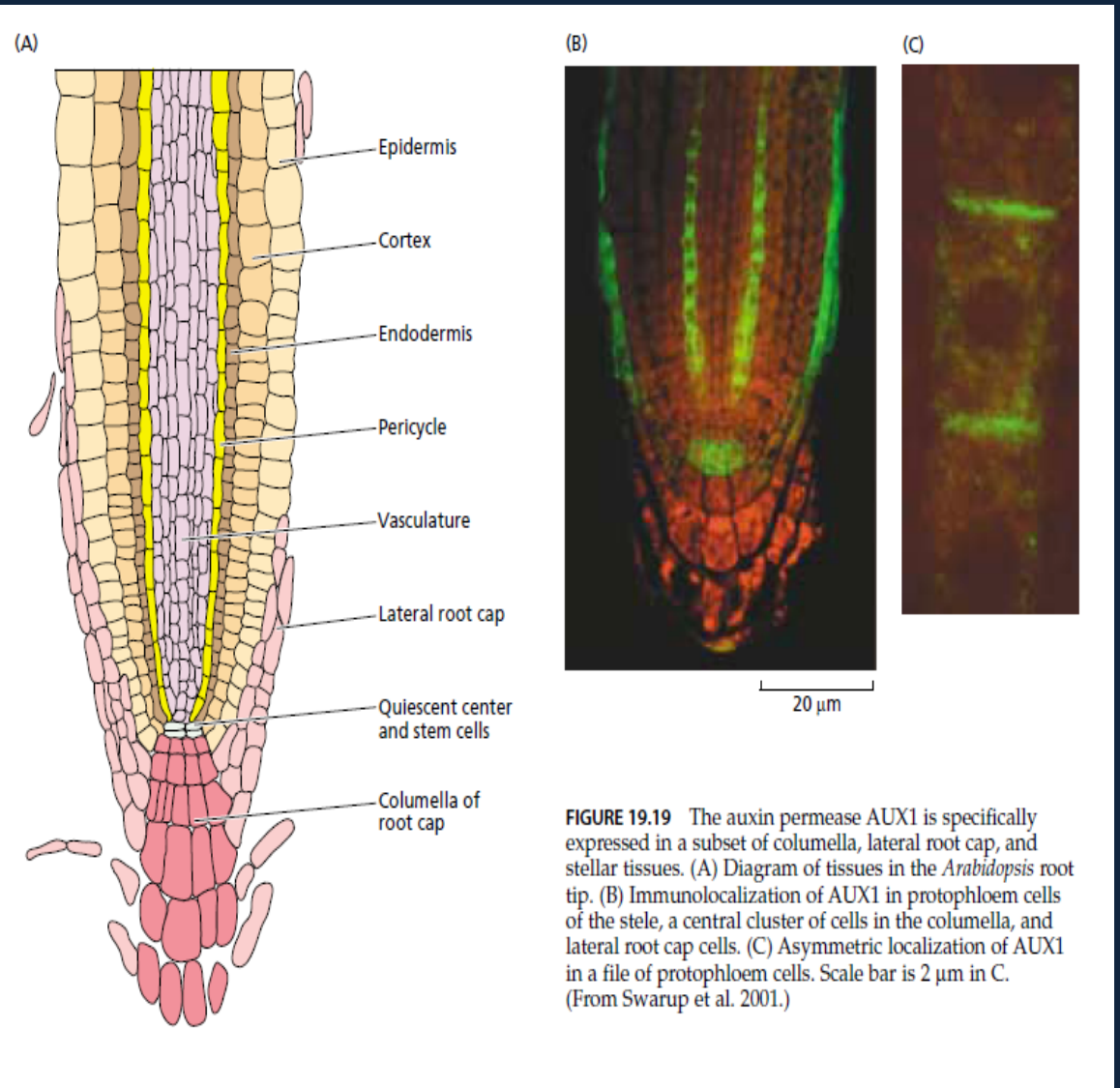
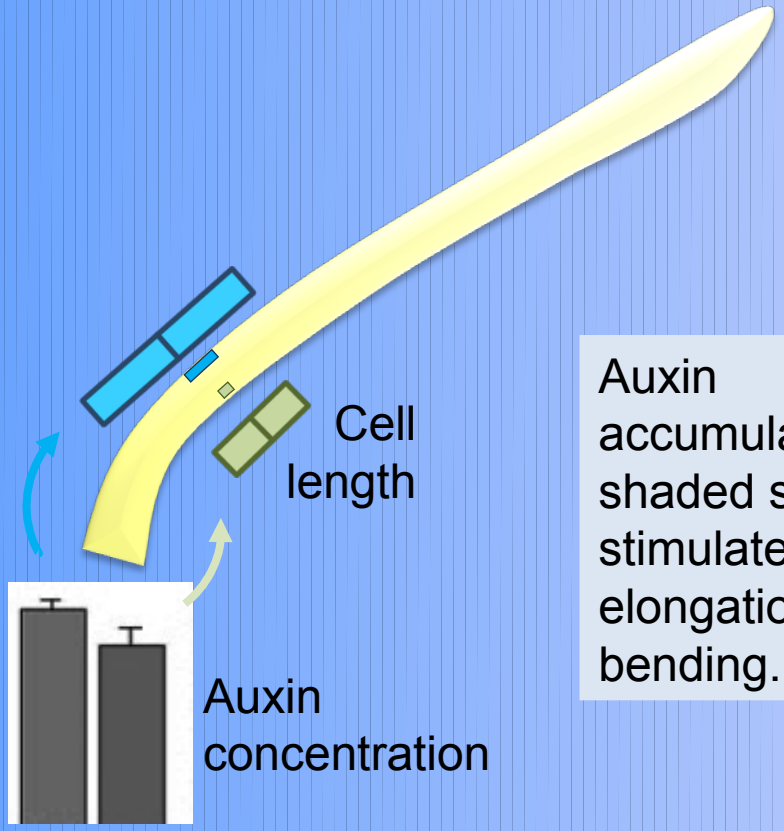


FIGURE 19.19 The auxin permease AUX1 is specifically expressed in a subset of columella, lateral root cap, and stellar tissues. (A) Diagram of tissues in the *Arabidopsis* root tip. (B) Immunolocalization of AUX1 in protophloem cells of the stele, a central cluster of cells in the columella, and lateral root cap cells. (C) Asymmetric localization of AUX1 in a file of protophloem cells. Scale bar is 2 µm in C. (From Swarup et al. 2001.)

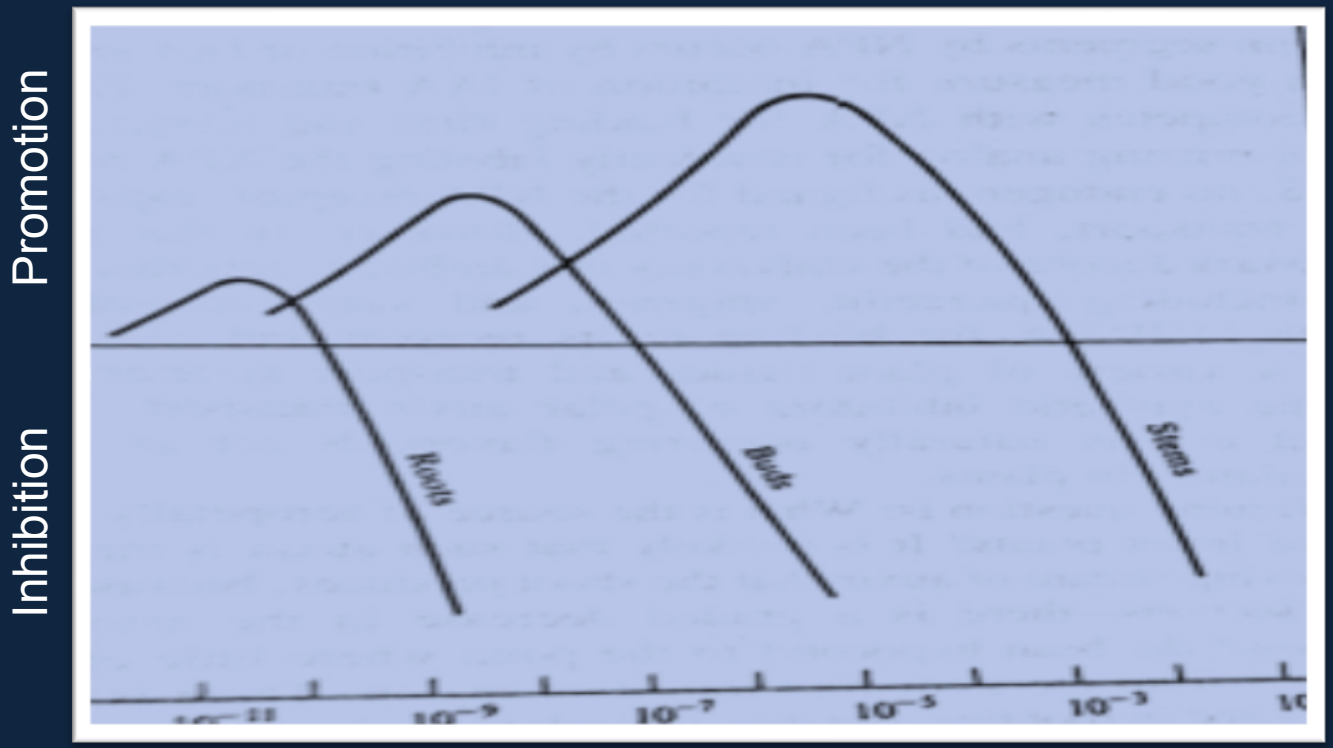
AUX1 permease is asymmetrically localized on the plasma membrane at the upper end of root protophloem cells (i.e., the end distal from the tip)

PHYSIOLOGICAL EFFECTS OF AUXIN: CELL ELONGATION

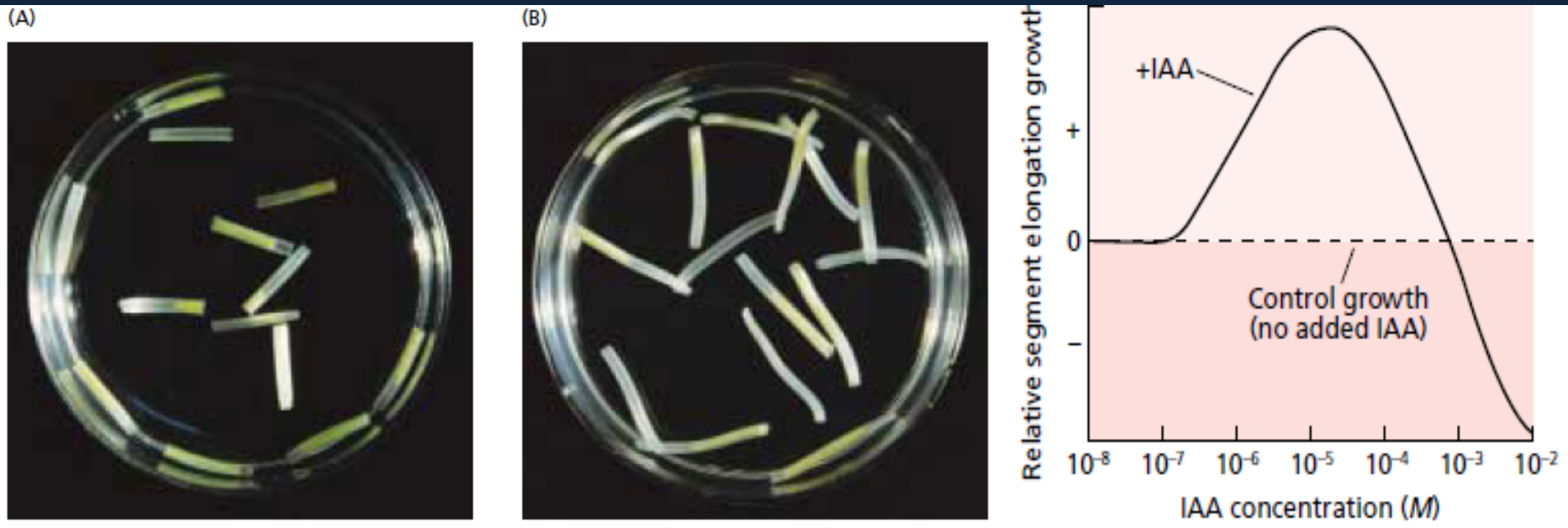


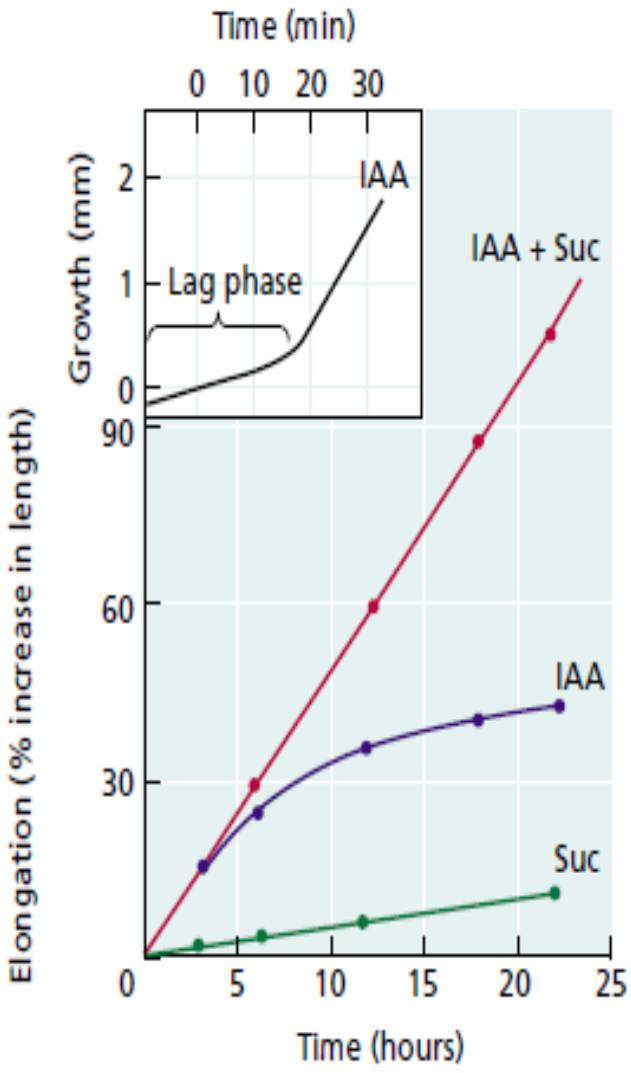
Auxin accumulation on shaded side stimulates elongation and bending.

Auxins Promote Growth in Stems and Coleoptiles, While Inhibiting Growth in Roots



Auxin concentration (M)


(A)
(B)
+IAA
Control growth
(no added IAA)
+
0
–
10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2}
IAA concentration (*M*)



Without auxin in the medium, the growth rate declines rapidly. Addition of auxin markedly stimulates the growth rate after a lag period of only 10 to 12 minutes.

- Auxin-induced growth is also sensitive to inhibitors of protein synthesis such as cycloheximide, suggesting that proteins with high turnover rates are involved. Inhibitors of RNA synthesis also inhibit auxininduced growth, after a slightly longer delay

- The length of the lag time for auxin-stimulated growth can be increased by lowering of the temperature or by the use of suboptimal auxin concentrations, the lag time cannot be shortened by raising of the temperature, by the use of supraoptimal auxin concentrations, or by abrasion of the waxy cuticle to allow auxin to penetrate the tissue more rapidly..

The lag time reflects the time needed for the biochemical machinery of the cell to bring about the increase in the growth rate.

Plant cells expand in three steps:

1. Osmotic uptake of water across the plasma membrane is driven by the gradient in water potential (ΔYw).
2. Turgor pressure builds up because of the rigidity of the cell wall.
3. Biochemical wall loosening occurs, allowing the cell to expand in response to turgor pressure.

