Plant Growth

Hormones

Chemical Messengers

What are the plant hormones?

Plant hormones are endogenous organic compounds active at very low concentration, produced in one tissue, and translocated to another point in the plant where their effect on growth and development are manifested.

Or

- Small;
- Organic compounds;
- Synthesized by the plant;
- Active in low concentration ($<10^{-6}$);
- Promote or inhibit growth and developmental responses;
- Often show a separation of the site of production and the site of action.

Techniques to study hormones

A. Bioassays: A bioassay examines the effect of a test substance on a plant tissue.

B. Immunological studies: Antibodies are made against the plant hormones and then used as specific probes to localize and quantify. The antibodies are usually coupled to radioisotopes or fluorescent dyes to make it easier to trace. An other method is Bio-sensers, both of these techniques is very sensitive and specific.

C. Instrumental Methods GC-MS; HPLC; high specificity and sensitivity.

Techniques to study hormones

A. Bioassays: A bioassay examines the effect of a test substance on a plant tissue.

Several, bioassays have been devised for auxins such as

* Avena curvature test.

** Avena section test.

*** Split pea stem curvature test.

**** Cress root inhibition test.

Techniques to study hormones

B. Immunological studies:

- Antibodies are made against the plant hormones and then used as specific probes to localize and quantify. The antibodies are usually coupled to radioisotopes or fluorescent dyes to make it easier to trace. An other metho is Bio-sensers, both of these techniques is very sensitive and specific.

- Bio-Sensers, A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction.

Techniques to study hormones

Component of Bio senser method Component

- **Analyte**: A substance of interest that needs detection. For instance, glucose is an 'analyte' in a biosensor designed to detect glucose.

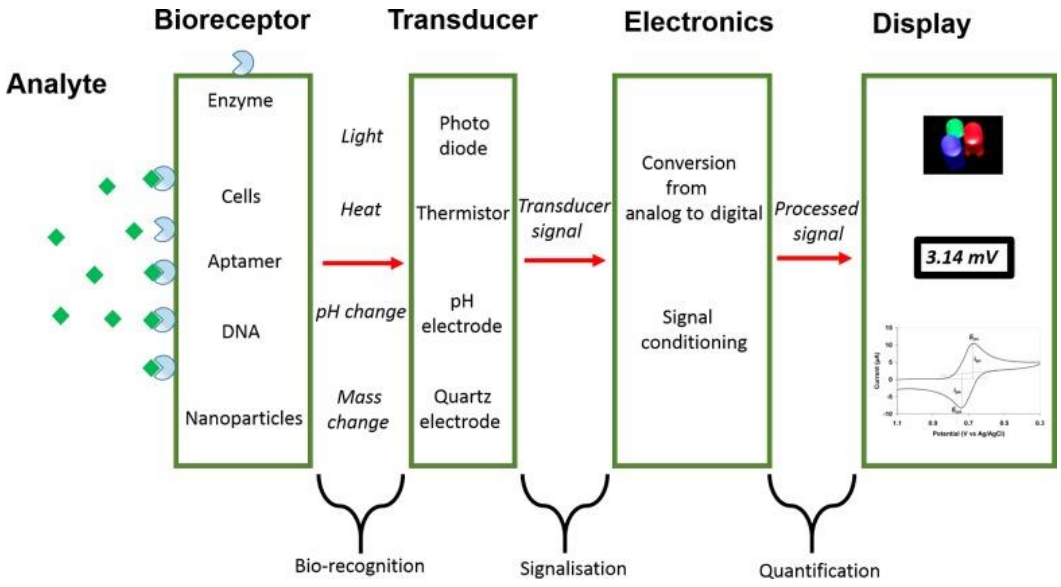
- **Bioreceptor**: A molecule that specifically recognizes the analyte is known as a bioreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio-recognition.

- **Transducer**: The transducer is an element that converts one form of energy into another. In a biosensor the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalisation. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions.

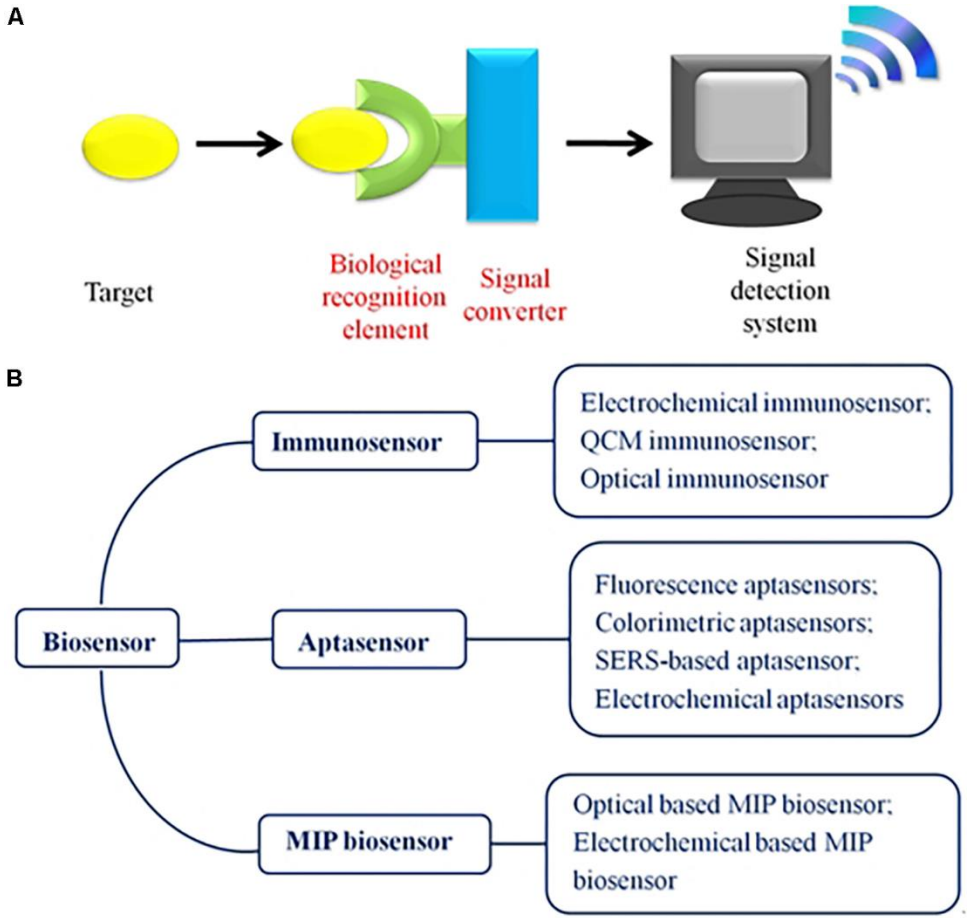
- **Electronics**: This is the part of a biosensor that processes the transduced signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then quantified by the display unit of the biosensor.

Techniques to study hormones

- **Display**: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user



Techniques to study hormones



Figure/ (A) Schematic illustration of the biosensor, including the following three parts: the bio-recognition element, the signal converter, and the signal measurement system. **(B)** Outline of the biosensors used for monitoring AFB1. According to the bio-recognition element, the biosensor is divided into aptasensors, immunosensors, and MIP biosensors in this review.

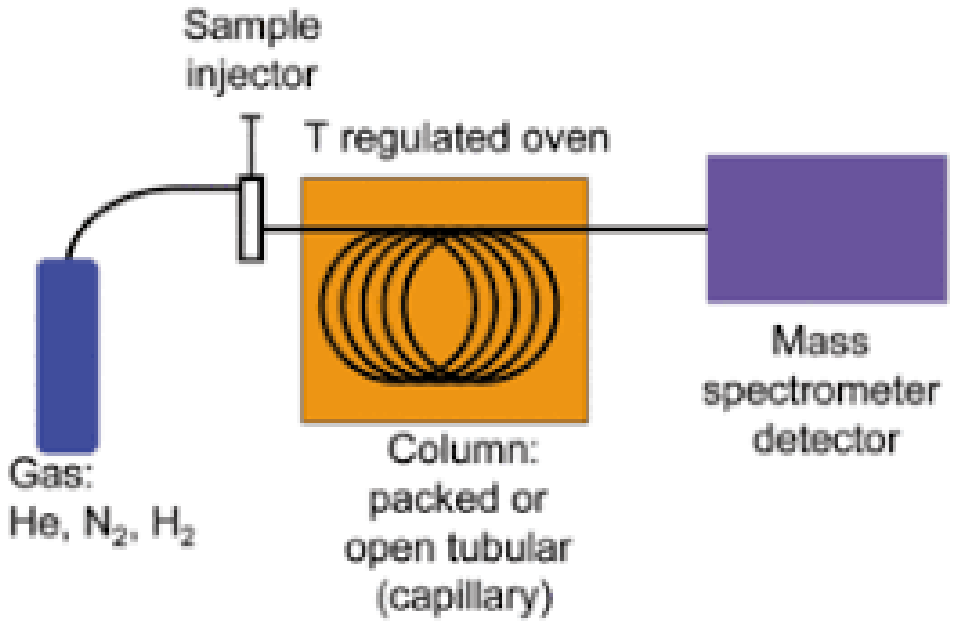
Techniques to study hormones

C. Instrumental Methods:-

- **GC-MS :-** Gas chromatography-mass spectrometry.
- **HPLC:-** High-performance liquid chromatography.
- **Nano-flow LC-ESI-IT-MS/MS:-** Nanoflow liquid chromatography-electrospray ionization – ion trap mass spectrometry

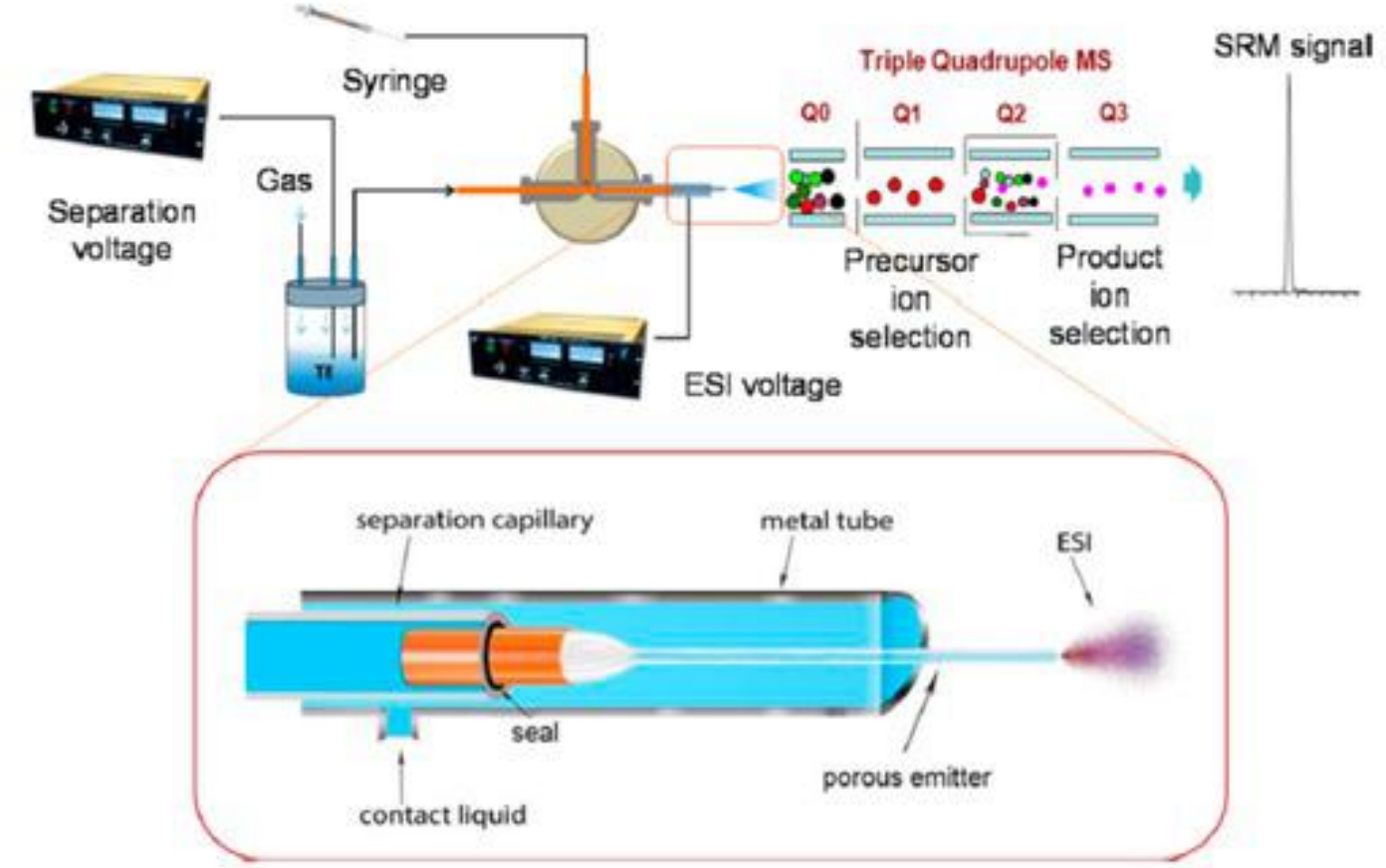
High specificity and sensitivity.

Techniques to study hormones GC/ MS



Techniques to study hormones

Nano-flow LC-ESI-IT-MS/MS



Mechanism of hormone action

Hormones act on target tissues to activate a receptor.
The general mechanism is:

Hormone

Target tissue/cell

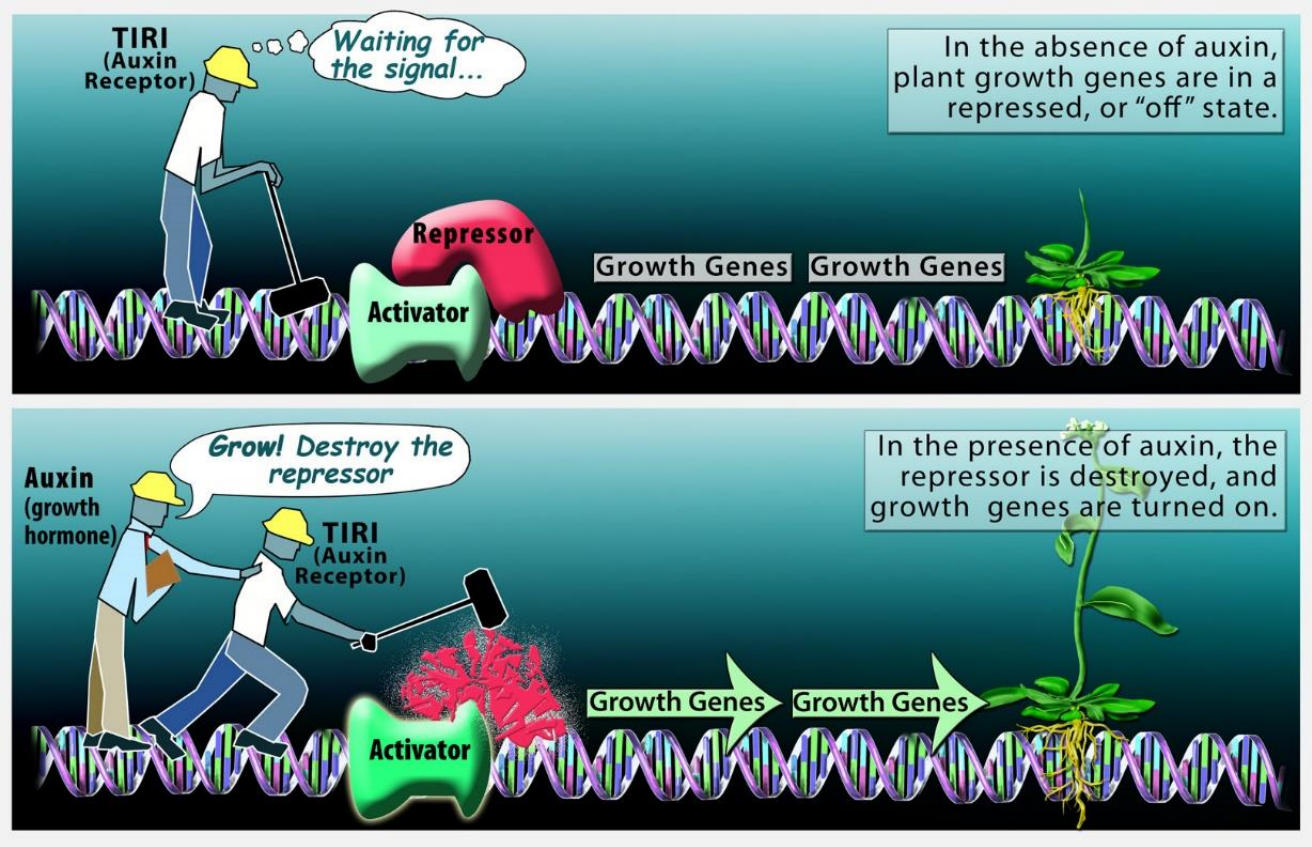
Receptor

Signal amplification

Response

For a response to occur:

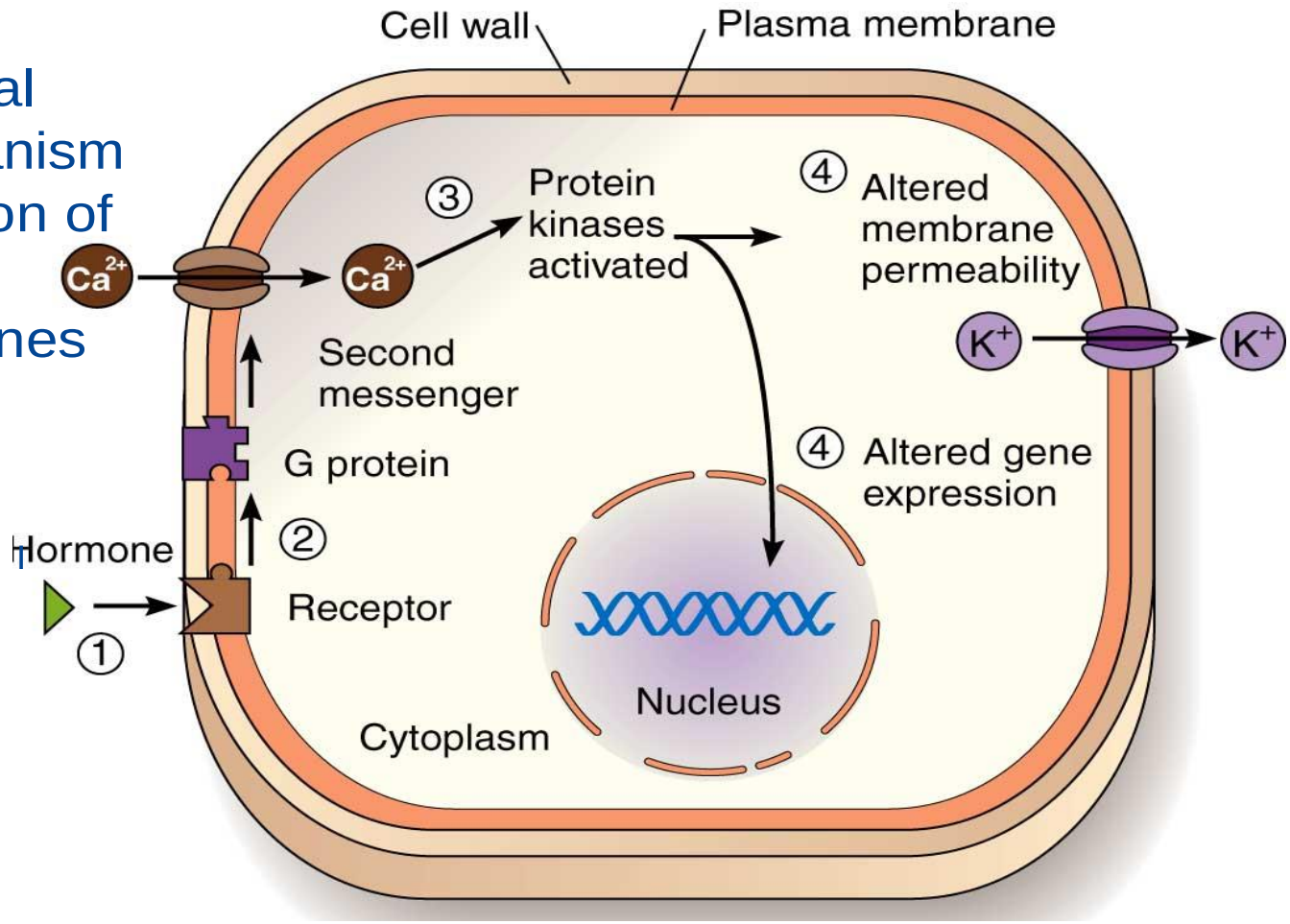
A. The hormone **must be present in sufficient quantity.**
B. The **target tissue must be sensitive** to the hormone.
C. The **target tissue recognizes** the hormone (**there must be a receptor to which the hormone can bind**).
D. The binding of the hormone/receptor **should initiate a change in the receptor** (**amplification**).
E. The activated receptor **initiates a physiological response.**



When a plant is not growing, **auxin** is not produced, and growth genes are repressed, or turned "off." But when conditions are favorable for growth, **auxin** is produced and binds to a molecule known as TIR1. The auxin-TIR1 complex in turn signals for the destruction of the repressor protein that keeps growth in check, and growth promoting genes are **turned "on."**

TIR is **TRANSPORT INHIBITOR RESPONSE**

General mechanism of action of plant hormones



G proteins, also known as **guanine nucleotide-binding proteins**, are a family of **proteins** that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to the inside.