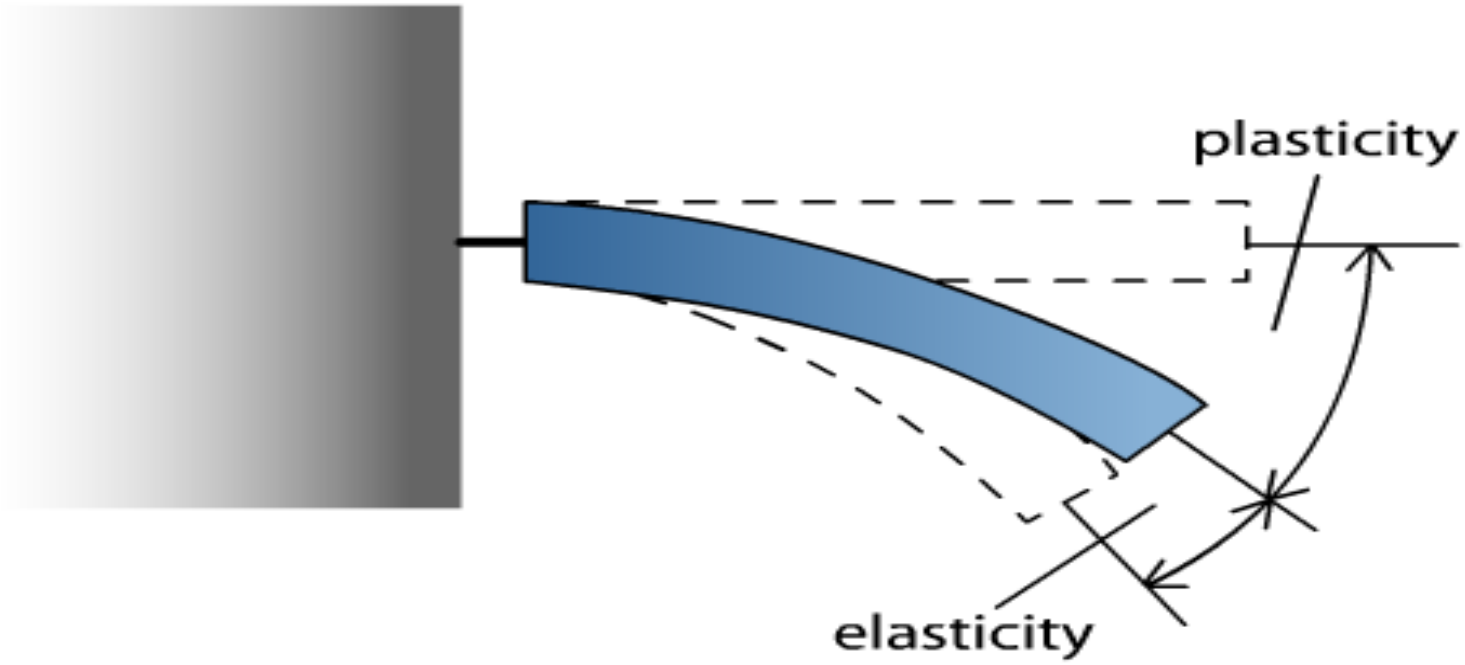
$$GR = m\,(Yp - Y)$$

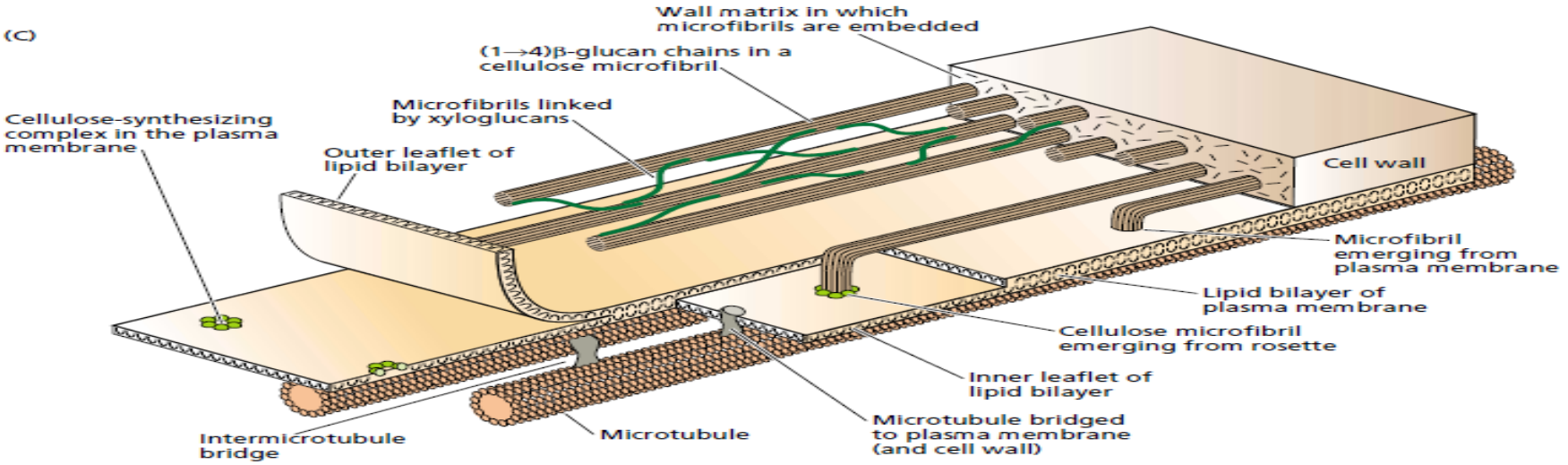
where *GR is the growth rate, Yp is the turgor pressure,*
Y is the yield threshold, and
m is the coefficient (wall extensibility) .

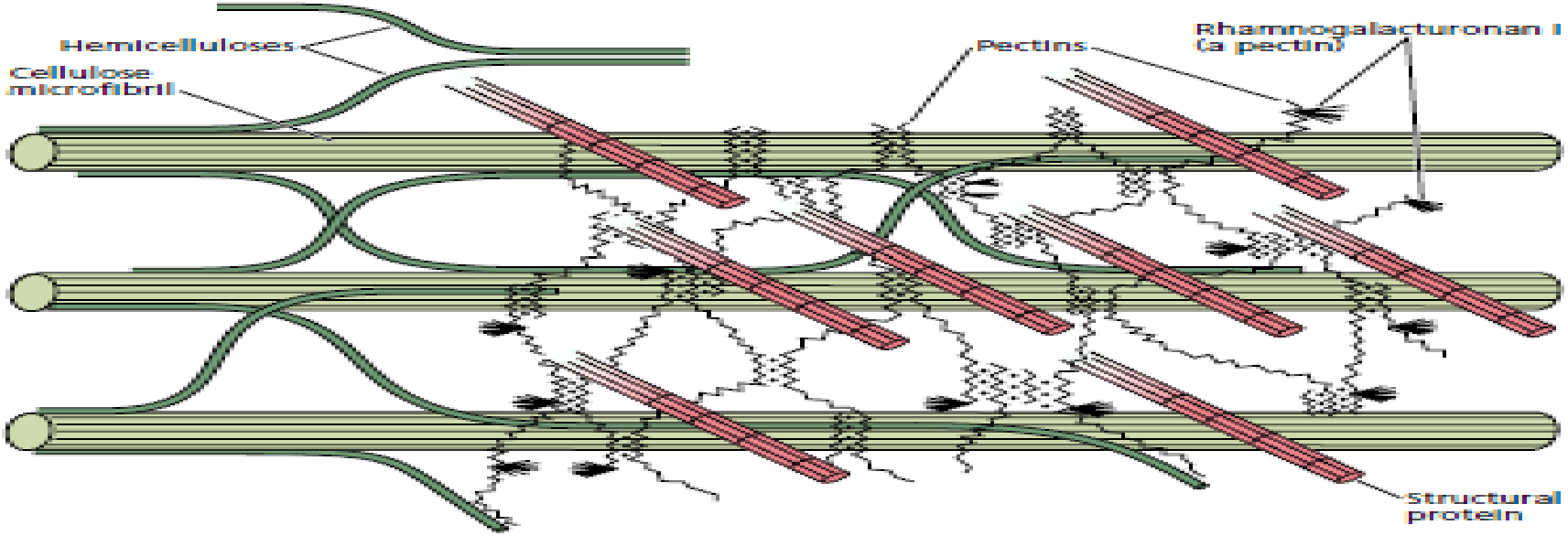
In principle, auxin could increase the growth rate by increasing *m, increasing Yp, or decreasing Y. Although* extensive experiments have shown that auxin does not increase turgor pressure when it stimulates growth, conflicting results have been obtained regarding auxininduced decreases in *Y. However, there is general agreement* that auxin causes an increase in the wall extensibility parameter, *m.*

Auxin and cell water relation ?

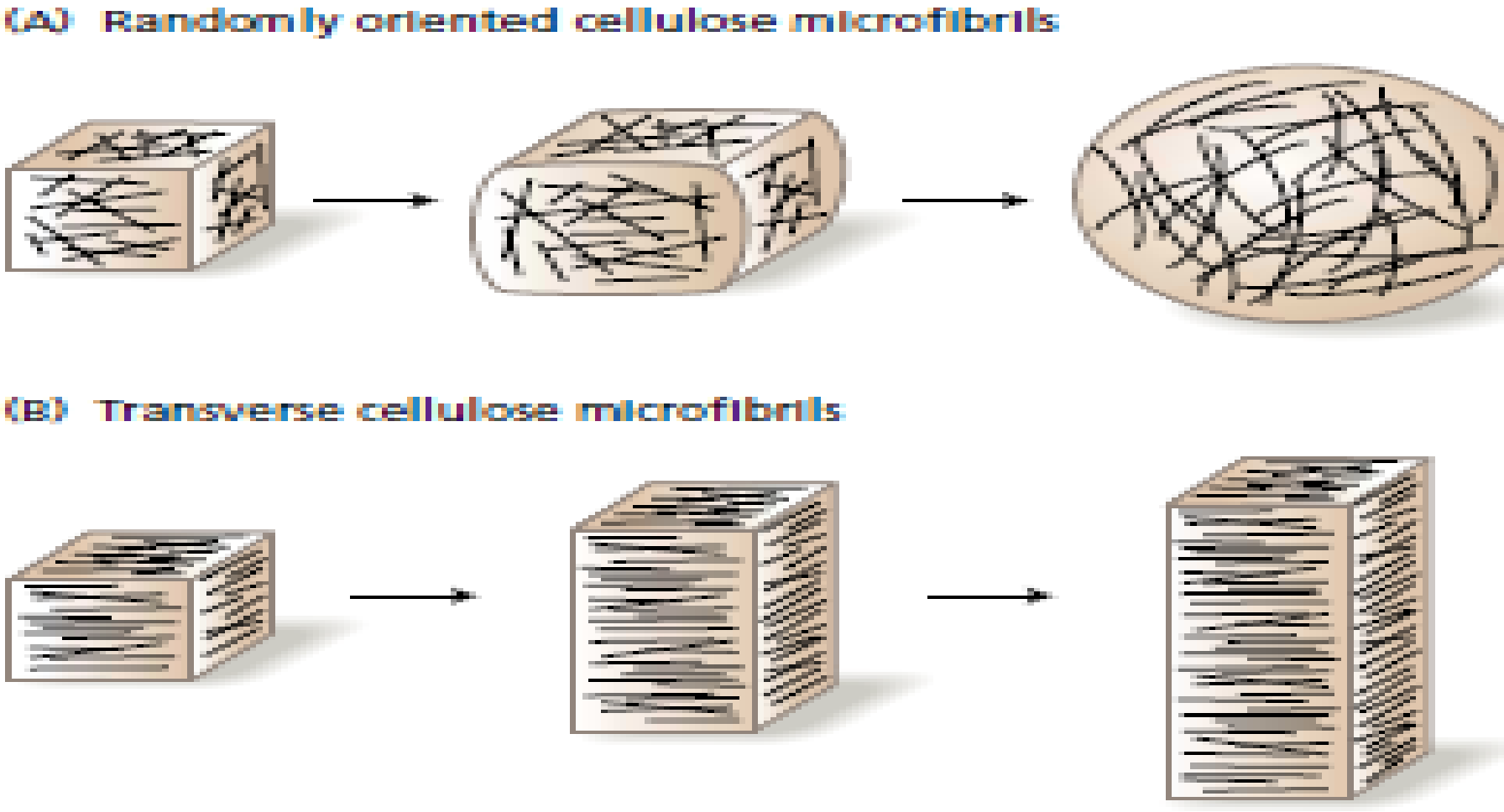
Auxin increase cell elasticity or plasticity?




(C)
Wall matrix in which microfibrils are embedded
(1→4)β-glucan chains in a cellulose microfibril
Microfibrils linked by xyloglucans
Cellulose-synthesizing complex in the plasma membrane
Outer leaflet of lipid bilayer
Cell wall
Microfibril emerging from plasma membrane
Lipid bilayer of plasma membrane
Cellulose microfibril emerging from rosette
Inner leaflet of lipid bilayer
Microtubule bridged to plasma membrane (and cell wall)
Microtubule
Intermicrotubule bridge


Hemicelluloses
Cellulose microfibril
Pectins
Rhamnogalacturonan I (a pectin)
Structural protein

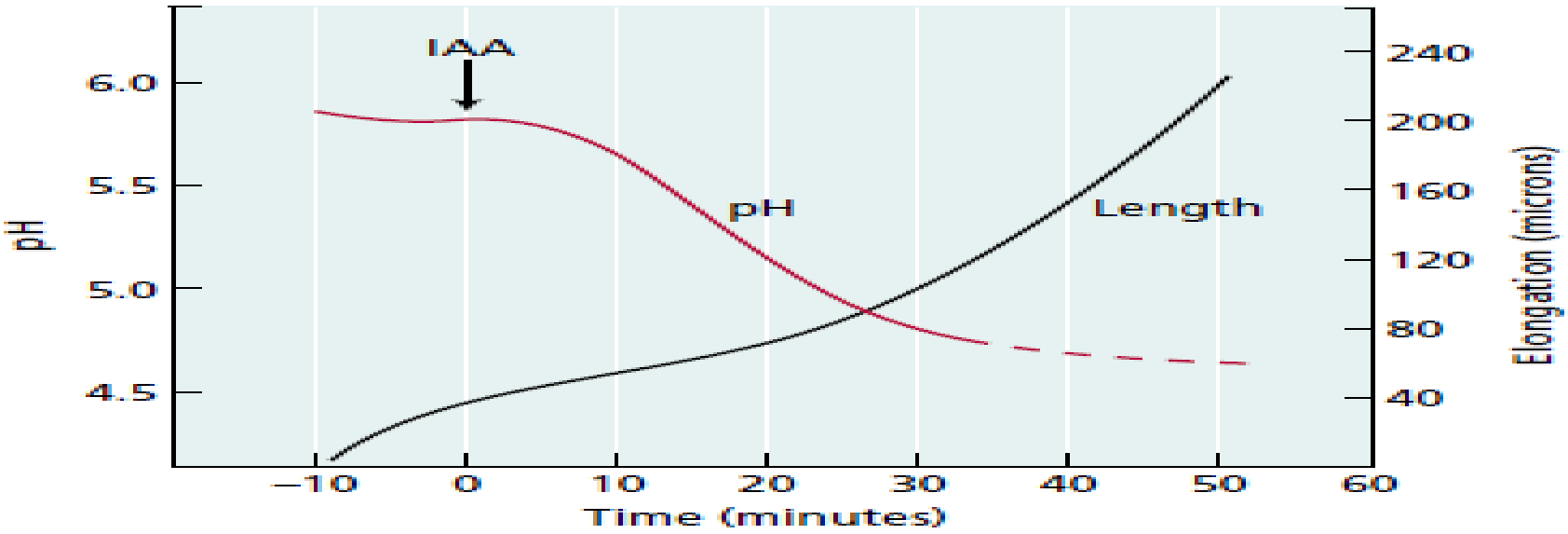
The orientation of newly deposited cellulose microfibrils determines the direction of cell expansion.



Auxin-Induced Proton Extrusion Acidifies the Cell Wall and Increases Cell Extension

The acid growth hypothesis allows five main predictions:

1. Acid buffers alone should promote short-term growth, provided the cuticle has been abraded to allow the protons access to the cell wall.
2. Auxin should increase the rate of proton extrusion (wall acidification), and the kinetics of proton extrusion should closely match those of auxin-induced growth.
3. Neutral buffers should inhibit auxin-induced growth.
4. Compounds (other than auxin) that promote proton extrusion should stimulate growth.
5. Cell walls should contain a “wall loosening factor” with an acidic pH optimum.

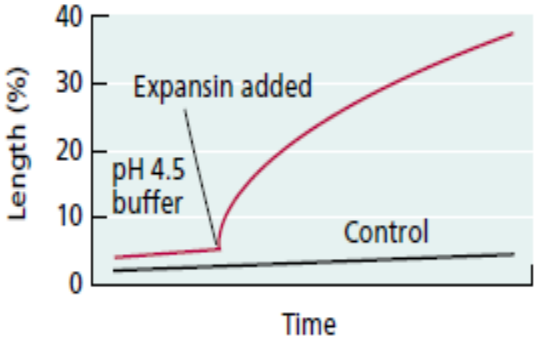


Auxin-induced growth has also been shown to be inhibited by neutral buffers, as long as the cuticle has been abraided.

Fusicoccin, a fungal phytotoxin, stimulates both rapid proton extrusion and transient growth in stem and coleoptile sections.

Wall loosening proteins called **expansins have been identified** in the cell walls of a wide range of plant species.

At acidic pH values, expansins loosen cell walls by weakening the hydrogen bonds between the polysaccharide components of the wall.