5 recognized groups of natural plant hormones and growth regulators discovered in Plant

1. Auxins
2. Cytokinins
3. Gibberellins
4. Ethylene
5. Abscissic acid

Hormones / Growth regulators

Class	Endogenous Hormone	Growth Regulators
Auxin	**Indole-3-acetic acid (IAA)**	**IBA, NAA, 2,4-D, others**
Cytokinin	**Zeatin, zeatin riboside**	**Kinetin, BA, 2iP, TDZ**
Gibberellin	**GAx…..125**	**GA3, GA4+7**
Abscisic acid	**Abscisic acid(ABA)**	
Ethylene	**Ethylene**	**Ethephon, ethrel**

IBA = **Indole butyric acid.**

NAA= **1-Naphthaleneacetic acid.**

2,4-D= **2,4-Dichlorophenoxyacetic acid.**

BA=**Benzyl adenine.**

2iP=**2-isopentenyl adenine.**

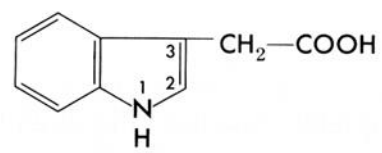
TDZ= **Thidiazuron.**

Auxins

Auxin is a general name for a group of hormones that are involved with growth responses. (The term "auxin" is derived from the Greek word "**to increase or grow**". This was the first group of plant hormones discovered.

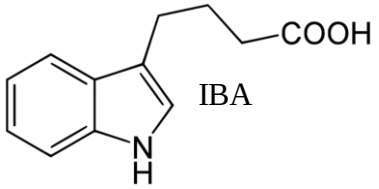
Three types:-

1- Naturally accruing auxin:- The most important auxin found in plants is indole-3-acetic acid (IAA).



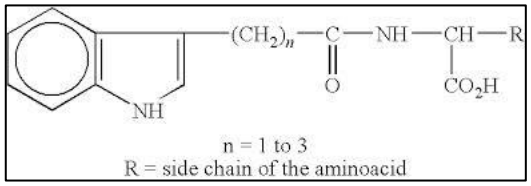
3-indoleacetic acid

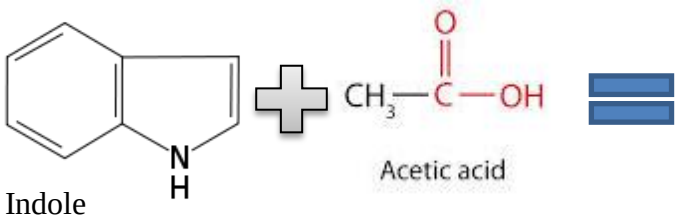
2- Synthetics with aixin activity:- There are a variety of substances that are not known to occur in plants that have auxin activity, like IBA, NAA, 2,4-D, 2,4,5-T.



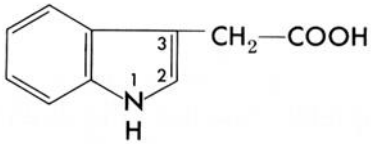
IBA

3- Conjugated forms :- Auxins, as do other hormones, occur in a free or conjugated (bound to sugars, alcohols or other molecules) form. In fact, up to 98% of the auxin may be bound.





Indole Acetic acid



3-indoleacetic acid

Biosynthesis of Indole-3-aacetic acid (IAA)

A. Site :- Auxin is made in actively growing tissue which includes young leaves, fruits, and especially the shoot apex. Made in cytosol of cells.

B. Routes :- There are two major routes to the production of IAA.

1. Tryptophan-dependent Pathways. The similarity of chemical structure of IAA and tryptophan suggested a connection between these. Considerable research has shown that tryptophan, one of the protein amino acids, is a precursor of auxin biosynthesis.
2. Tryptophan-independent Pathway - this route doesn't involve tryptophan directly as an intermediate to the formation of auxin.

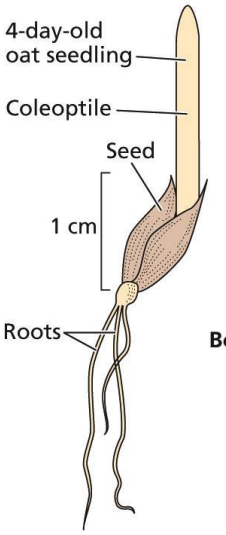
Transport:- Basipetal (or Polar) Transport

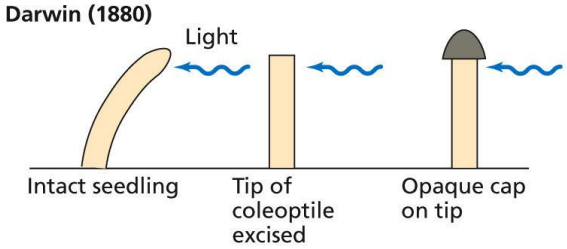
Auxin is transported in a basipetal (towards the base) direction. In other words, auxin moves from the shoot tip towards the roots.

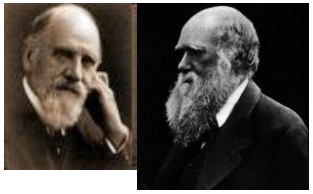
Function:-

- Cell elongation and expansion.
- Stop or lower the lateral bud growth.
- Stimulate root initiation (lateral roots, adventitious).
- Stimulation of abscission (young fruits) or delay of abscission.
- Tropic responses; hormone implicated in tropisms (photo-, gravi-, thigmo-).
- Apical dominance.
- Inhibition of root growth, *etc.*

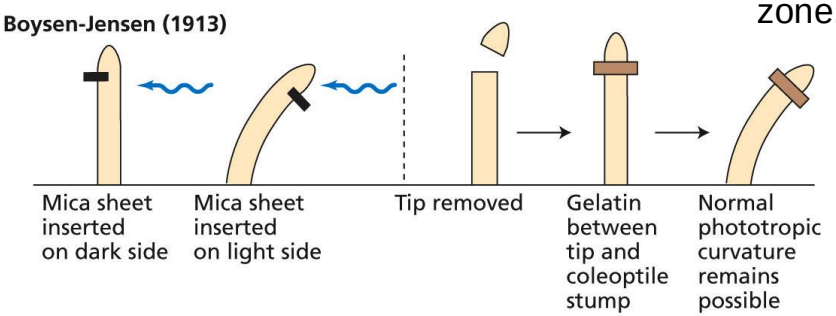
Auxin: earliest experiments





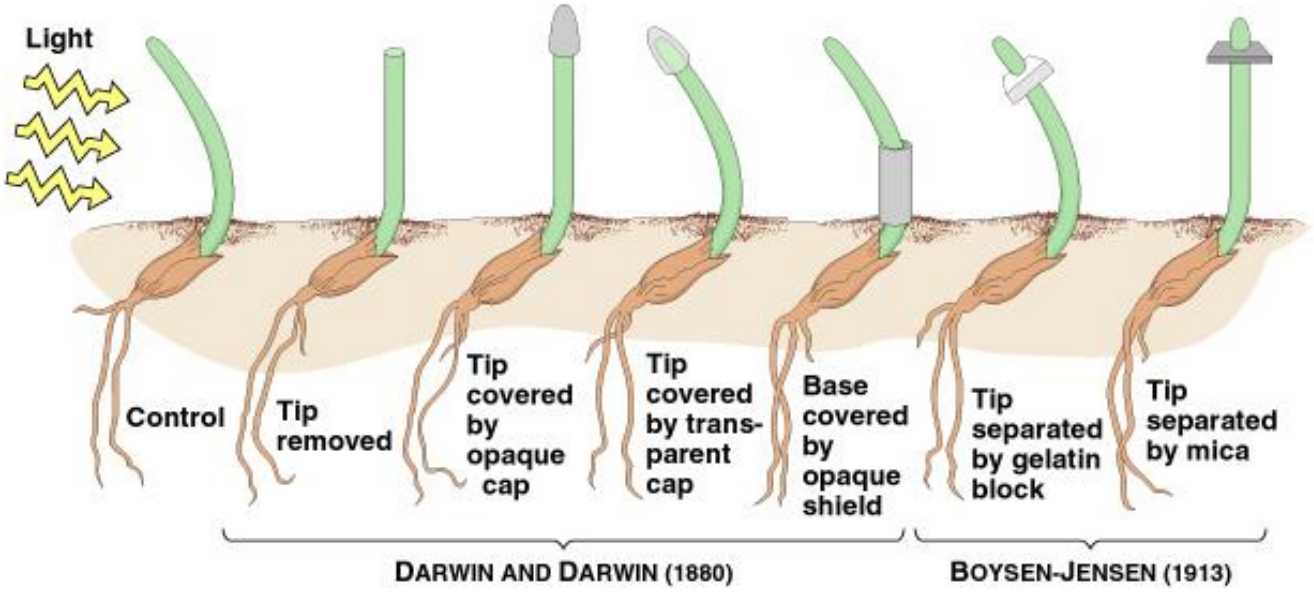


Darwins 1880, stimulus in tip and transmitted to growth zone.



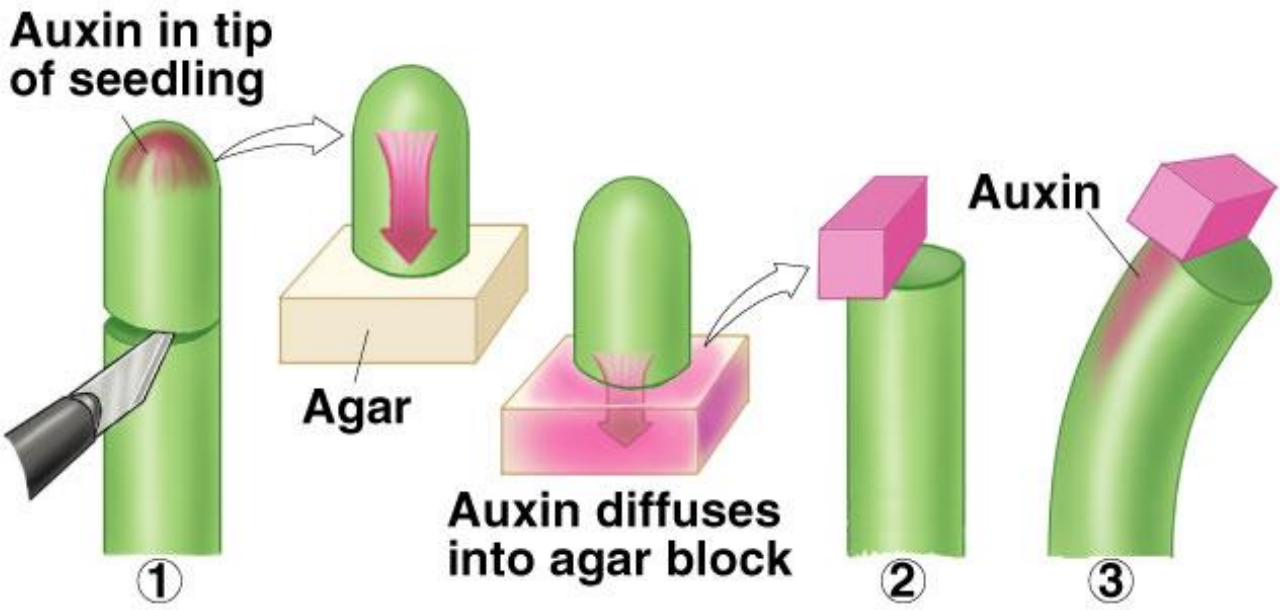
Boysen-Jensen 1913, Stimulus passe through permeable membrane

Early experiment on phototropism showed that stimulus (light) released chemicals that influenced growth



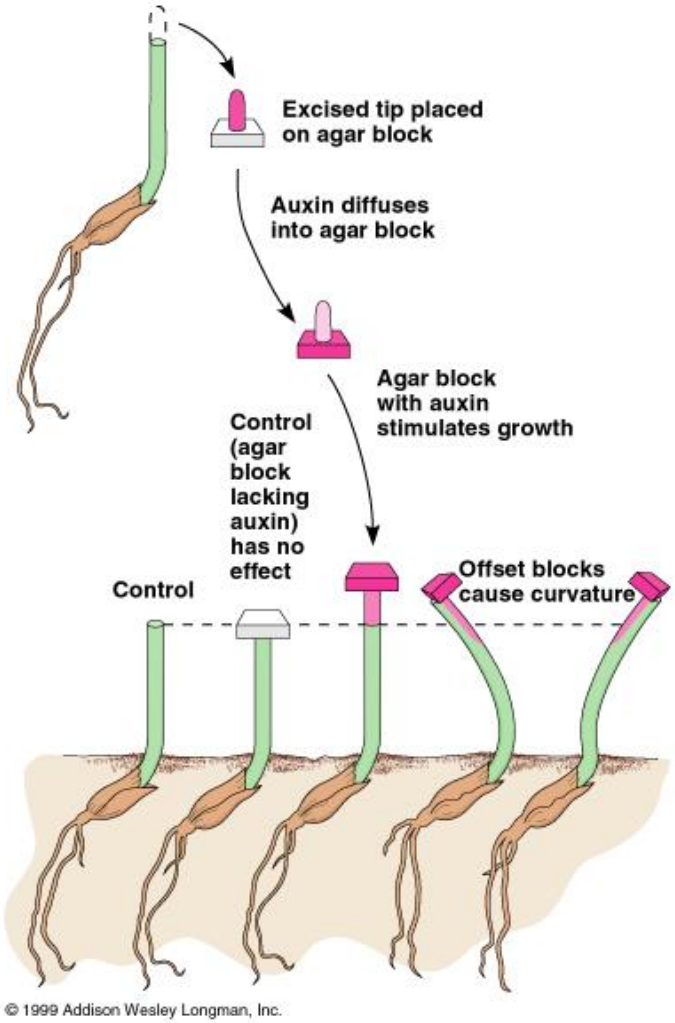
Results on growth of coleoptiles of oats suggested that the reception of light in the tip of the shoot stimulated a bending toward light source.

Auxin increases the plasticity of plant cell walls and is involved in stem elongation.



Frits Went (1926) determined auxin enhanced cell elongation.

Demonstration of transported chemical

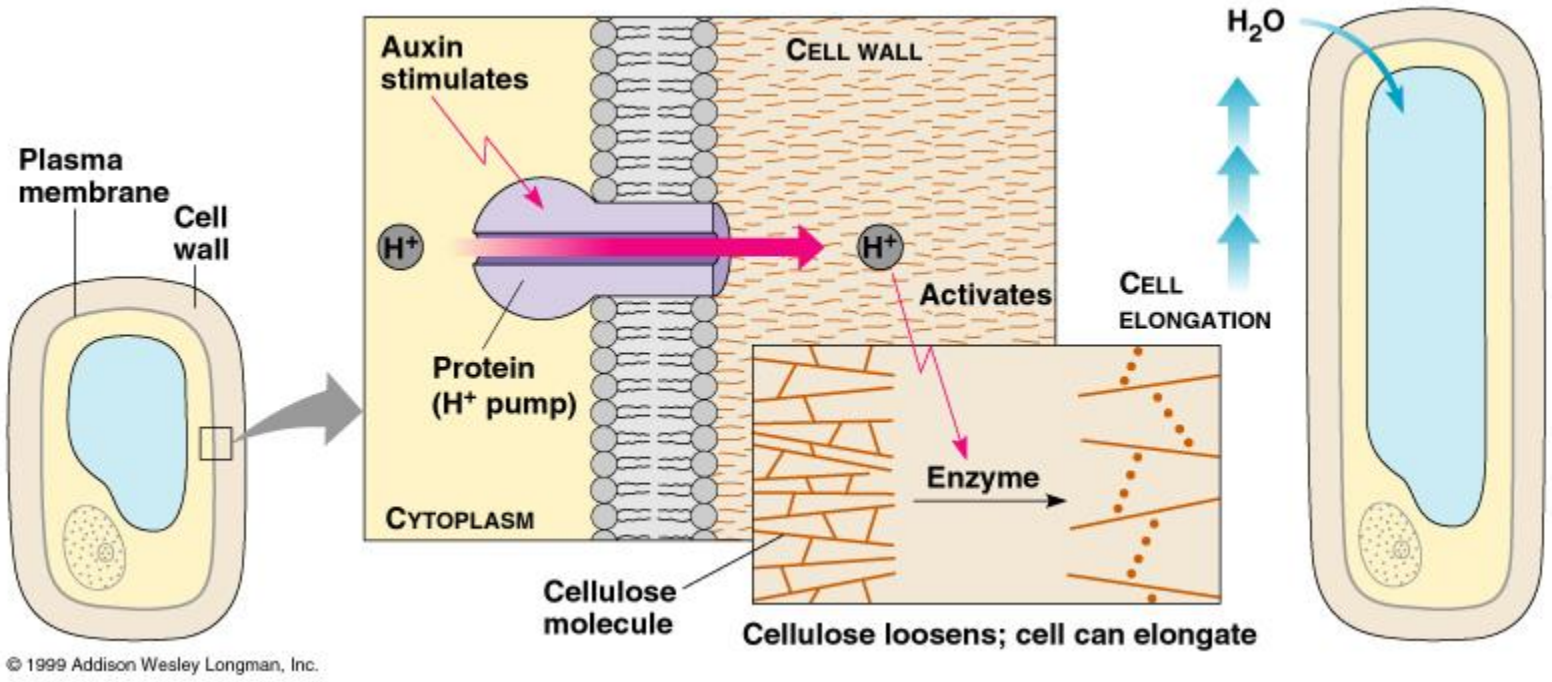


Auxin:-

- Discovered as substance associated with phototropic response.

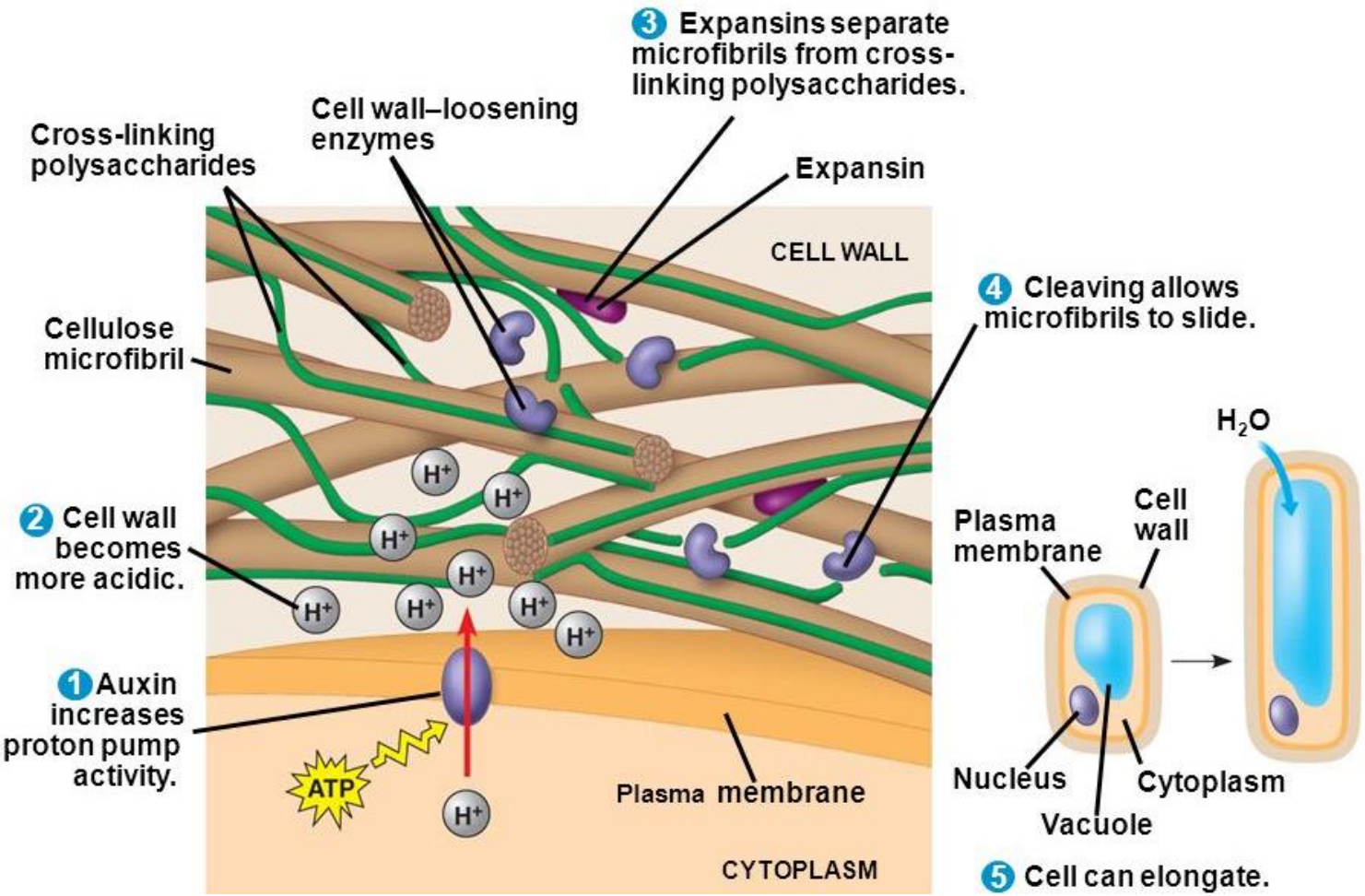
- Occurs in very low concentrations.
  - Isolated from human urine, (40mg 33 $gals^{-1}$)
  - In coleoptiles (1g 20,000 $tons^{-1}$)
- Differential response depending on dose.