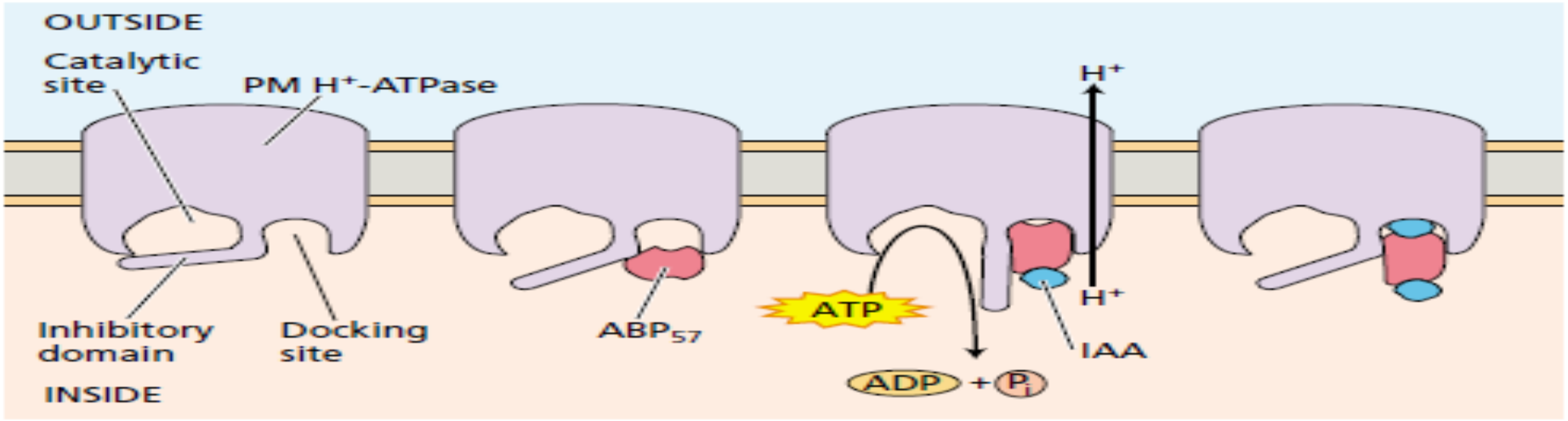


Auxin-Induced Proton Extrusion May Involve Both Activation and Synthesis:

In theory, auxin could increase the rate of proton extrusion by two possible mechanisms:
1.Activation of preexisting plasma membrane H+- ATPases

2. Synthesis of new H+-ATPases on the plasma membrane

Model for the activation of the plasma membrane (PM) H+-ATPase by ABP and auxin.



ABP57 binds PM H+-ATPase at docking site.

IAA binding causes conformational change in ABP57. ABP57 then interacts with inhibitory domain of PM H+-ATPase activating the enzyme.

Binding of IAA to second site decreases interaction with H+-ATPase inhibitory domain; the enzyme is inhibited.

the plasma membrane (PM)

H+-ATPase synthesis.

Protein synthesis inhibitors, such as cycloheximide, to rapidly inhibit auxin induced proton extrusion and growth suggests that auxin might also stimulate proton pumping by increasing the synthesis of the H+-ATPase.

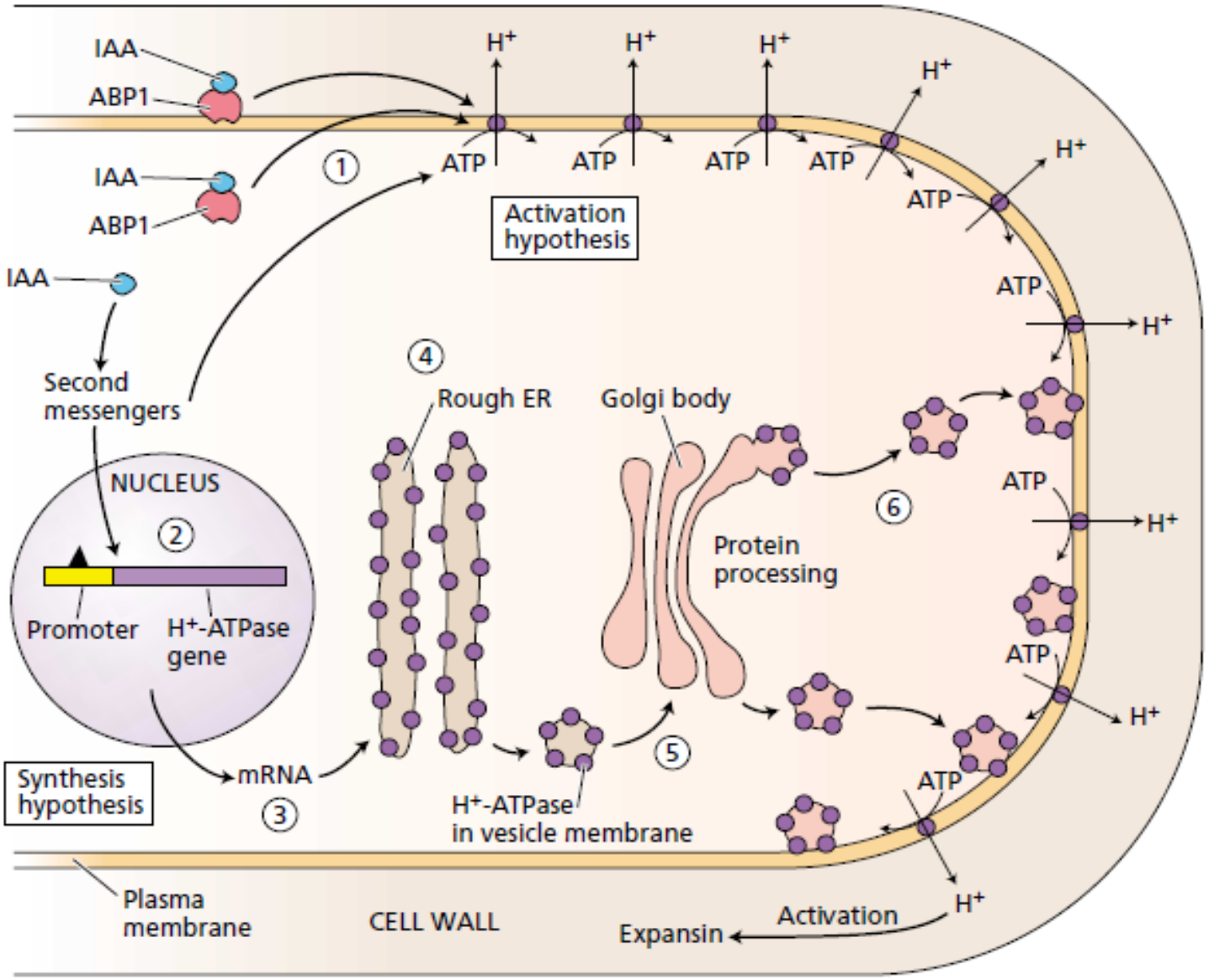
An increase in the amount of plasma membrane ATPase in corn coleoptiles was detected immunologically after only 5 minutes of auxin treatment, and a doubling of the H+-ATPase was observed after 40 minutes of treatment. A threefold stimulation by auxin of an mRNA for the H+-ATPase was demonstrated specifically in the nonvascular tissues of the coleoptiles.

In summary, the question of activation versus synthesis is still unresolved, and it is possible that auxin stimulates proton extrusion by both activation and stimulation of synthesis of the H+-ATPase.

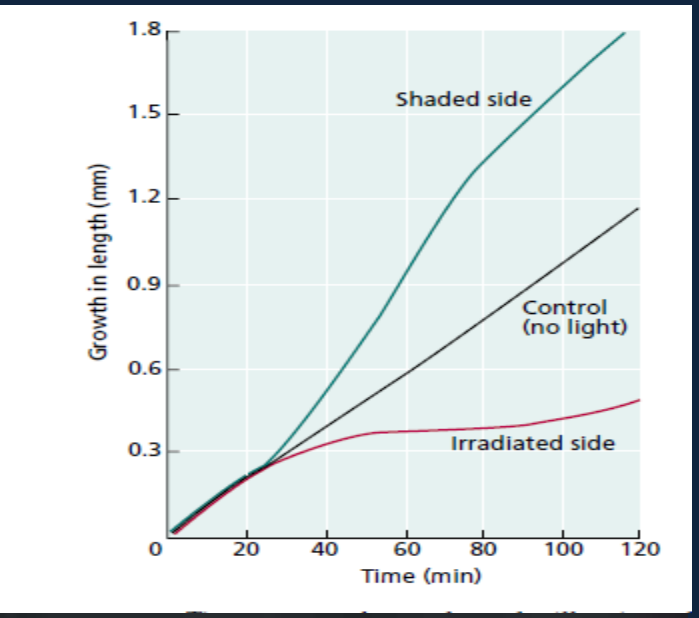
Current Model of Auxin induced cell elongation:

Activation hypothesis: Auxin binds to an auxin-binding 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, could 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.



Phototropism Is Mediated by the Lateral Redistribution of Auxin

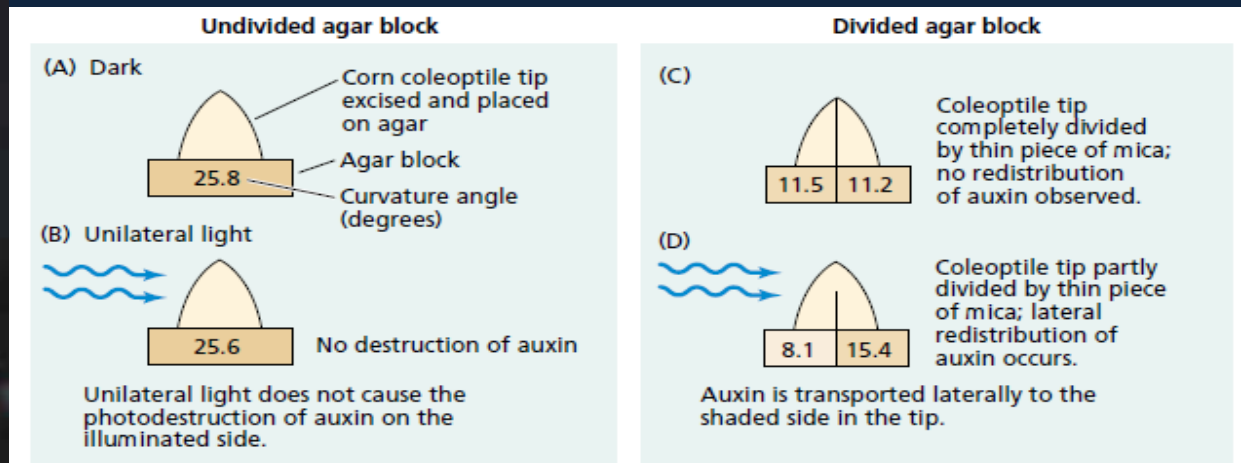


Cholodny–Went model of phototropism, the tips of grass coleoptiles have three specialized functions:

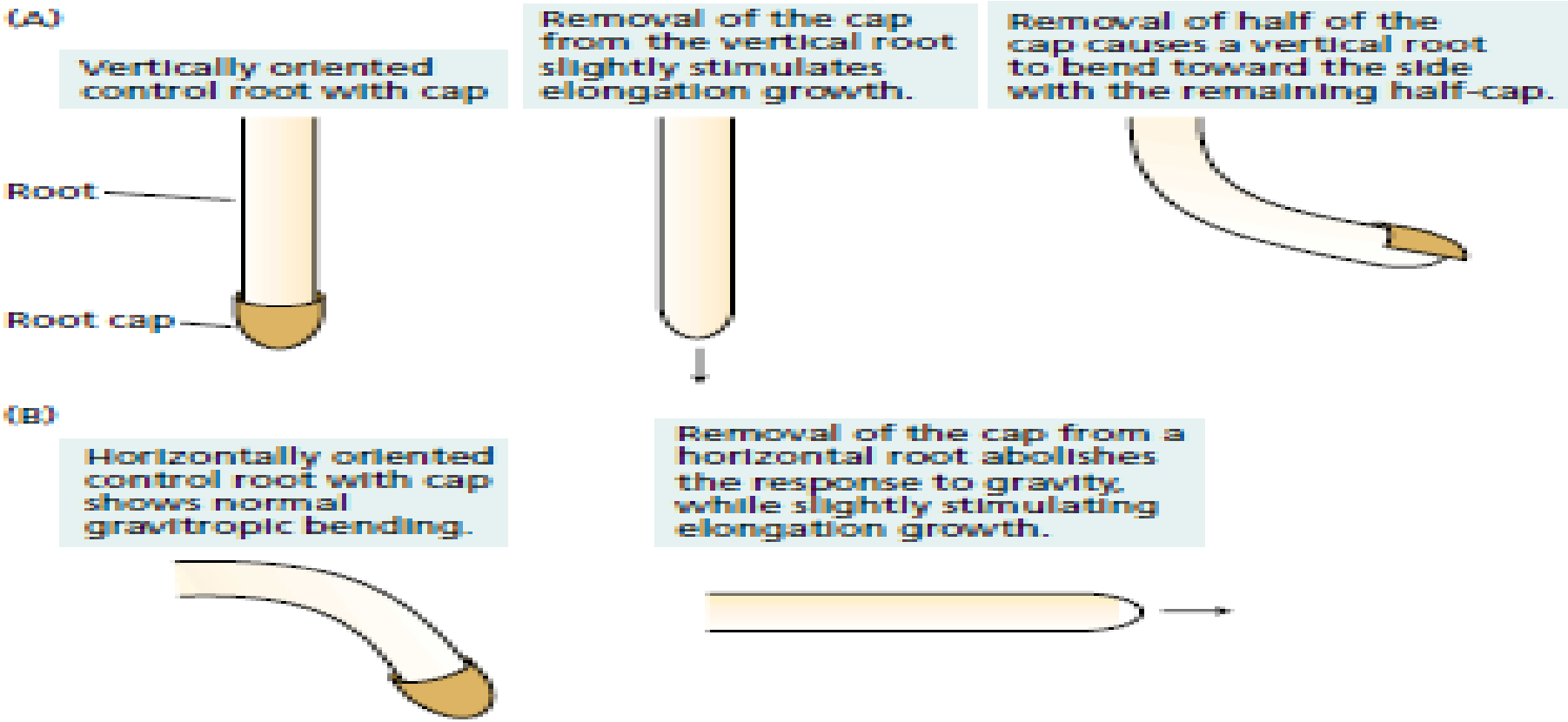
1. The production of auxin
2. The perception of a unilateral light stimulus
3. The lateral transport of IAA in response to the phototropic stimulus

According to the current hypothesis, the gradient in phototropin phosphorylation induces the movement of auxin to the shaded side of the coleoptile

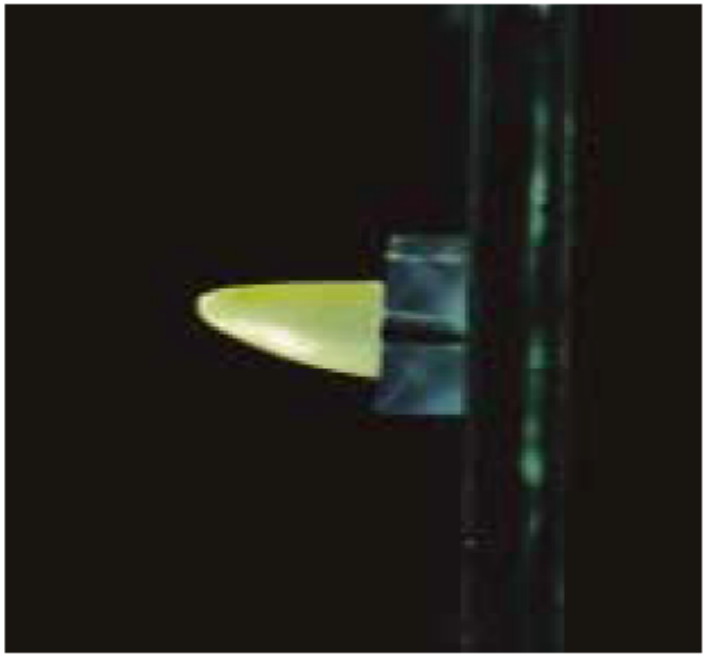
Consistent with both the Cholodny–Went hypothesis and the acid growth hypothesis, the apoplastic pH on the shaded side of a phototropically bending stem or coleoptile is more acidic than the side facing the light



Gravitropism Also Involves Lateral Redistribution of Auxin



(A)



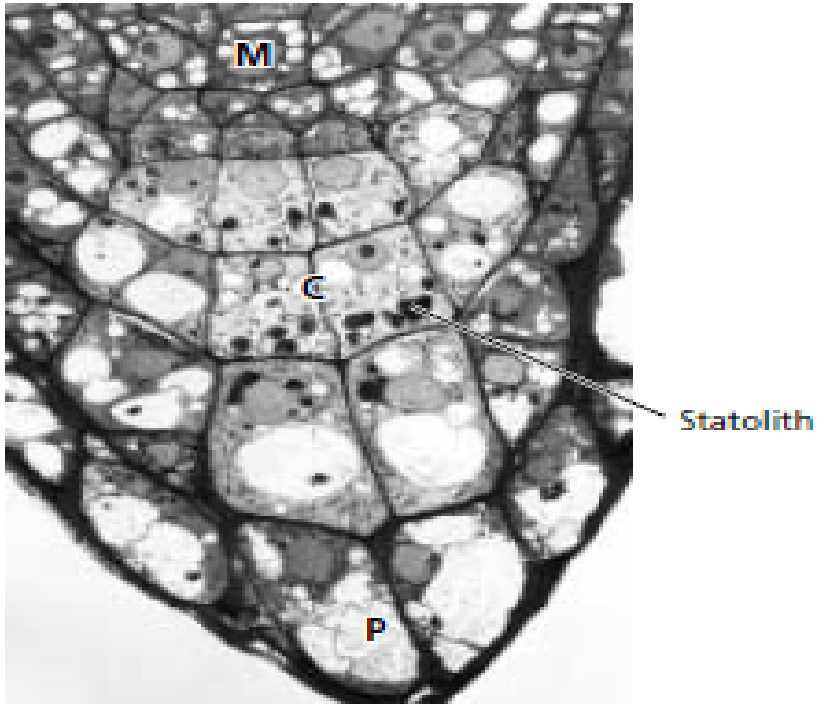
(B)

In vertical seedlings, SAUR gene expression is symmetrically distributed. Within 20 minutes after the seedling is oriented horizontally, SAURs begin to accumulate on the lower half of the hypocotyl. Under these conditions, gravitropic bending first becomes evident after 45 minutes, well after the induction of the SAURs. ***The existence of*** *a lateral gradient in SAUR gene expression is indirect evidence for the existence of a lateral gradient in auxin detectable within 20 minutes of the gravitropic stimulus.*

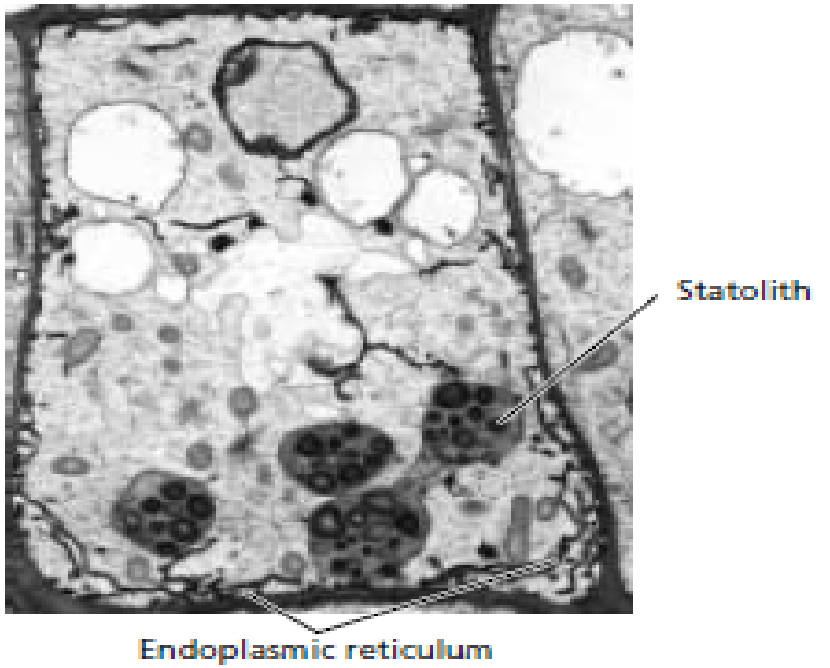
Statoliths Serve as Gravity Sensors in Shoots and Roots

Unlike unilateral light, gravity does not form a gradient between the upper and lower sides of an organ. All parts of the plant experience the gravitational stimulus equally. How do plant cells detect gravity? The only way that gravity can be sensed is through the motion of a falling or sedimenting body.

*Statoliths (**statocytes**) and starch sheath*



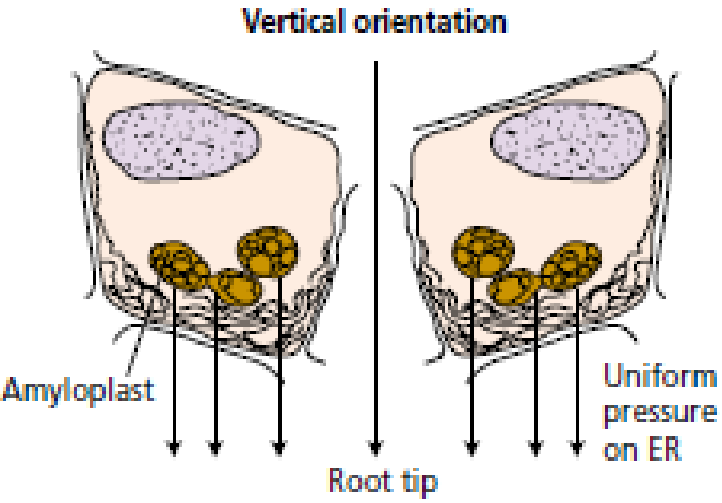
(B)



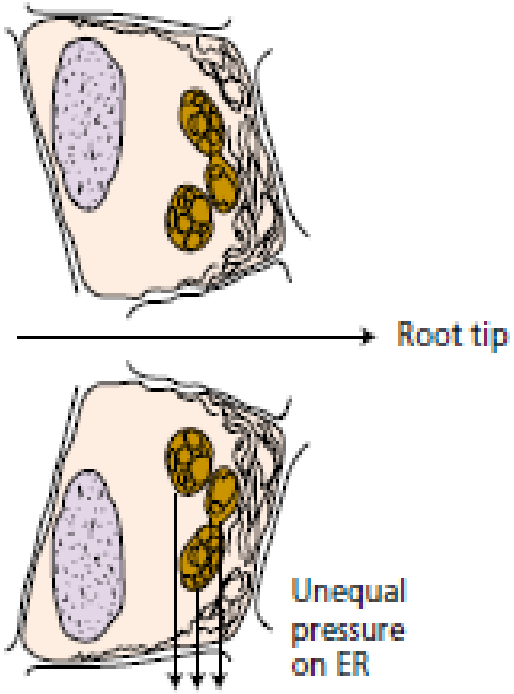
apical meristem (M),
columella (C),
and peripheral (P) cells.

Enlarged view of a columella cell, showing the amyloplasts resting on top of endoplasmic reticulum at the bottom of the cell.

(C)



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.

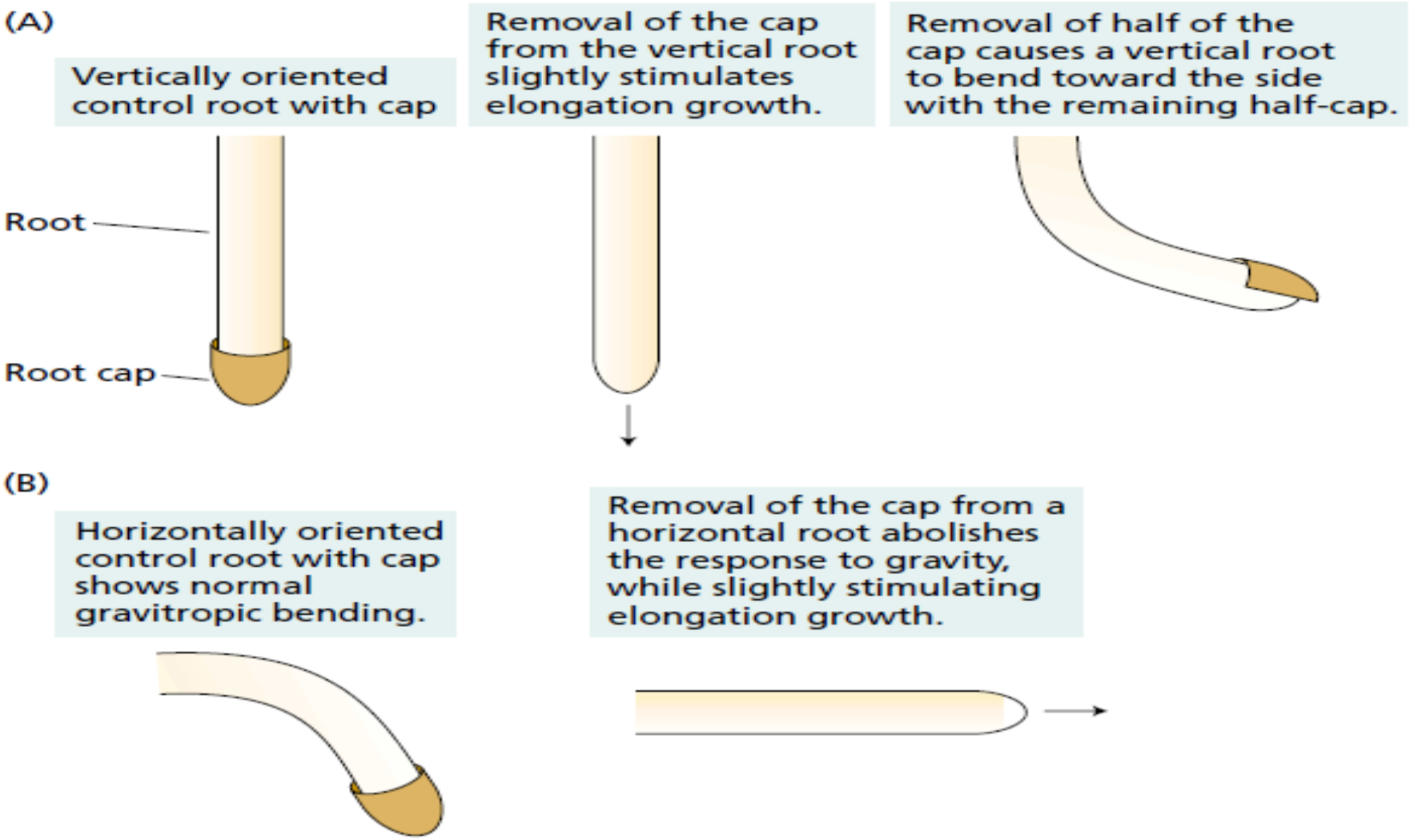
*The **starch–statolith hypothesis of gravity perception in** roots is supported by several lines of evidence.*

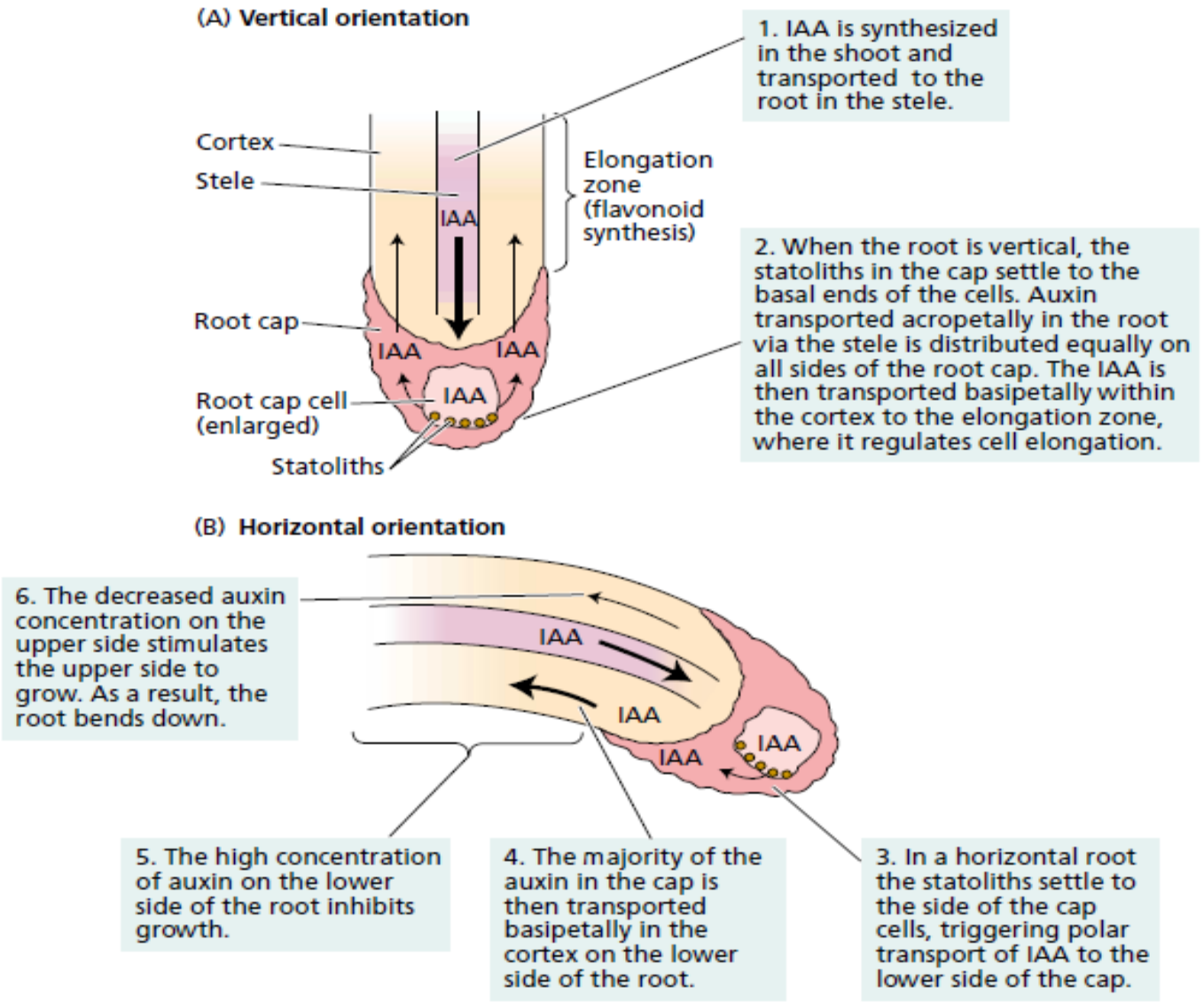
Amyloplasts are the only organelles that consistently sediment in the columella cells of different plant species, and the rate of sedimentation correlates closely with the time required to perceive the gravitational stimulus.

The gravitropic responses of starch-deficient mutants are generally much slower than those of wild-type plants.

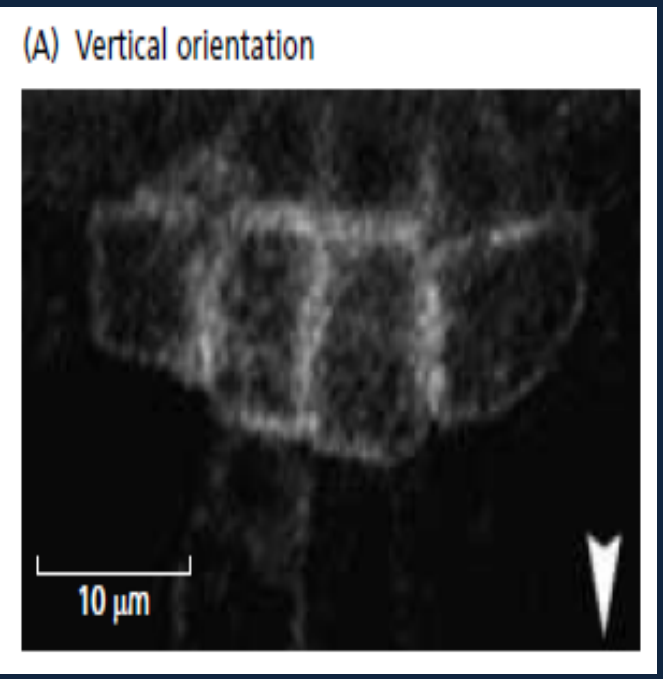
Nevertheless, starchless mutants exhibit some gravitropism, suggesting that although starch is required for a normal gravitropic response, starch-independent gravity perception mechanisms may also exist.

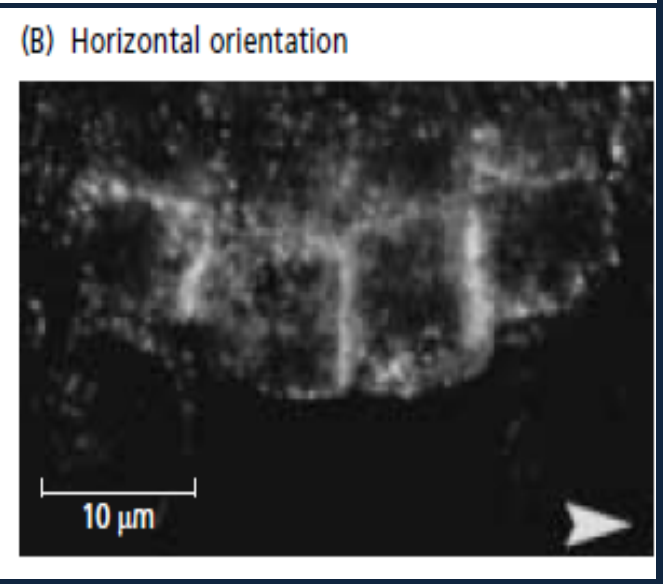
Because the cap is some distance away from the elongation zone where bending occurs, a chemical messenger is presumed to be involved in communication between the cap and the elongation zone.