Loosening of cell wall

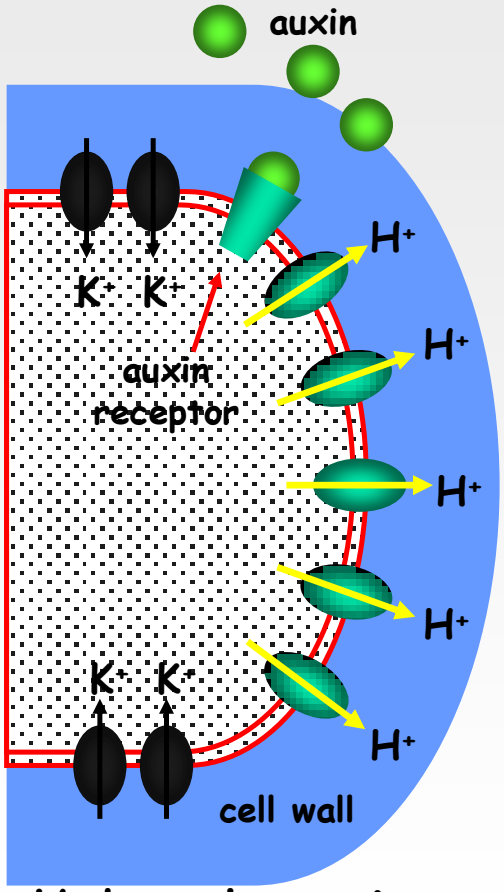


Cell Elongation: Acid growth hypothesis

auxin works by causing responsive cells to actively transport hydrogen ions from the cytoplasm into the cell wall space



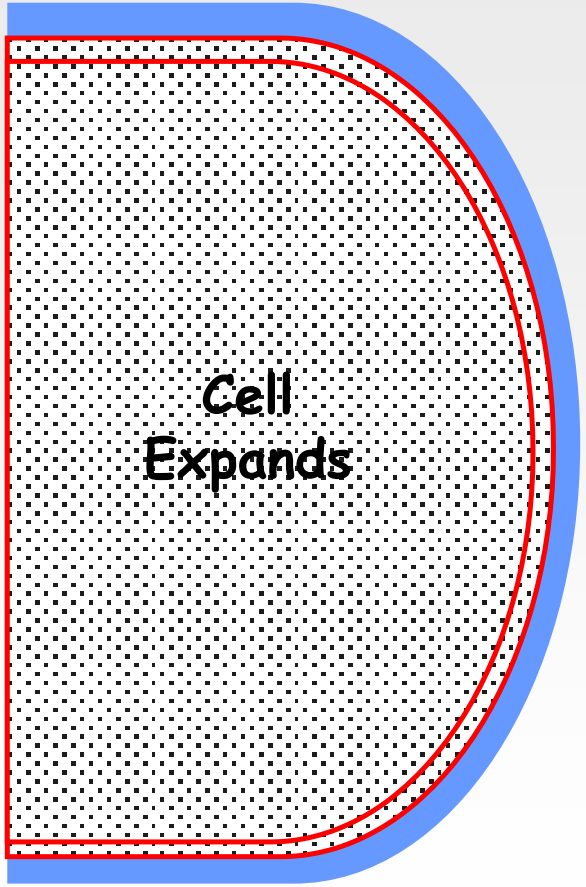
Auxin: Acid growth hypothesis



auxin binds to receptor

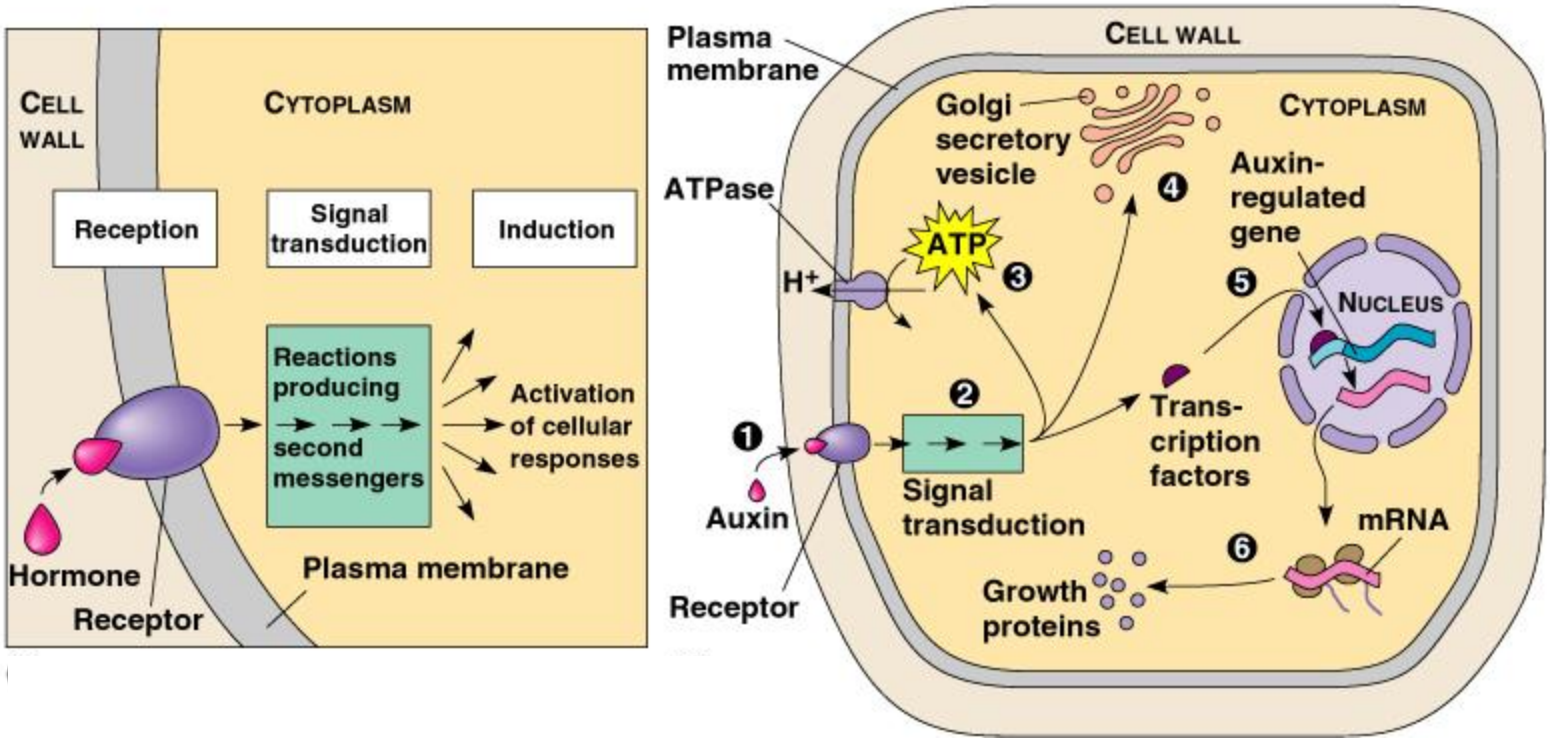
produces and activates pumps: H^+ pumped out of cell, K^+ pumped in to maintain turgor

wall pH drops from 5.5 to 4.5 – breaks bonds in wall matrix loosens cell wall

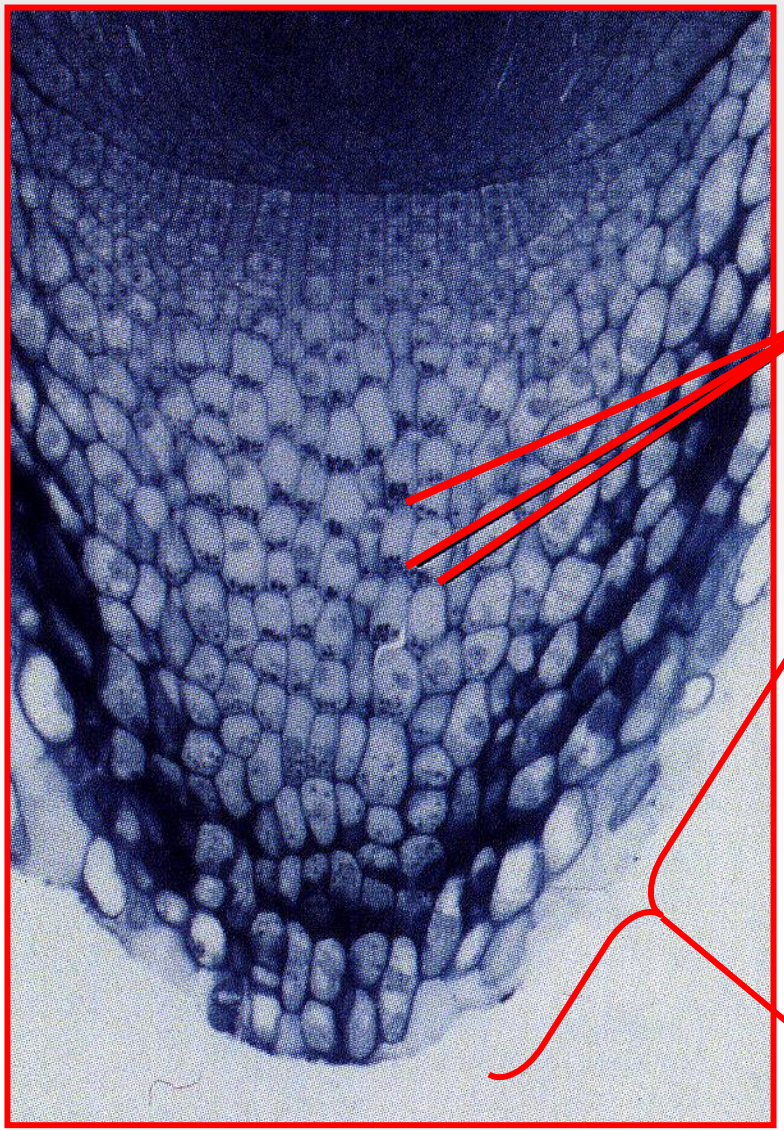


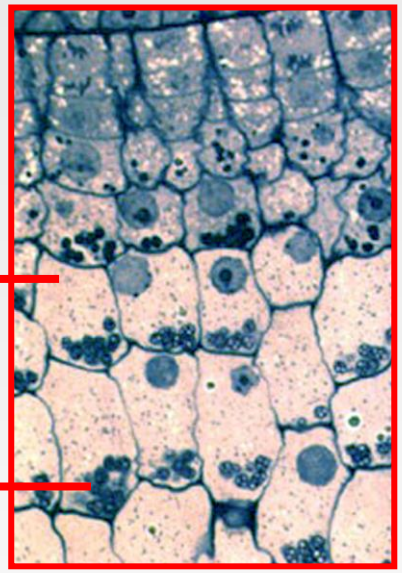
pH drop also activates EXPANSINS
= proteins in cell walls - promote wall loosening

Signal-transduction pathways in plants



Auxin interacts with calcium ions which in turn calmodulin, a protein, which regulates many processes in plants, animals, and microbes.