(A) Vertical orientation
1. IAA is synthesized in the shoot and transported to the root in the stele.
Cortex
Stele
Elongation zone (flavonoid synthesis)
IAA
Root cap
IAA
IAA
Root cap cell (enlarged)
IAA
Statoliths
2. When the root is vertical, the statoliths in the cap settle to the basal ends of the cells. Auxin transported acropetally in the root via the stele is distributed equally on all sides of the root cap. The IAA is then transported basipetally within the cortex to the elongation zone, where it regulates cell elongation.
(B) Horizontal orientation
6. The decreased auxin concentration on the upper side stimulates the upper side to grow. As a result, the root bends down.
IAA
IAA
IAA
IAA
5. The high concentration of auxin on the lower side of the root inhibits growth.
4. The majority of the auxin in the cap is then transported basipetally in the cortex on the lower side of the root.
3. In a horizontal root the statoliths settle to the side of the cap cells, triggering polar transport of IAA to the lower side of the cap.

PIN3 Is Relocated Laterally to the Lower Side of Root Columella Cells





As noted previously, PIN proteins are constantly being cycled between the plasma membrane and intracellular secretory compartments. This cycling allows some PIN proteins to be targeted to specific sides of the cell in response to a directional stimulus.

In a vertically oriented root, PIN3 is uniformly distributed around the columella cell. But when the root is placed on its side, PIN3 is preferentially targeted to the lower side of the cell . As a result, auxin is transported polarly to the lower half of the cap.

Auxin Regulates Apical Dominance

The axillary buds are suppressed in the intact plant because of apical dominance.

Removal of the terminal bud releases the axillary buds from apical dominance.

Applying IAA in lanolin paste (contained in the gelatin capsule) to the cut surface prevents the outgrowth of the axillary buds.

Kenneth V. Thimann and Folke Skoog originally proposed that auxin from the shoot apex inhibits the growth of the axillary bud directly—the so-called direct inhibition model.

Objection:

No significant differences in IAA content in the nodes of buds that were arrested or growing after decapitation have been reported.

If shoot tip is bent down, the laterals are released from arrest with no change in endogenous IAA.

Nutritive theory

?

- Auxin increases after decapitation.

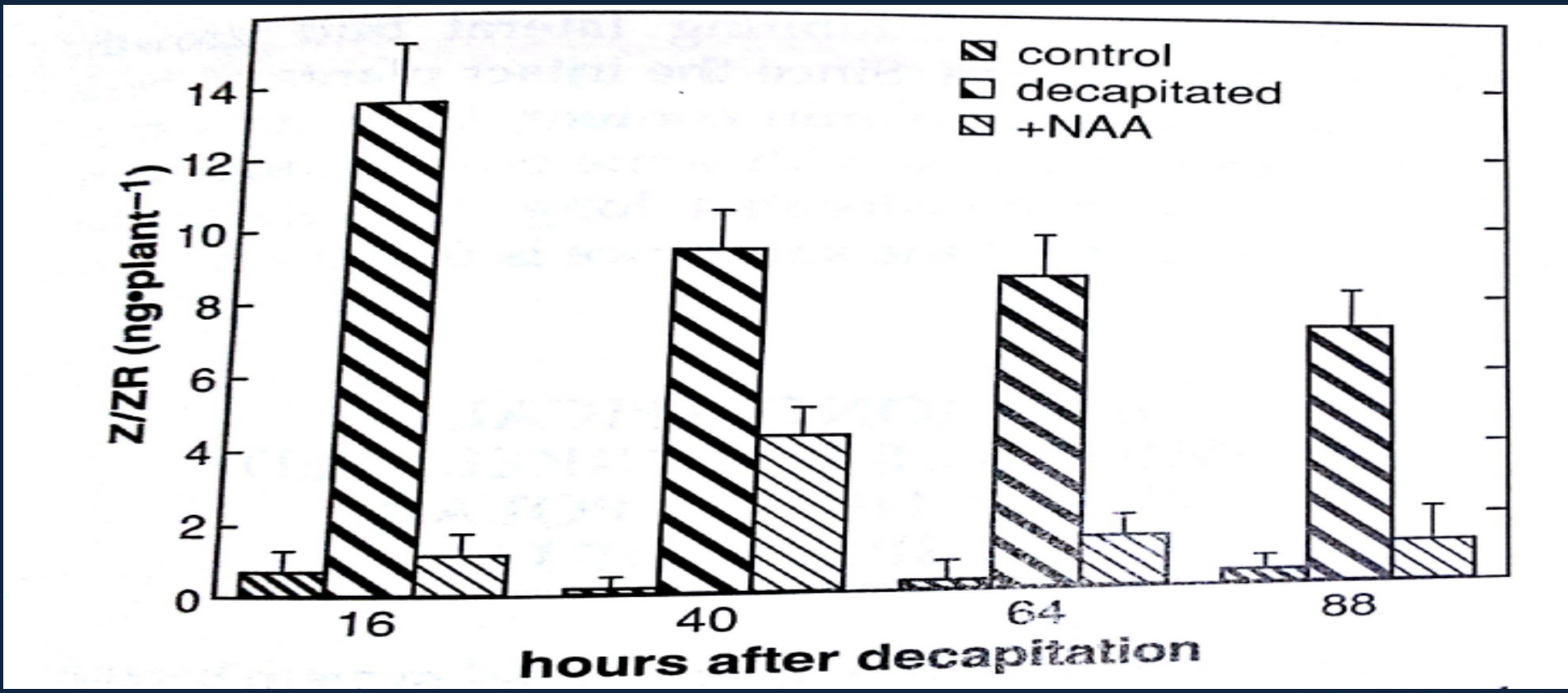
- Direct application of cytokinins to axillary buds stimulates bud growth in many species, overriding the inhibitory effect of the shoot apex. Auxin makes the shoot apex a sink for cytokinin synthesized in the root, and this may be one of the factors involved in apical dominance.

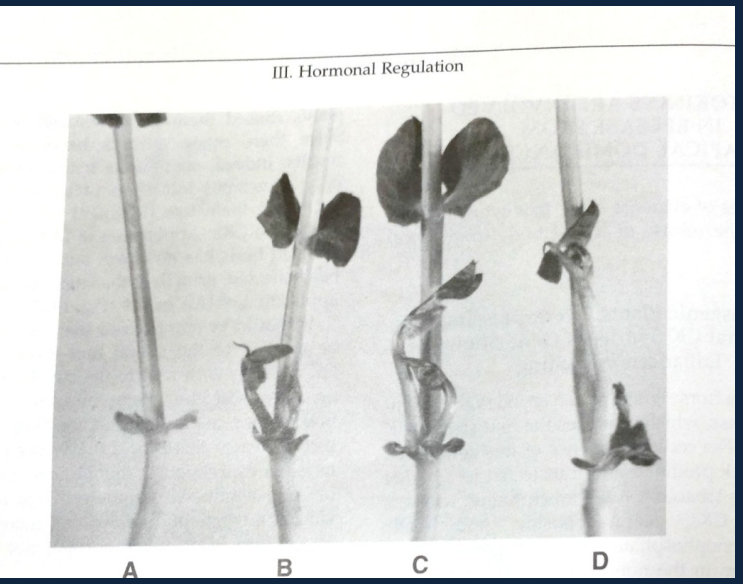
- Finally, ABA has been found in dormant lateral buds in intact plants. When the shoot apex is removed, the ABA levels in the lateral buds decrease. High levels of IAA in the shoot may help keep ABA levels high in the lateral buds. Removing the apex removes a major source of IAA, which may allow the levels of bud growth inhibitor to fall.

- Other factors viz. light, distance from apical tip, reproductive phase.

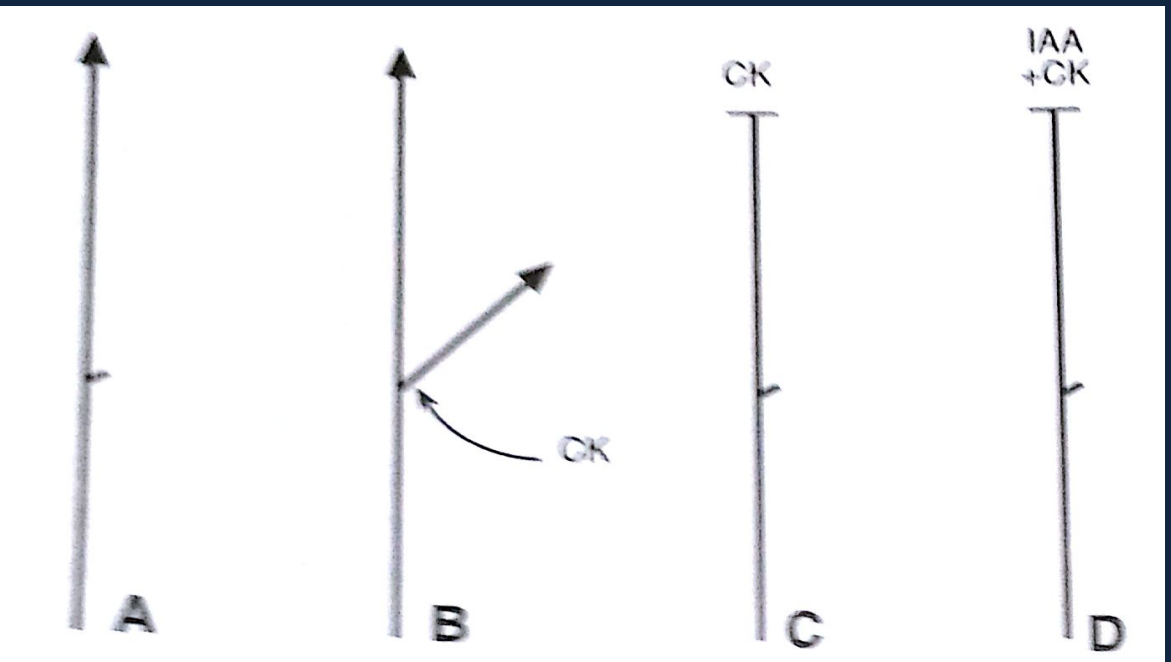
CK are involved in release from apical dominance:

Exogenous Ck application
Transgenic studies





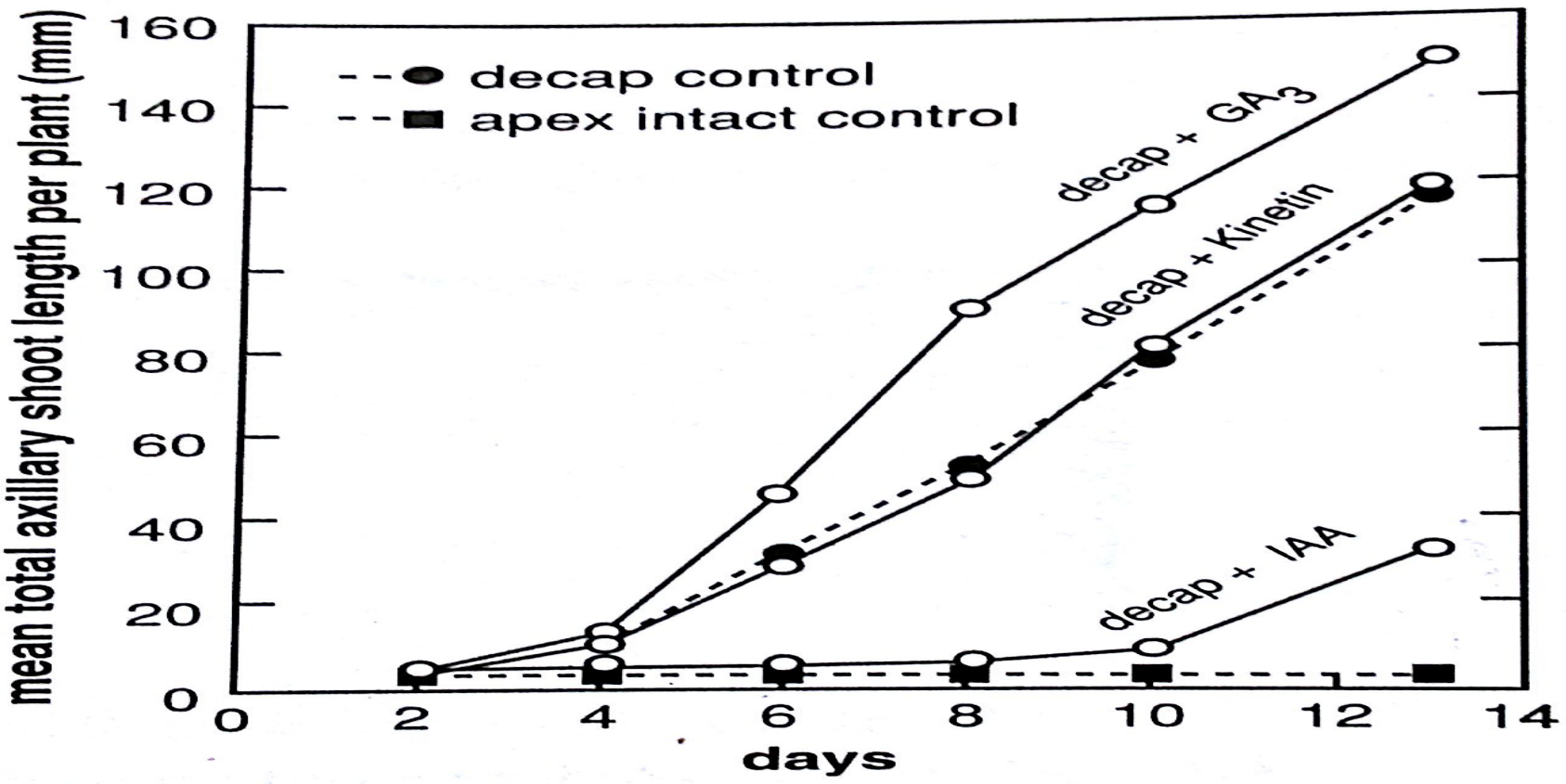
A. Untreated control
B. Bud treated with KN
C. KN followed by GA
D. KN followed by IAA



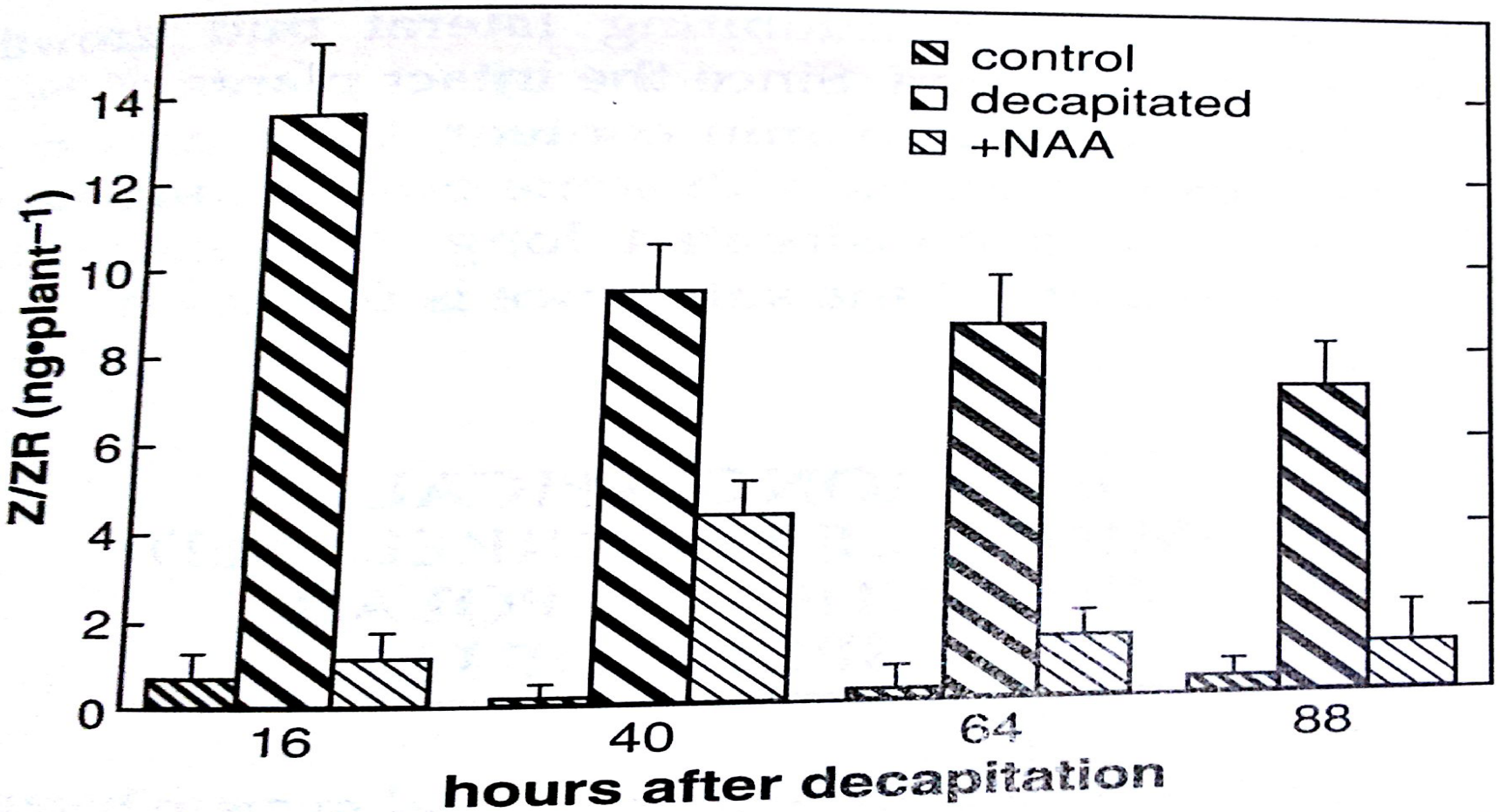
Ratio of IAA and CK.

Abundance of IAA could reduce free CK or vice versa.

Such inhibition is caused only by auxins.

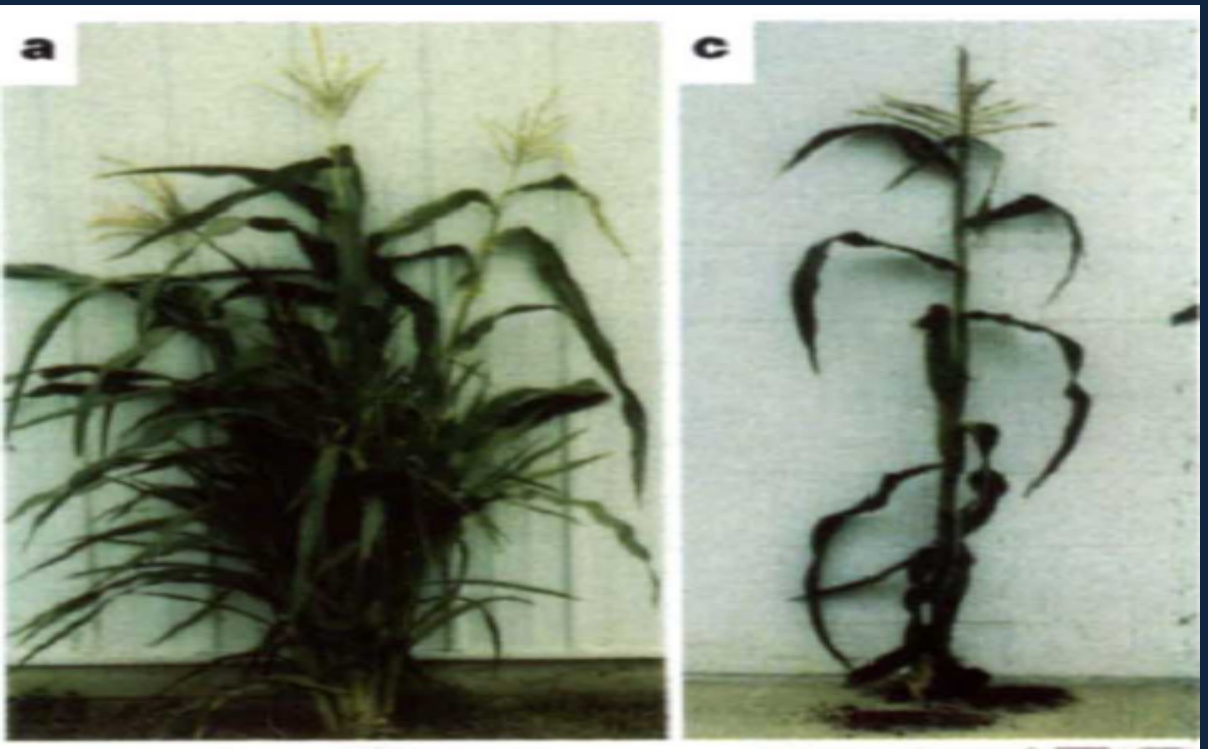


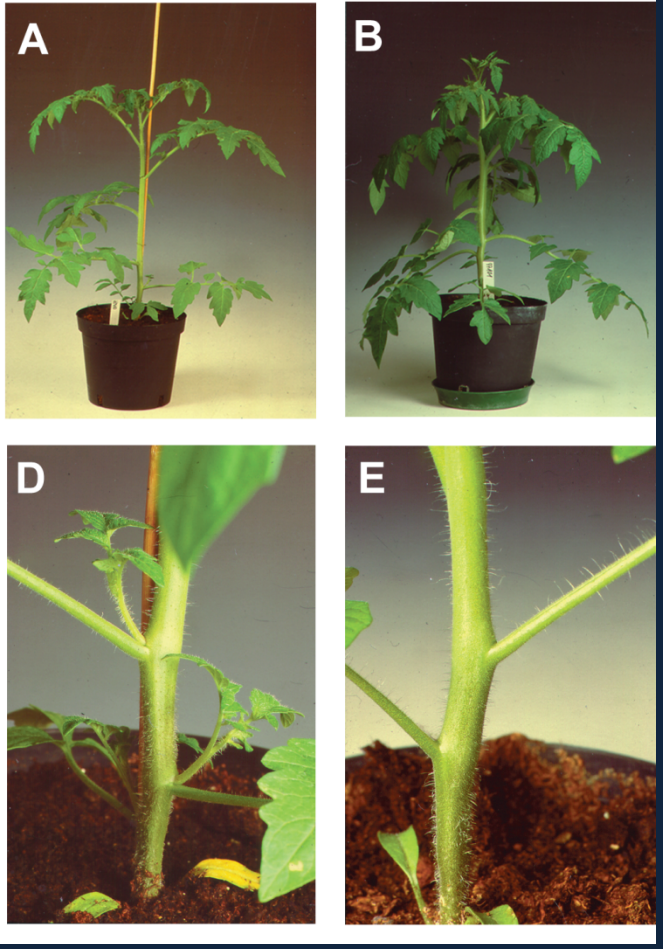
Amount of zeatin/zeatin riboside in the xylem exudates of P. vulgaris at different times after decapitation.



The developmental program of the plant takes priority over regulation of hormones.

✓ Plant transformed with ipt gene, axillary buds show increased growth but such growth usually occurs in upper nodes or later stages of plant development.
✓ Plants transformed by the iaaL gene drop free IAA levels, but may not show enhanced branching until the time of flowering.
✓ The tb1 (for teosinte branched 1) mutant of maize shows a much larger number of tillers than normal maize. The Tb1 gene is also expressed in maize plants at nearly twice the level as in Teosinte.



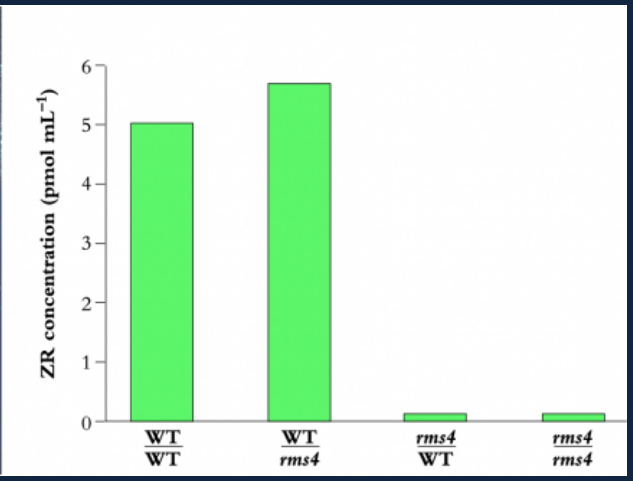


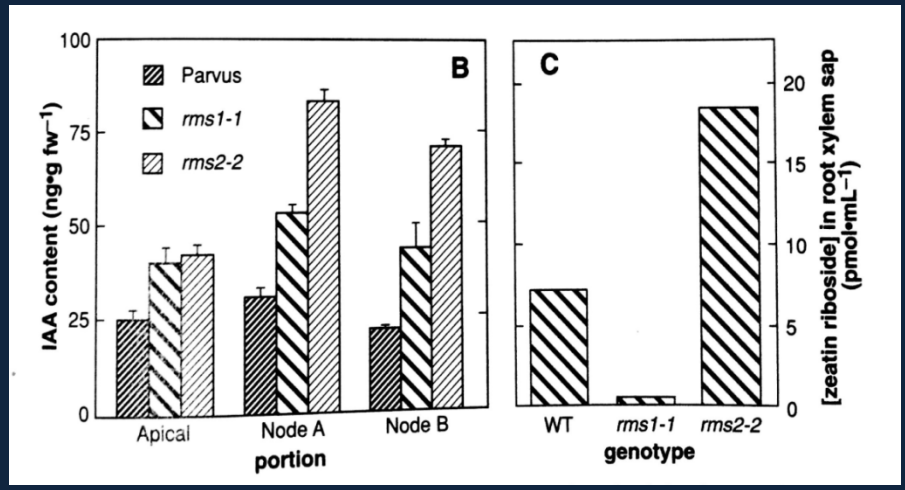
Phenotypes of wild type and *ls* mutant of tomato

✓ The ls (for lateral suppressor) mutant of tomato fails to produce axillary buds in leaf axils during vegtative growth although on transition to flowering lateral buds are formed. The Ls gene encodes a protein that shares sequence similarly to the GRAS family of plant transcriptional regulators.

✓ rms (for ramosus, latin for having many branches) mutant of pea showed enhanced branching, but no correlation with IAA content in tissues proximal to axillary buds and CK levels in the xylem sap.

In pea, the *rms4* mutant is highly brached and has extremely low levels of cytokinins moving from root to shoot in the xylem sap. The conventional theory of apical dominance regulation suggests that *high* cytokinin levels would be associated with increased branching. The evidence here from reciprocal grafts between *rms4* and its wild type is that the extent of shoot branching governs the export of cytokinins from the root rather than vice versa.





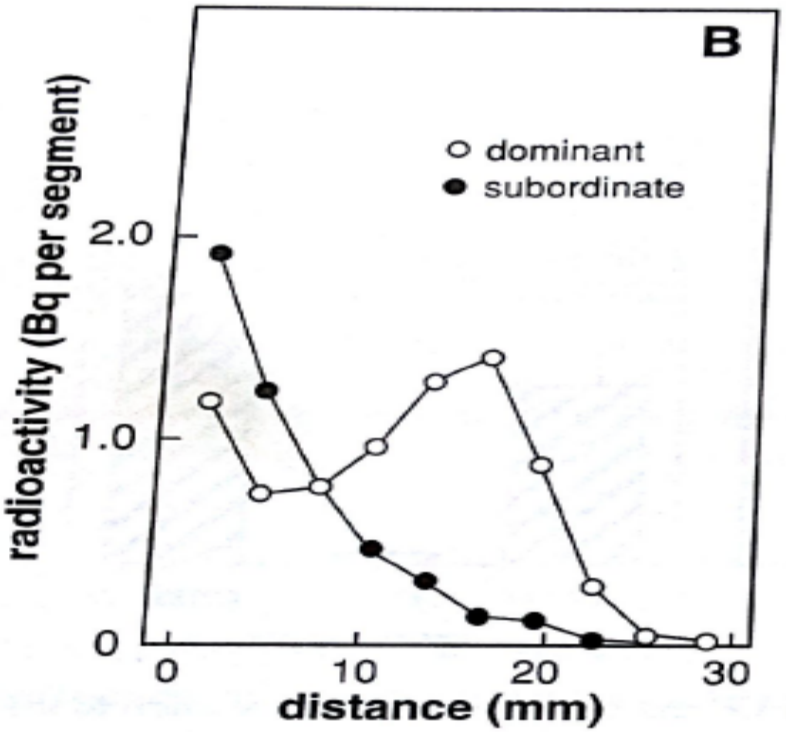
The deduction is that the normal Rms4 gene is acting in the shoot only, and two consequences of the rms4 mutation are enhanced branching and downregulation of root cytokinin export.

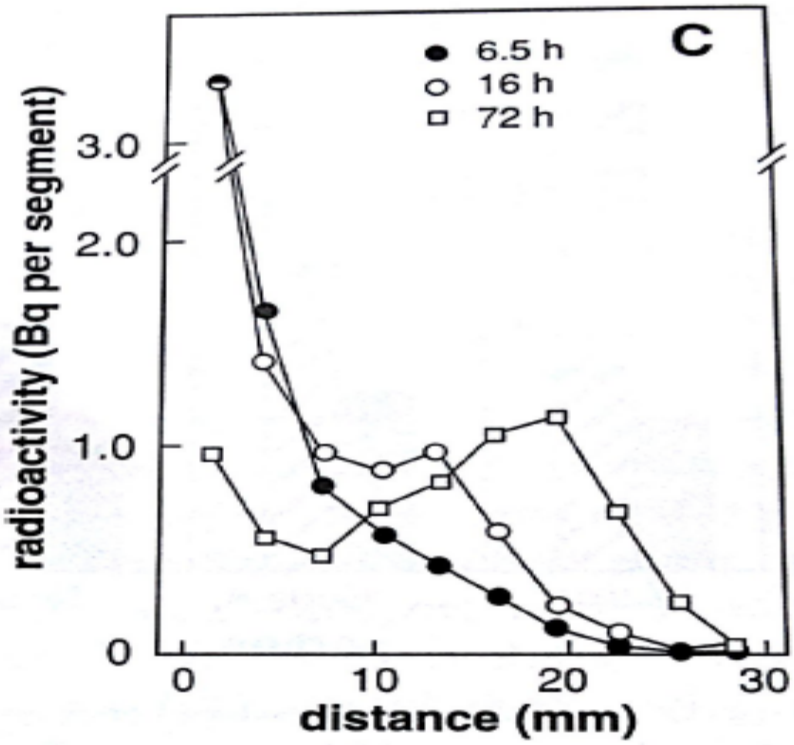
Other hormones in apical dominance:

Other hormones do not seems to play a role.

Exogenous ABA inhibits cell division and growth at apical tip, release the lateral buds from inhibition.

Polar transport of IAA in growing lateral buds.





B. Transport of IAA in segments from dominant and subordinate shoots. In the dominant shoots much more radiolabel translocated over longer distance than the subordinate shoot.

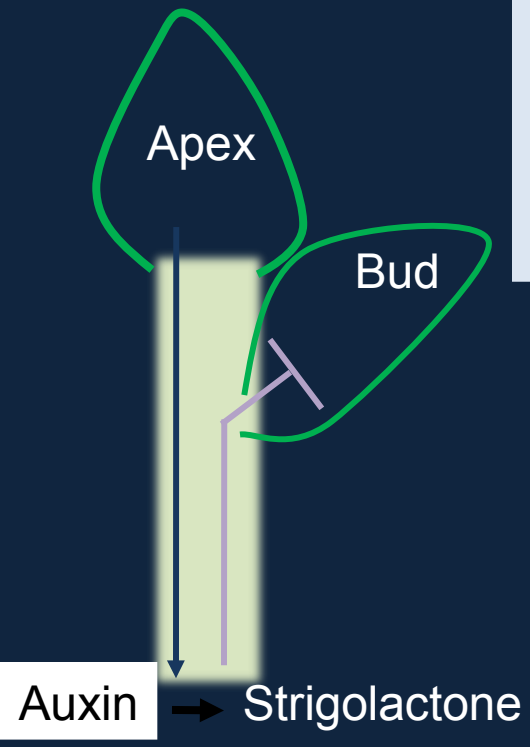
C. Transport of IAA in segments from subordinate shoot taken at different hrs after decapitation of the dominant shoot.

“Canalization”

A Root-Derived Hormone, Strigolactone, Is Involved in the Suppression of Branching in Shoots

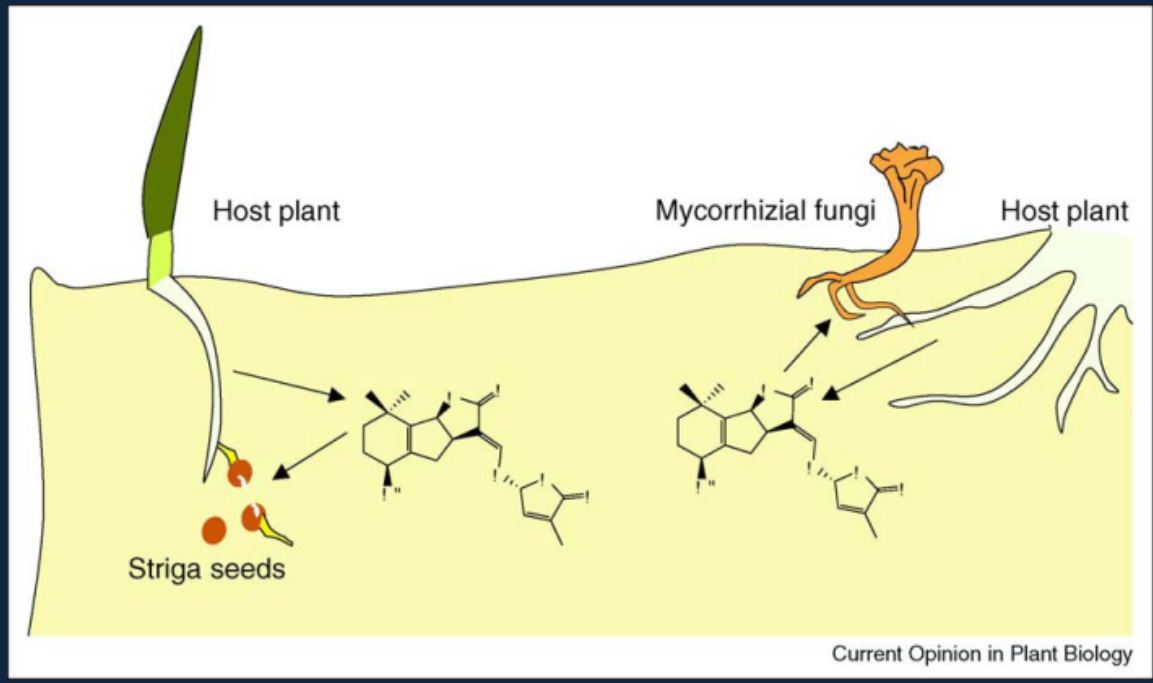
Evidence for a root-derived signaling molecule that is involved in the suppression of branching in the shoot comes from grafting studies in mutants with highly branched phenotypes. The branching genes *MAX4*(***M****ORE* ***AX****ILLARY* *BRANCHING****4***), *RMS1* (***R****A****M****OSU****S1***), and *HTD* (***H****IGH-****T****ILLERING* ***D****WARF*) in Arabidopsis, pea, and rice, respectively, are involved in the production of this root derived signal, which was found to be a carotenoid derivative (Sorefan et al. 2003; Foo et al. 2005). Recent work has identified this signal as a novel plant hormone, strigolactone, which also promotes the germination of root parasitic weeds and the symbiosis with arbuscular mycorrhizal fungi (Gomez-Roldan et al. 2008; Umehara et al. 2008).