statocytes
in root cap

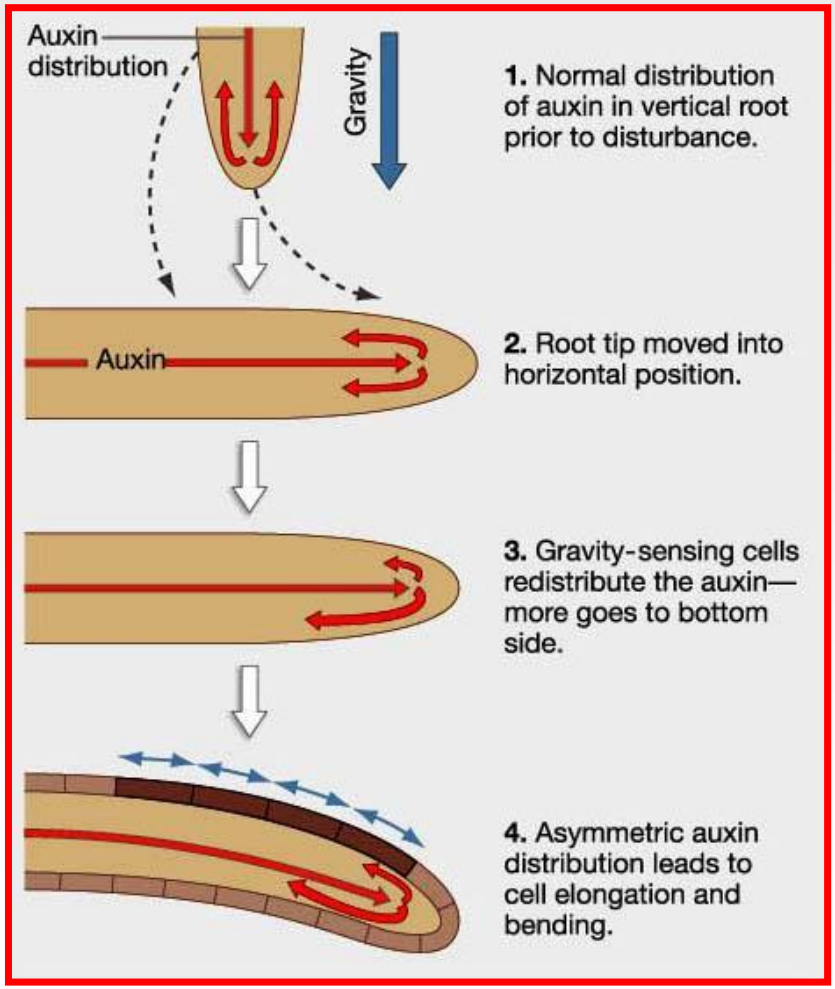
statoliths

= amyloplasts
with starch

heavier than
cytoplasm so
sediment to
lower side of
cell

root cap

Gravitropism mode of action



auxin moves downward in vertical root

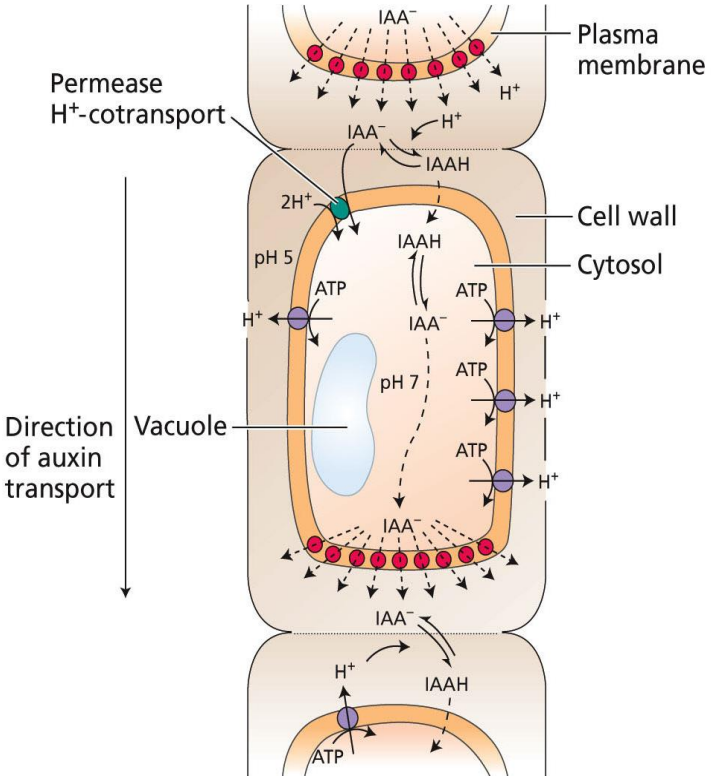
if shifted to horizontal, statocytes have their statoliths sediment

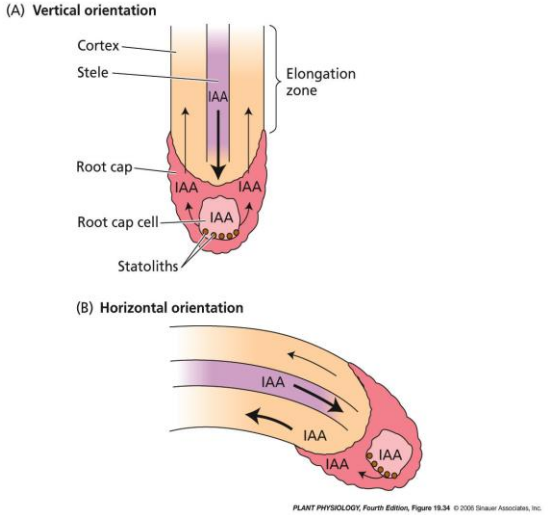
- believe they contact membrane-bound receptor molecules

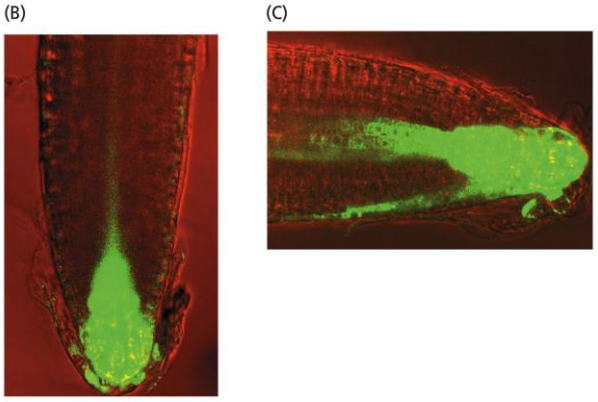
statolith movement triggers auxin redistribution to lower side of root

greater concentration of auxin on lower side of root actually inhibits cell elongation, so root bends downward