Strigolactones inhibit branch outgrowth



Auxin transported from the shoot to the root induces strigolactone synthesis, which indirectly inhibits bud outgrowth.

In a rice mutant that does not produce strigolactones, tillers (lateral branches) grow out as shown.



Strigolactones, synthesized from carotenoids, are produced in plant roots. They attract mycorrhizal fungi and promote the germination of parasitic plants of the genus *Striga*.

Summary of apical dominance

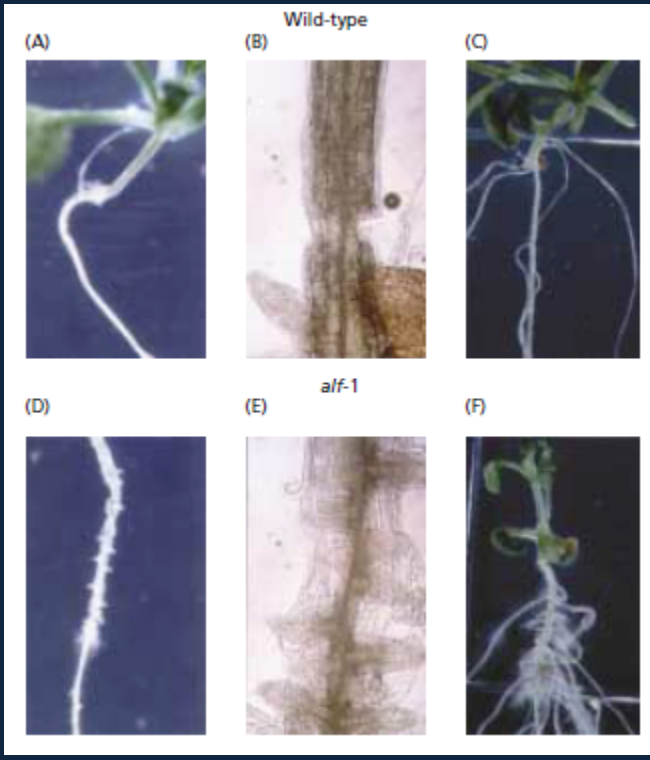
- ✓It is a complex phenomenon which is determined genetically.
- ✓Not all plants shows complete apical dominance.
- ✓It is regulated by relative concentration of IAA and CK.
- ✓Translocation of IAA and CK is more important.
- ✓Transgenic/branching mutant studies indicated that it is controlled by genetic make up and developmental programme of the plant.
- ✓Some factor other than CK may be interacting with auxin.
- ✓There is strong correlation b/w apical dominance of shoot and polar basipetal movement of IAA.
- ✓Relocation of auxin efflux carriers.

Auxin Promotes the Formation of Lateral and Adventitious Roots

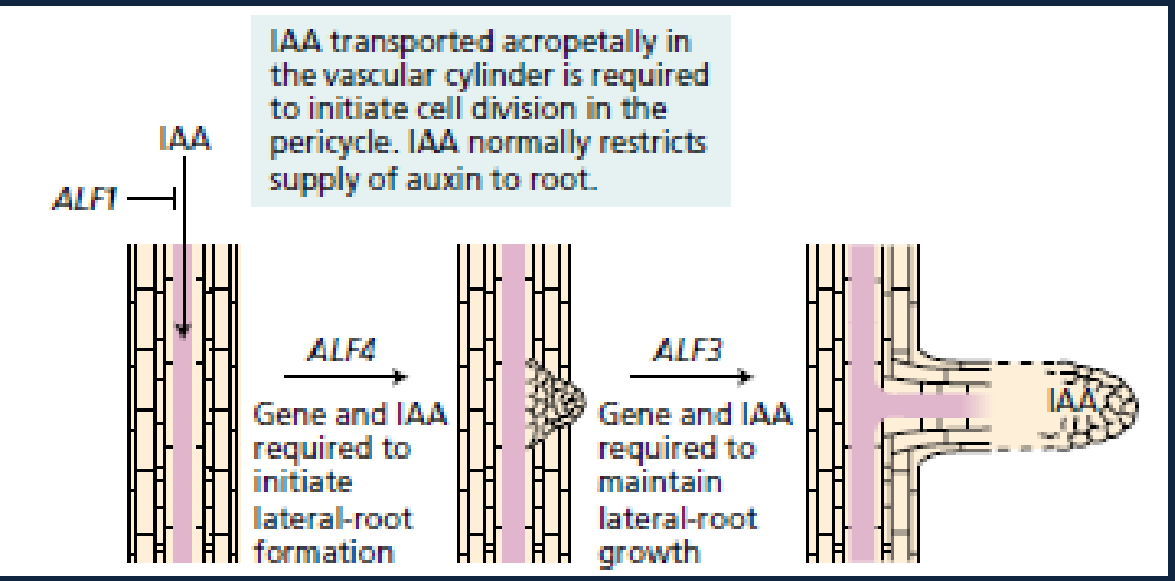
Elongation of the primary root is inhibited by auxin. *Initiation* of lateral (branch) roots and adventitious roots is stimulated by high auxin levels.

These roots originates from small groups of cells in the pericycle . Auxin stimulates these pericycle cells to divide. The dividing cells gradually form into a root apex, and the lateral root grows through the root cortex and epidermis.

Arabidopsis mutants, alf (aberrant lateral root formation), have provided some insights into the role of auxin in the initiation of lateral roots. The *alf1 mutant exhibits extreme proliferation* of adventitious and lateral roots, coupled with a 17-fold increase in endogenous auxin.



Root morphology of *Arabidopsis (A–C) wild-type* and *alf1 seedlings (D–F) on hormone-free medium. Note the proliferation* of root primoridia growing from the pericycle in the *alf1* seedlings (D and E).



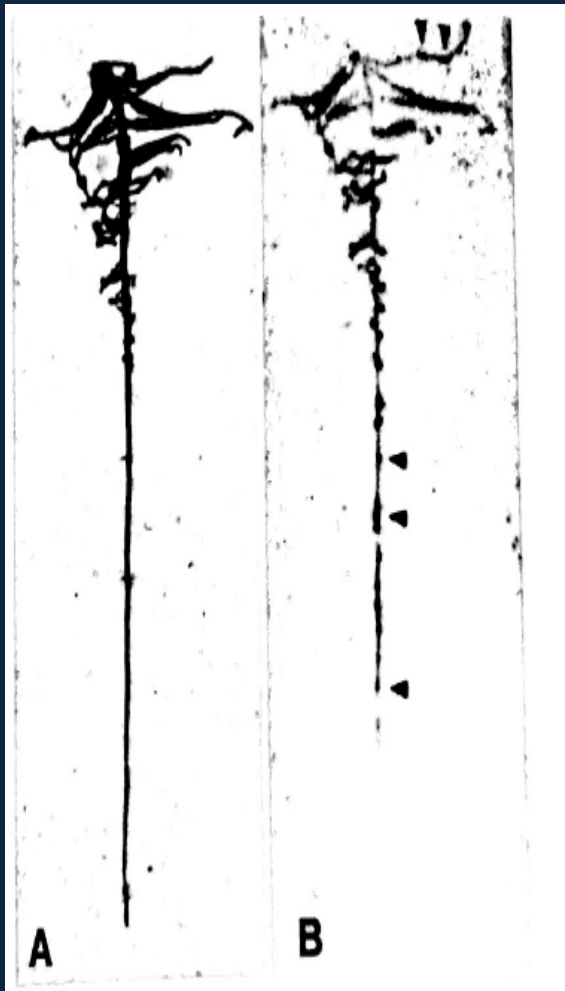
1. Induction of cell division in quiescent pericycle cells or parenchyma cells of vascular tissue.
2. Organization of root apex.
3. Growth of the primoridum.

1. IAA transported acropetally (toward the tip) in the stele is required to initiate cell division in the pericycle.
2. IAA is required to promote cell division and maintain cell viability in the developing lateral root.

"Evidence suggested that acropetal movement is essential for root initiation while basipetal for gravitropic response."

Effect of localized NPA application on Arabidopsis rooting

Treatment	Free IAA concentration (ng/g fresh weight)	Lateral root number
Control agar	25.8 + / – 4.7	15.5 + / – 0.8
NPA agar	17.6 + / – 2.2	5.9 + / – 0.5

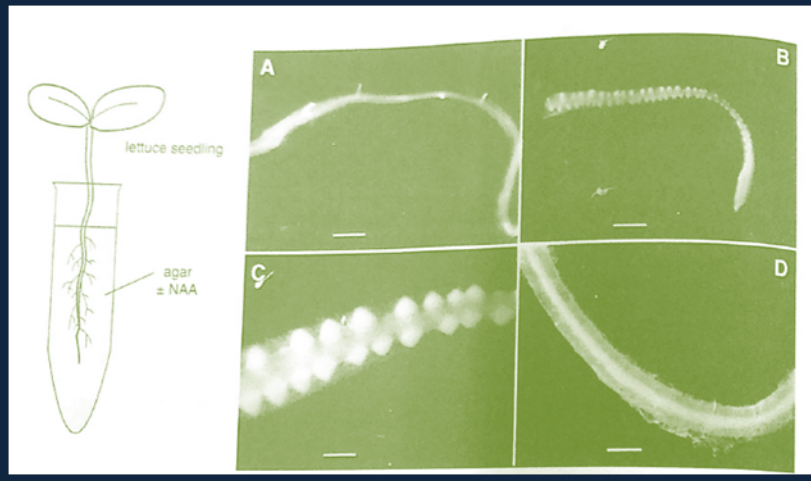


Transport of radioactive IAA, applied at shoot apex of Pea seedling.

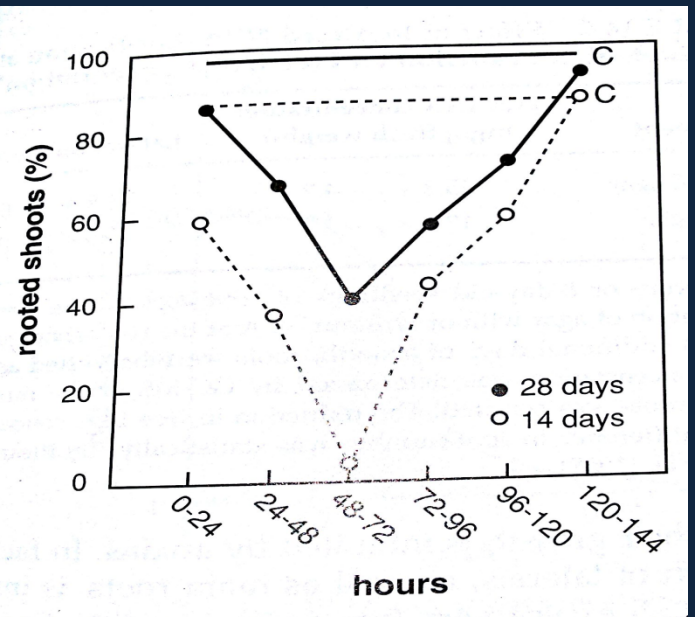
IAA and CK in rooting: Evidence from Surgical experiments

Application of Auxins and Cytokinins

Inhibitory effect of CK depends on time of application and concentration.



A. Only agar
B. + NAA
C. Enlargement of root growth
D. NAA+CK



Inhibition of rooting in apple micro cuttings by BA.

Root initiation and Root growth have different auxin concentration optima.

Root growth of laterals as well as main roots are promoted at very low conc. (10^{-9} M) however for induction 10^{-6} or 10^{-5} M is required .

(?)

Ethylene may be involved in induction of rooting.

- Ethylene has promotive effect only in presence of auxin.

- Experiment viz hypocotyl + ethylene, Application of ethylene inhibitors, Flooded condition, auxin transport inhibitors etc.

- It is thought that ethylene may alters the sensitivity of tissues to endogenous auxin.

- Auxin is also known inducer of ethylen biosynthesis.
-
- *never ripe* mutant of Tomato cutting?

Commercial application of auxin in rooting.

Species differences is correlated with endogenous IAA content.

Age related differences.

Differences in different auxins.

Duration of treatment.

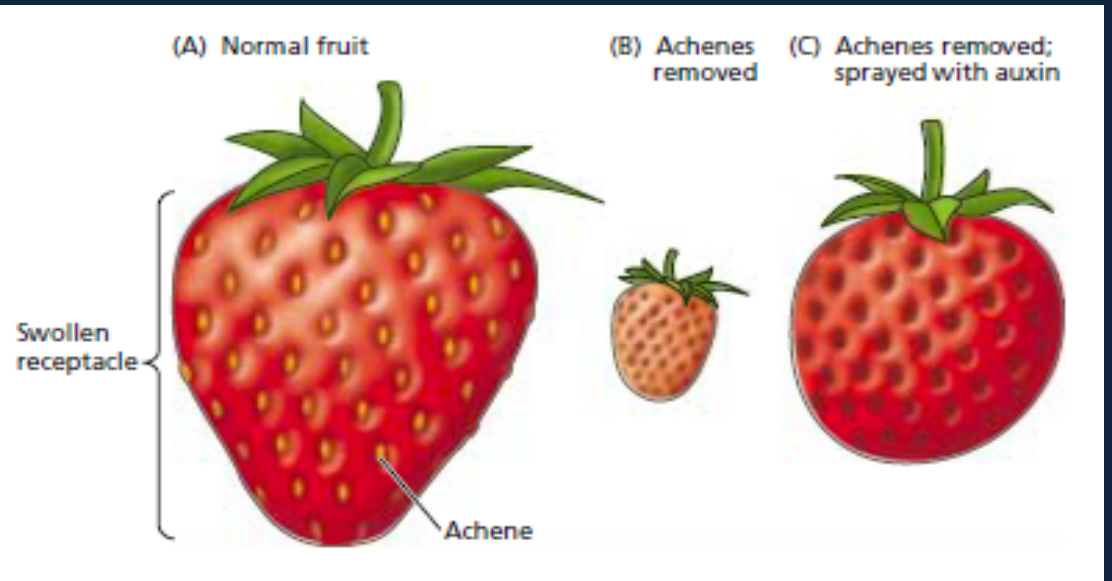
Time of the year when cutting are taken.

K Thimann treated Canada hemlock with IAA for 24 hrs. First roots did not appear until after 3 months.
(3 months!)

Auxin Transport Regulates Floral Bud Development:

Treating *Arabidopsis plants with the auxin transport* inhibitor NPA causes abnormal floral development, suggesting that polar auxin transport in the inflorescence meristem is required for normal floral development.

Auxin Promotes Fruit Development



Auxin is produced in pollen and in th endosperm and the embryo o developing seeds, and the initia stimulus for fruit growth may resu from pollination.

Auxin Induces Vascular Differentiation

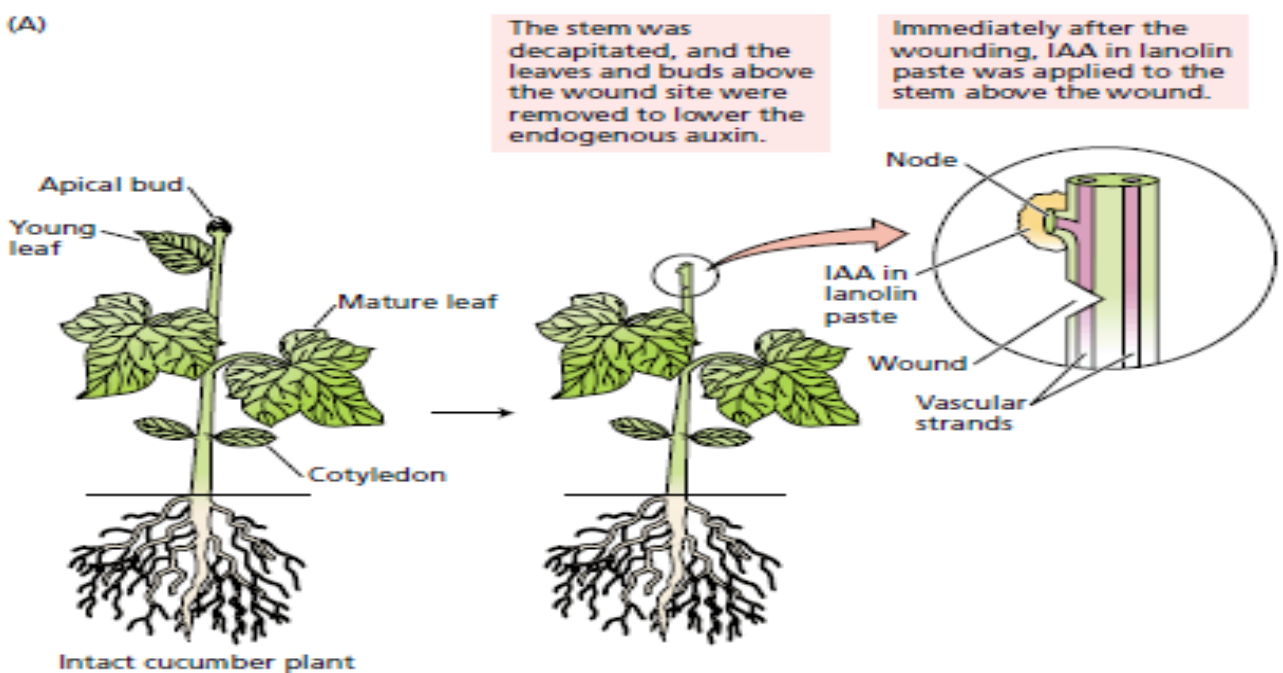
• Apical bud and vascular differentiation.

(?)

• Procambial strand typically differentiated in acropetal manner

• The relative amounts of xylem and phloem formed are regulated by the auxin concentration: High auxin concentrations induce the differentiation of xylem and phloem, but only phloem differentiates at low auxin concentrations.

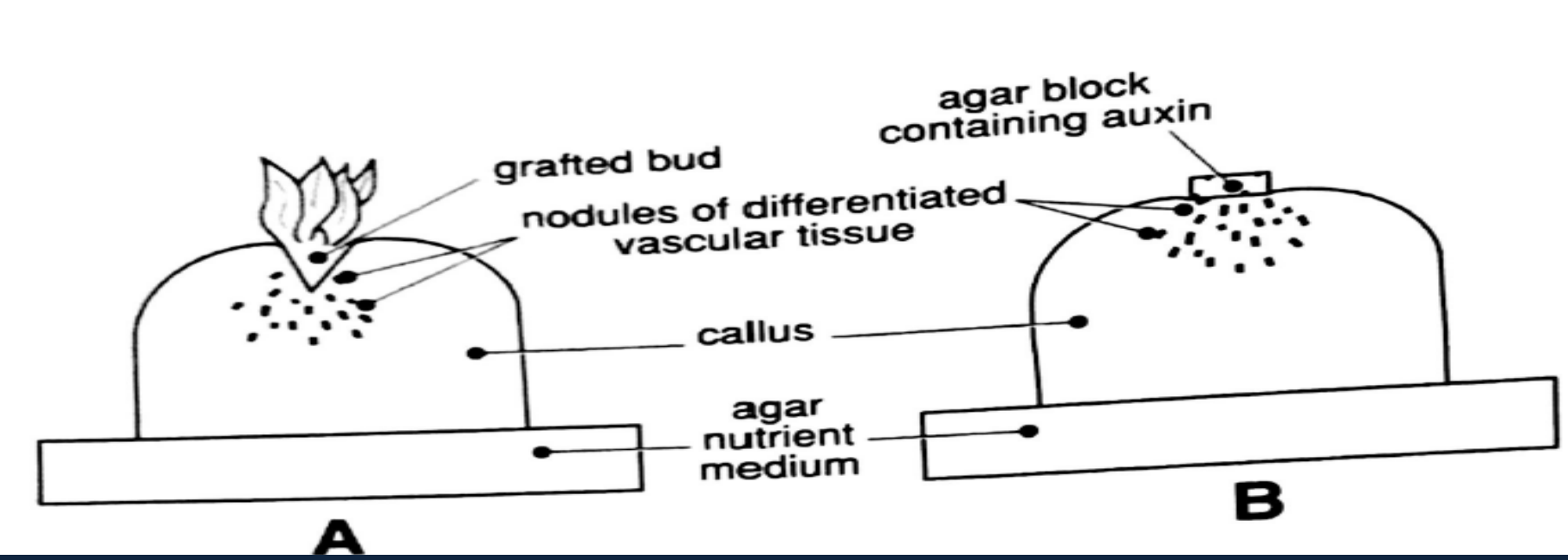
• Direction of secondary growth in woody plants



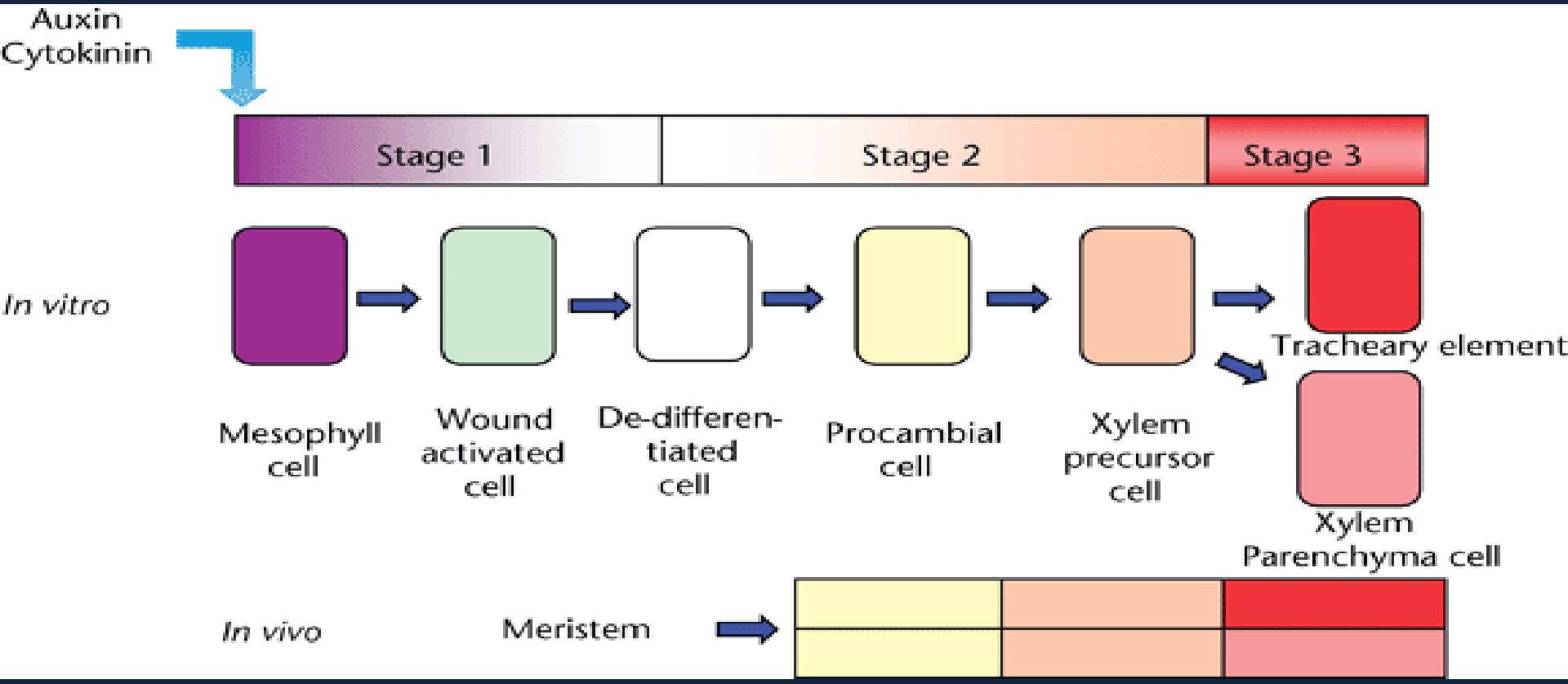
Xylem differentiation occurs around the wound, following the path of auxin diffusion.

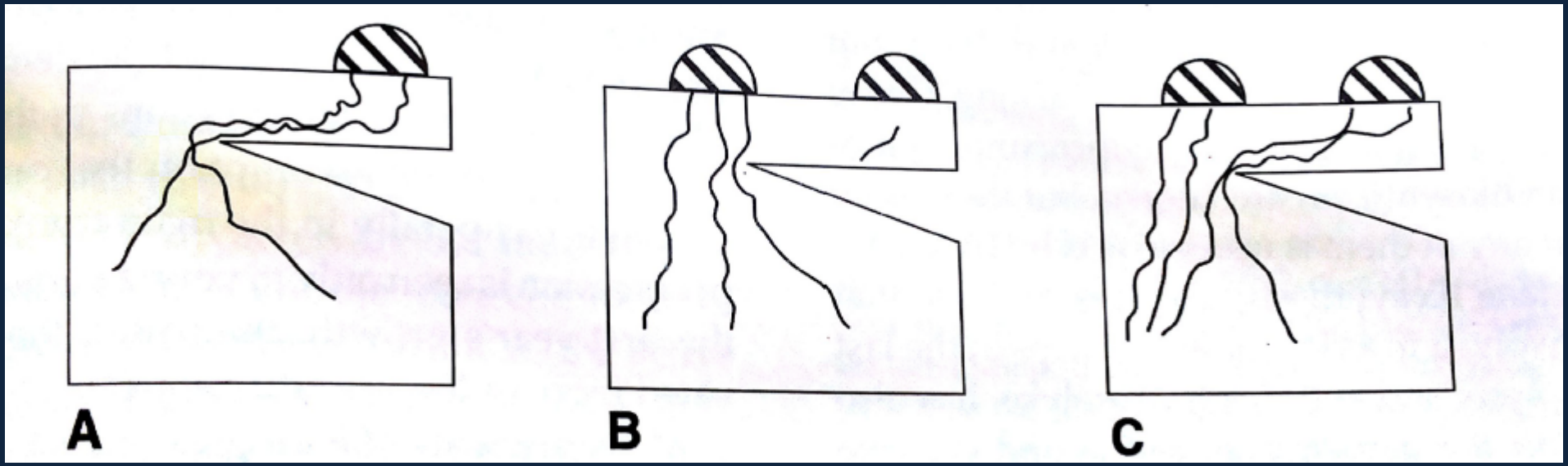
IAA in Xylogenesis:

- Explants cells differentiate in tracheary elements in presence of auxin.
  - Secondary wall growth
  - Lignification
  - Autolysis



Tracheary elemnt differentiation

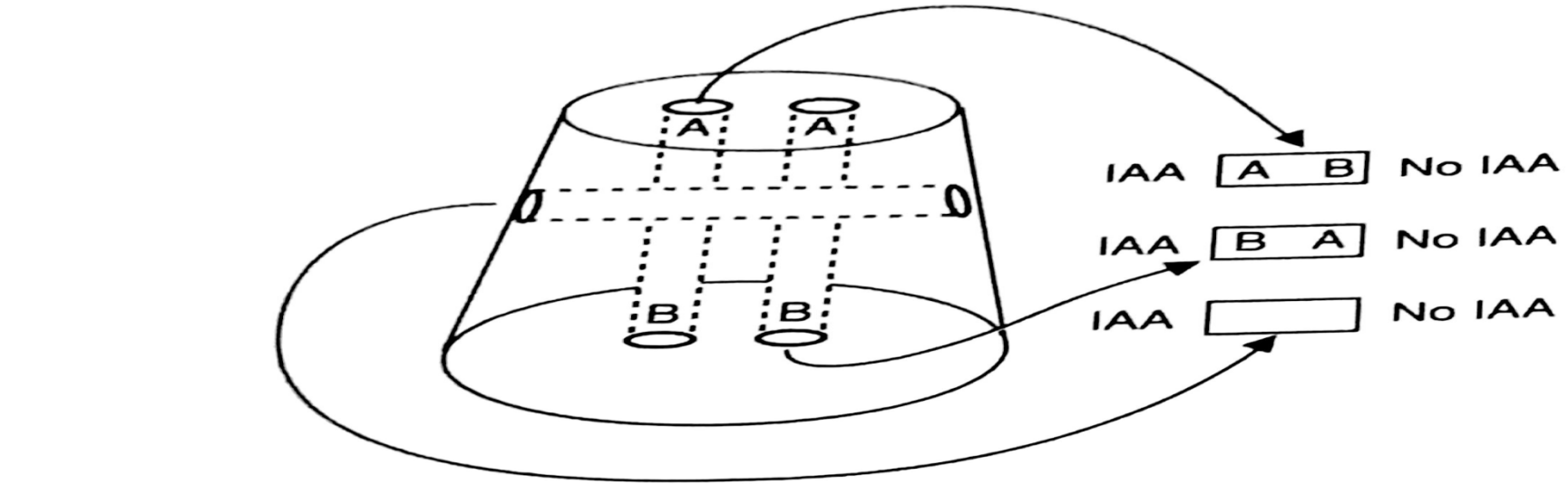


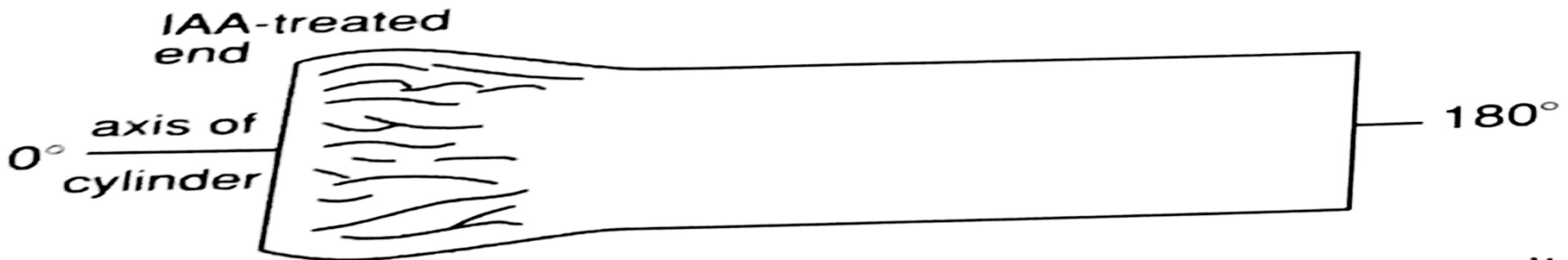


A. Auxin is applied as a single source and vascular differentiation
B. Two source of auxin, the source on left inhibits the flow of auxin from the source on right.
C. If the source on right, applied few day before it induces differentiation.

Tracheary strands differentiated close to the end where IAA was applied

Diffusive movement of IAA delineates the files of cells that become competent for polar basipetal transport, a phenomenon referred as canalization.
Simply acquisition of competence for polar transport.





Auxin may:

Precise role of IAA is unclear.

Expansion, deposition of secondary cell wall in precise patterns, lignification, hydrolysis of nucleus, cytoplasmic contents .

It may responsible for:

Regulates microtubules arrangments.

Auxin linked to lignification.

Auxin induce ethylene biosynthesis which triggers cell death and autolysis.

AUXIN SIGNAL TRANSDUCTION PATHWAYS

ABP1 Functions as an Auxin Receptor

Despite being localized primarily on the endoplasmic reticulum (ER), a small amount of ABP1 is secreted to the plasma membrane outer surface where it interacts with auxin to cause protoplast swelling and H+- pumping

It is unlikely that ABP1 mediates all auxin response pathways because expression of a number of auxin-responsive genes is not affected when protoplasts are incubated with anti-ABP1 antibodies. It is also unclear what role the ABP1 in the ER plays in auxin-responsive signal transduction. .

Calcium and Intracellular pH Are Possible Signaling Intermediates

Auxin increases the level of free calcium in the cell.

In plants, auxin induces a decrease in cytosolic pH of about 0.2 units within 4 minutes of application. The cause of this pH drop is not known. Since the cytosolic pH is normally around 7.4, and the pH optimum of the plasma membrane H+-ATPase is 6.5, a decrease in the cytosolic pH of 0.2 units could cause a marked increase in the activity of the plasma membrane H+-ATPase. The decrease in cytosolic pH might also account for the auxin-induced increase in free intracellular calcium, by promoting the dissociation of bound forms.

Cell cycle regulation

Auxin-Induced Genes Fall into Two Classes: Early and Late

Genes whose expression is stimulated by the activation of preexisting transcription factors are called **primary response genes or early genes.**

Early-gene expression cannot be blocked by inhibitors of protein synthesis such as cycloheximide

In general, primary response genes have three main functions:
(1)Some of the early genes encode proteins that regulate the transcription of s**econdary response genes, or late genes, that are required for the long-term responses to** the hormone. Because late genes require de novo protein synthesis, their expression can be blocked by protein synthesis inhibitors.
(2)Other early genes are involved in intercellular communication, or cell-to-cell signaling.
(3)Another group of early genes is involved in adaptation to stress.

Thank You