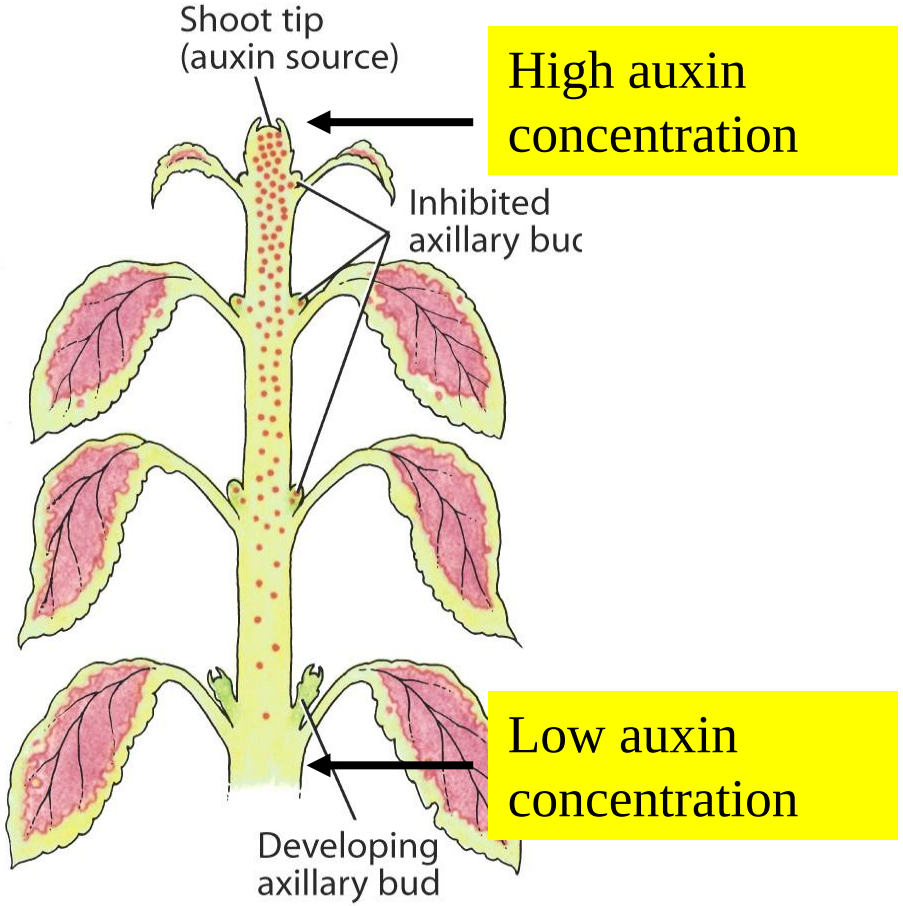
Polar auxin transport

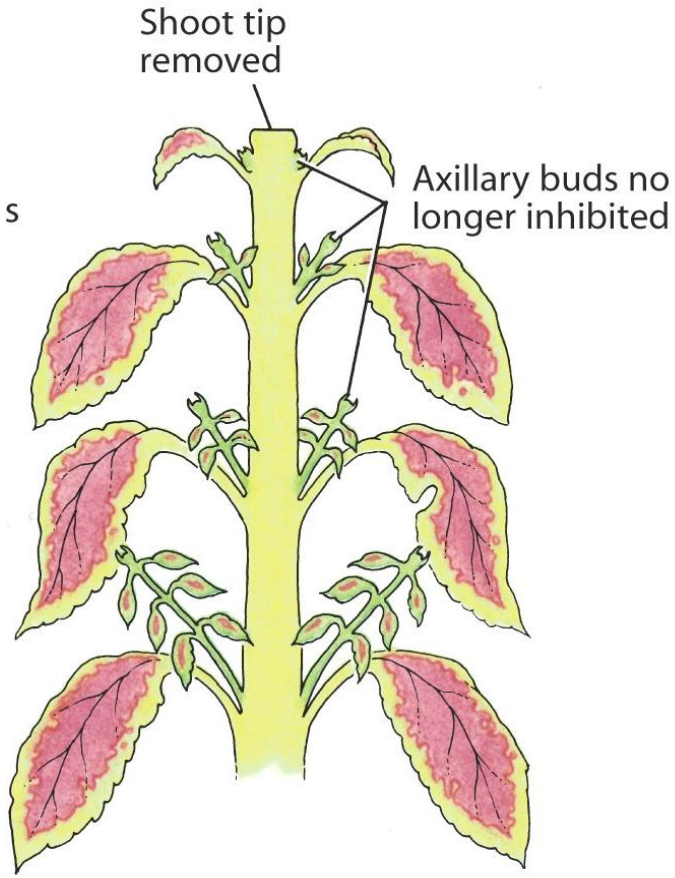




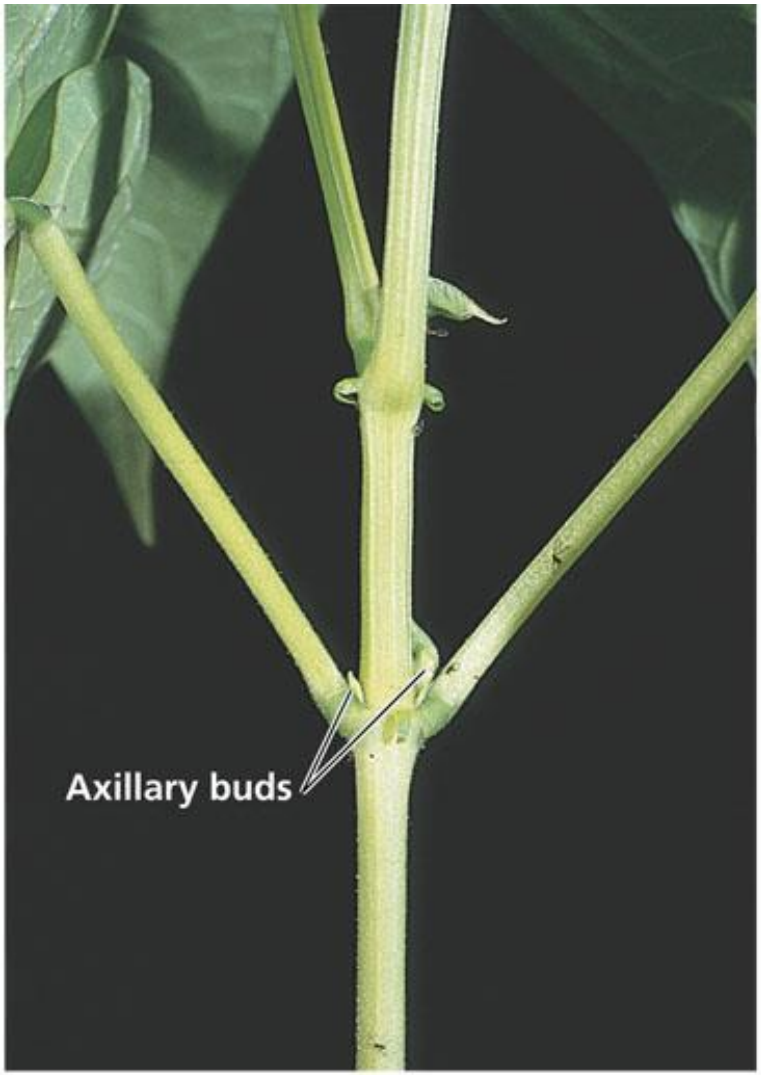


Evidence for the role of auxin in apical dominance





Plant (b) has apical bud removed so axillary buds grow

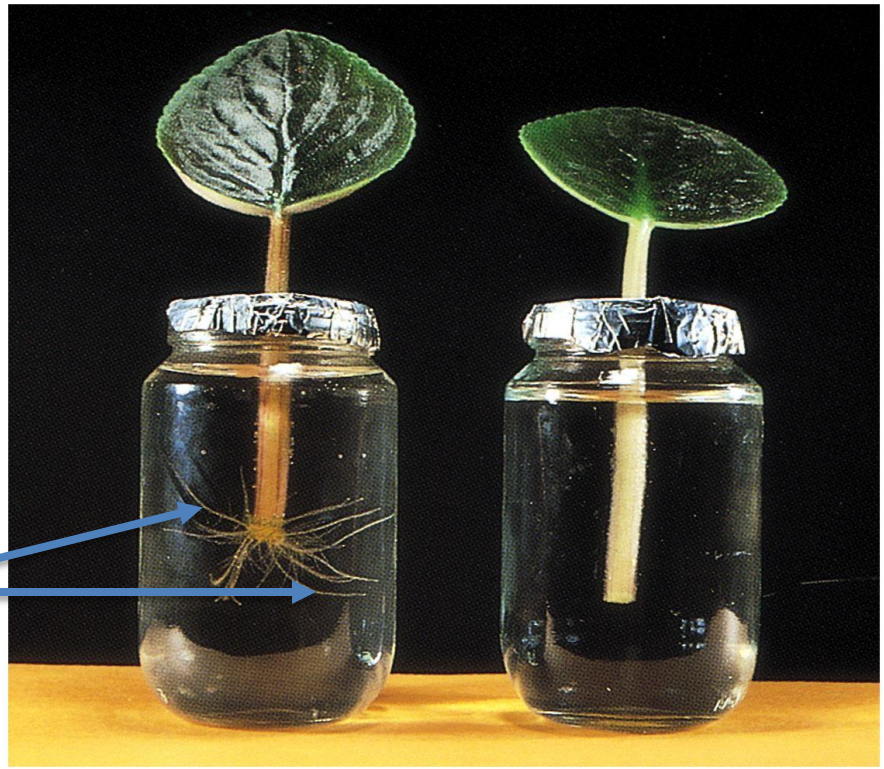


(a) Intact plant

(b) Plant with apical bud removed

Evidence for the role of auxin in adventitious root formation

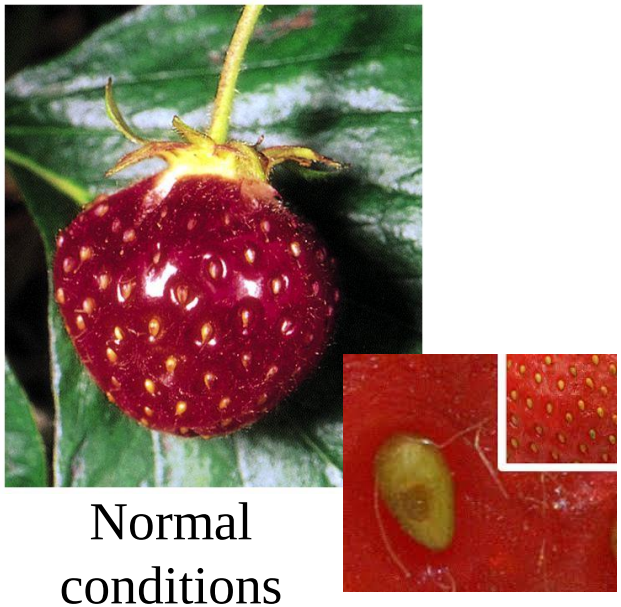
With synthetic auxin



Adventitious roots growing from stem tissue

Without synthetic auxin

Evidence for the role of auxin in formation of fruit and structures of similar function



Normal conditions

All achenes removed

Band of achenes removed

Without seed formation, fruits do not develop. Developing seeds are a source of auxin.

What do you expect?

Auxin replacement restores normal fruit formation and can be used commercially to produce seedless fruits. However, too much auxin can kill the plant and thus synthetic auxins used commercially as herbicides.

Cytokinins

- Called "cytokinins" because they stimulate cell division (*i.e.*, cytokinesis)
- Haberlandt (1904) noted that non-dividing potato parenchyma cells would revert to actively dividing ones in the presence of phloem sap. This observation suggested a soluble material was responsible for cell division.

- Folke Skoog (1940's) and colleagues at Univ. of Wisconsin found that cultured tobacco pith tissue explants would proliferate only if they were supplemented with various substances such as autoclaved herring sperm or coconut milk.

- Miller (1956) identified the first cytokinin, called kinetin, in the herring sperm.

- Cytokinins occur in most plants including mosses, ferns, conifers, algae and diatoms.

Chemistry

A. Natural:-

- adenine derivatives (amino purines).
- approximately 40 different structures known.
- Zeatin (Z), which was first isolated from maize (*Zea mays*) is the most common cytokinin.
- Other naturally occurring cytokinins include, dihydrozeatin (DHZ).

B. Synthetic cytokinins:-

- kinetin : probably byproduct of zeatin degradation
- benzyl adenine (benzylaminopurine; BA).

Synthesis

- **Site:**

1- primarily in the meristematic region of the roots. This is known in part because roots can be cultured (grown in artificial medium in a flask) without added cytokinin, but stem cells cannot.

2- developing embryos

3- crown gall tissues

- **Transport**

1- xylem

2- phloem

Actions

A. Control morphogenesis in plant tissue cultures, cytokinin is required for the growth of a callus (an unorganized, tumor-like mass of cells

callus + auxin + no cytokinin	→	little growth of callus
callus + auxin + cytokinin	→	callus grows well, undifferentiated

ratio of cytokinin and auxin are important in determining the fate of the callus:

callus + low cytokinin/auxin	→	callus grows well, forms roots
callus + high cytokinin/auxin	→	callus grows well, forms meristem & shoots

some tissues become habituated during repeated cell culture – loose the requirement for cytokinin in the growth medium.

B. Regulates the cell cycle/cell division (hence, the name "cytokinins).

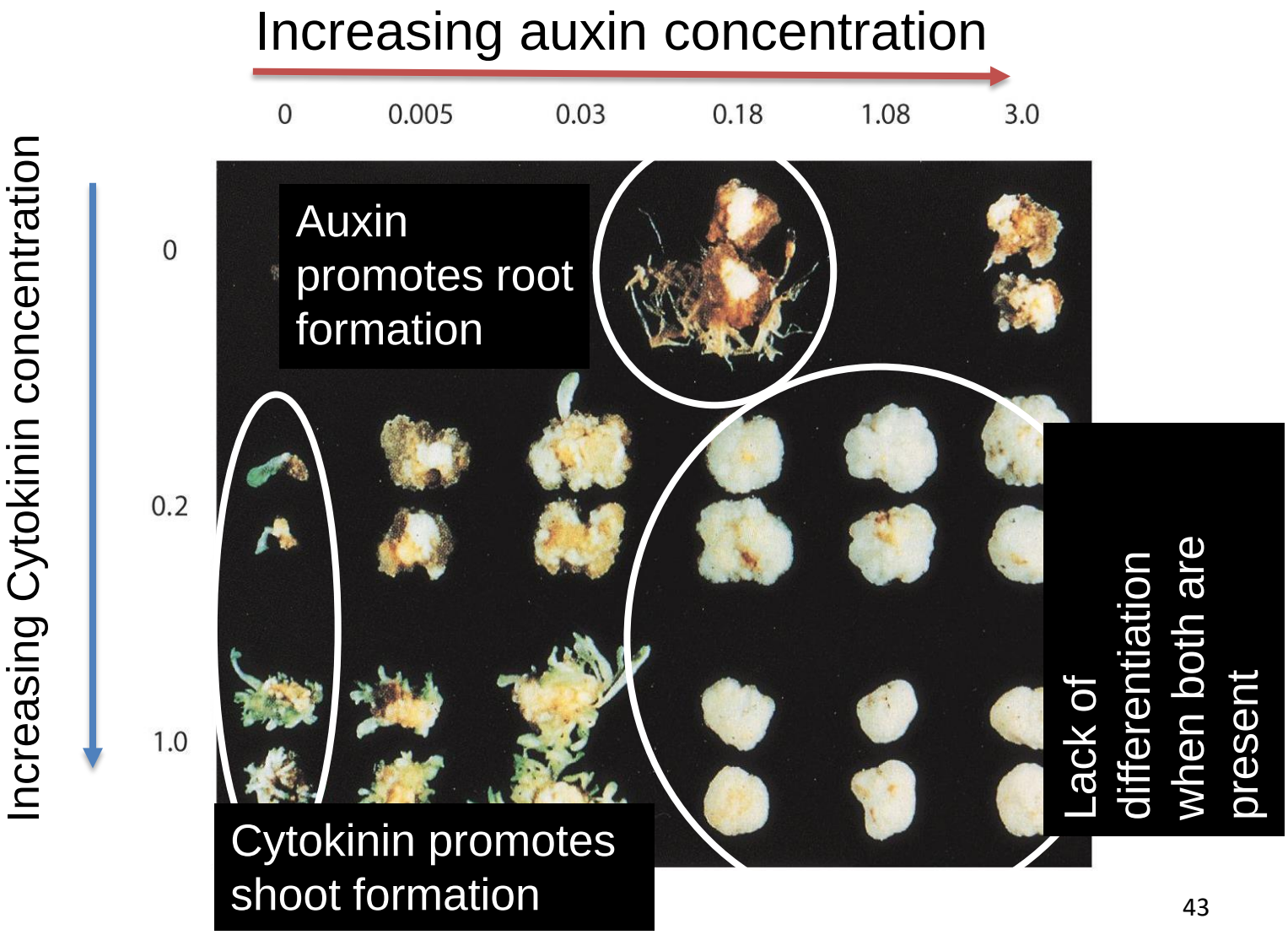
C. Delay senescence; senescence is the programmed aging process that occurs in plants (and other organisms for that matter). loss of chlorophyll, RNA, protein and lipids. Cytokinin application to an intact leaf markedly reduces the extent and rate of chlorophyll and protein degradation and leaf drop correlation between cytokinin levels and senescence.

D. Greening; promotes the light-induced formation of chlorophyll and conversion of etioplasts to chloroplasts(greening process).

E. Promote lateral bud development , some results suggest that apical dominance may be related to cytokinin, too.

F. Promote cell expansion, the mechanism is associated with increased plasticity of the cell wall.

Cytokinin and auxin
complexity of plant-hormone effects and interactions



Cytokinin delays leaf senescence (ageing and reabsorption of aged organs)

Transgenic Untreated

Genetic modification to increase cytokinin biosynthesis

Gibberellins or Gibberellic acid (GA)

Production

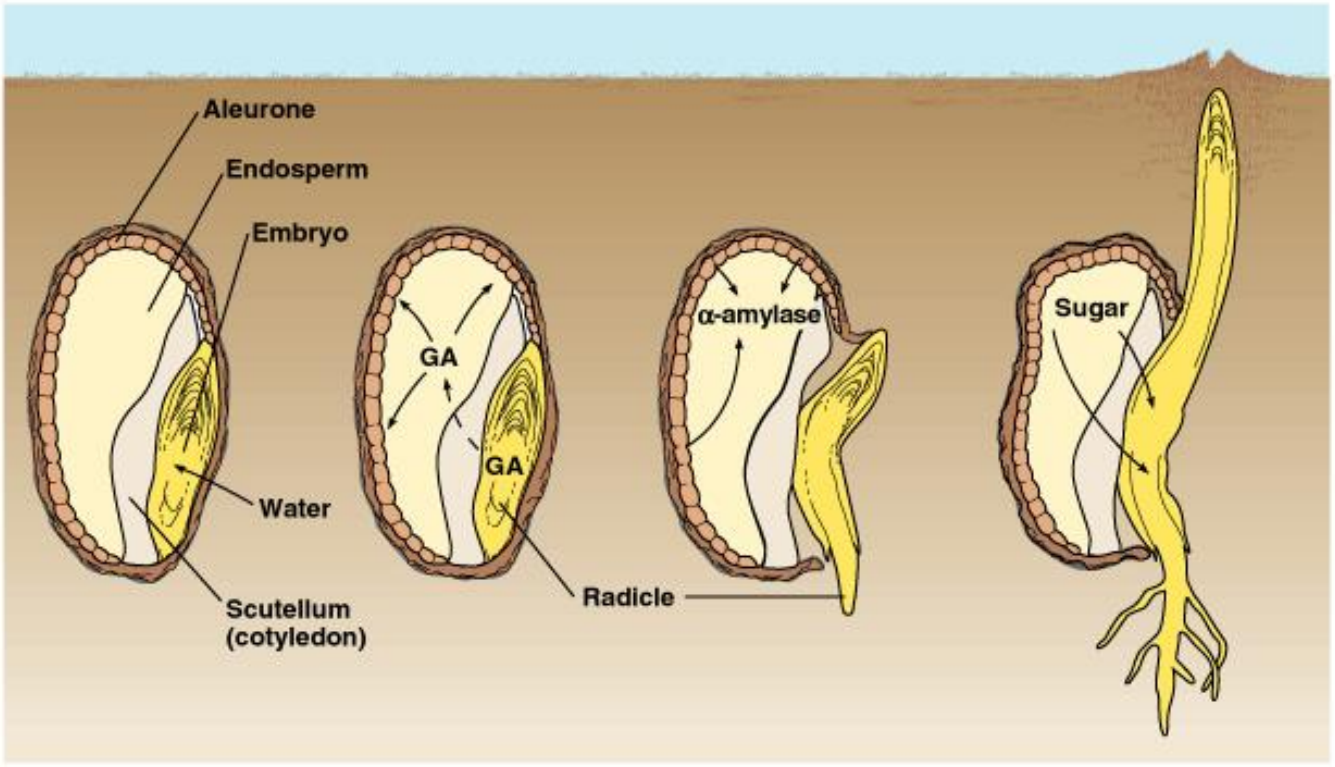
- In young, developing shoots and seeds

Some known actions

- Cell division
- Cell elongation
- Stimulate seed germination
- Stimulate flowering
- Stimulate fruit development
- Overcomes dormancy in seeds and buds. Treating dormant seeds with GA stimulates germination

Mobilization of food reserves

In grass seed germination:- GA is produced by the cotyledon of the embryo → stimulates the production of amylase by the aleurone layer → amylase hydrolyzes starch to simple sugars →absorbed by cotyledon and translocated to embryo for growth.



Commercial Applications

- increase size of grapes (spray at time of blooming and fruit set stage)
- increase distance between grapes in a cluster to minimize fungi/disease
- Delay senescence - spray on fruit like oranges
- Minimize lodging .

Abscisic Acid (ABA)

Chemistry

- a single structure, not a family of related structures like the gibberellins
- found in all green plants, also in some mosses, algae, and fungi

Bioassays/Analysis

A. Bioassays – there are several including:
inhibition of seed germination
inhibition of GA induced alpha- amylase production

B. Analysis – Gas chromatography, HPLC, and immunoassay

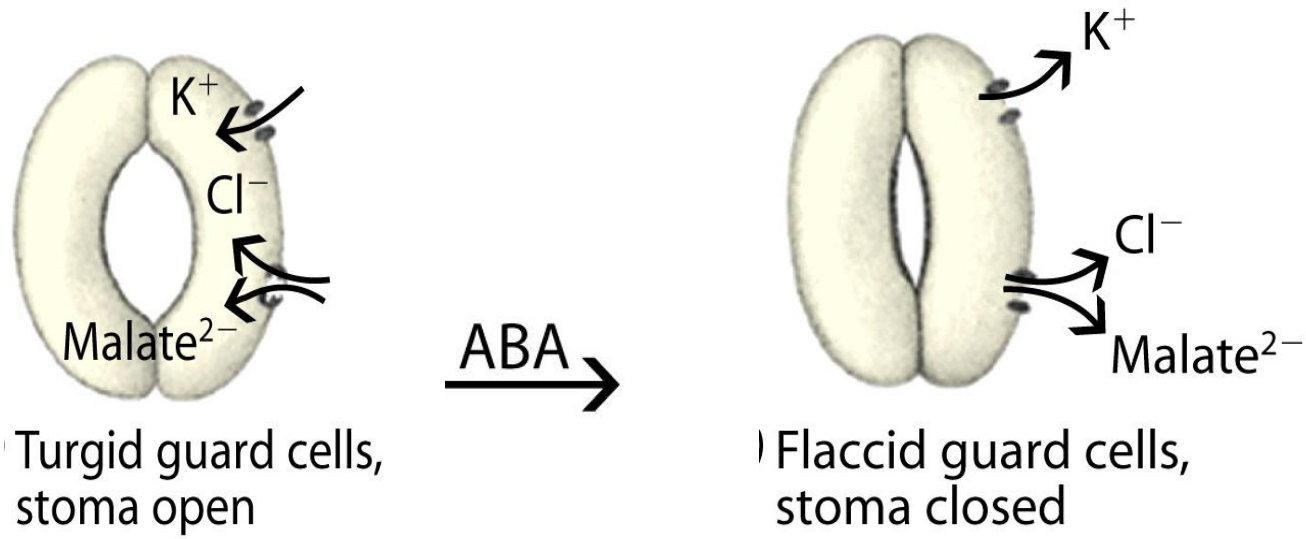
Production

- Mature leaves, especially under stress.
- Roots, then transported to shoots.

Some known actions

- Stress response.
- Stimulate stomatal closure.
- Inhibit premature germination of seeds.
- Embryogenesis.
- Seed dormancy maintenance.

ABA induces stomatal closure simplified diagram



Solutes (*e.g.* potassium and chloride ions) accumulate in guard cells causing water to accumulate in guard cells, making them turgid

ABA is one signal that causes guard cells to release solutes and thus release water, making them flaccid and closing the stoma (pore) between them

Ethylene

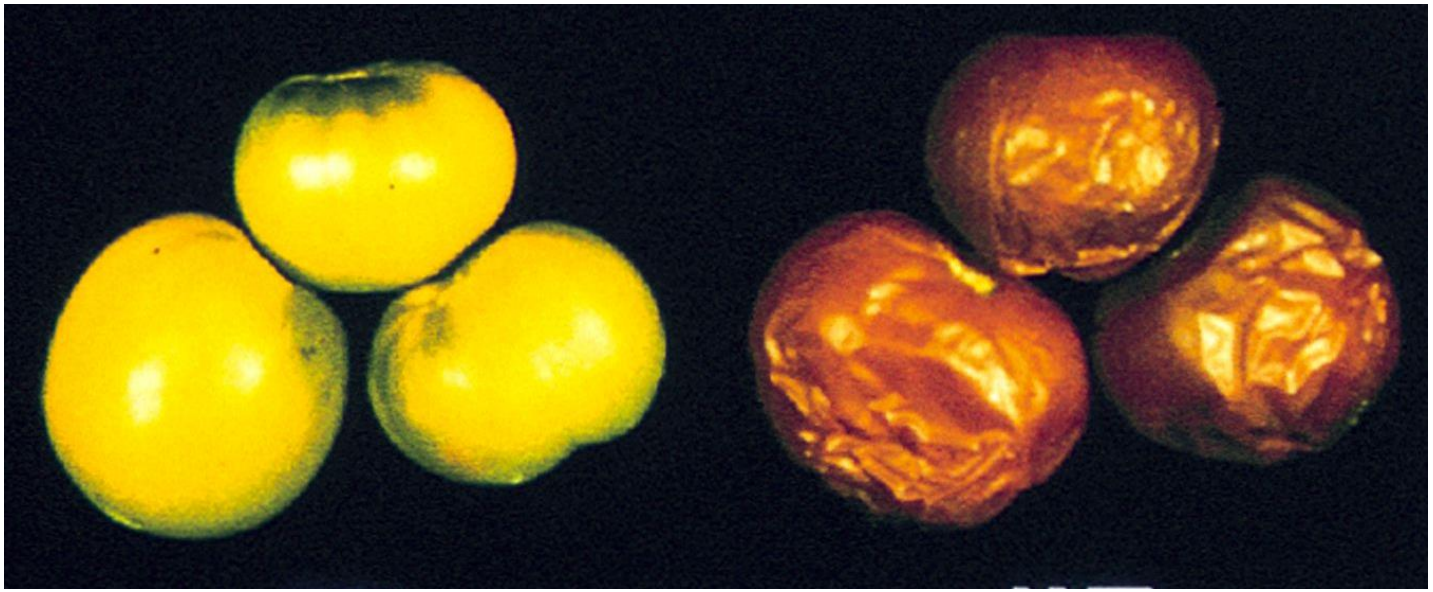
Production

- In most tissues under stress, senescence, or ripening.

Some known actions

- Fruit ripening.
- Leaf and flower senescence .
- Leaf and fruit abscission (controlled separation of plant part from the main body).
- Floral sex determination in monoecious species, promote female.

Experimenting with plant response to ethylene commercial uses



Mutated ethylene receptor

Normal ethylene receptor levels

Both are 100 days after picking