Cytokinins

Regulators of cell division

Mediate many aspects of light-regulated development :

- Chloroplasts differentiation
- Development of autothropic metabolism
- Leaf and cotyledon expansion

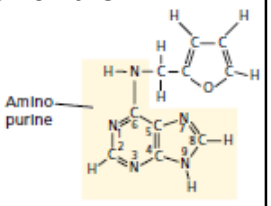
Cytokinins regulate many cellular processes BUT the control of cell divisions is central in plant growth and development

THE DISCOVERY, IDENTIFICATION, AND PROPERTIES OF CYTOKININS

- Many substances (yeast extract, tomato juice, liquid endosperm of coconut = coconut water) → to initiate proliferation of normal stem tissue
- Phillip White's nutrient medium + auxin + 10 to 20% coconut water → support the continued cell division of mature, differentiated cells → formation of callus tissue (Caplin and Steward 1948).
- coconut water : rich in proteins, amino acids, sugars, vitamins, minerals and growth factors **cytokinin *zeatin***, it plays a role in supporting tissue growth
- The **first cytokinin** to be discovered : synthetic **analog kinetin**.

Kinetin Was Discovered as a Breakdown Product of DNA

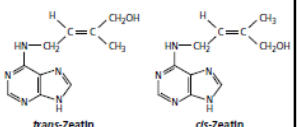
- 1940s and 1950s (Folke Skoog *et al.*) : **nucleic acid base adenine** had a slight effect to initiate proliferation of culture tobacco pith tissue
- **autoclaved** herring sperm **DNA** had a powerful cell division–promoting effect → **kinetin** : adenine (aminopurine) derivative, **6-furfurylaminopurine** (Miller *et al.* 1955)
- Kinetin : by-product of the heat-induced degradation of DNA
- deoxyribose sugar of adenosine is converted to a furfuryl ring and shifted from the 9 position to the 6 position on the adenine ring

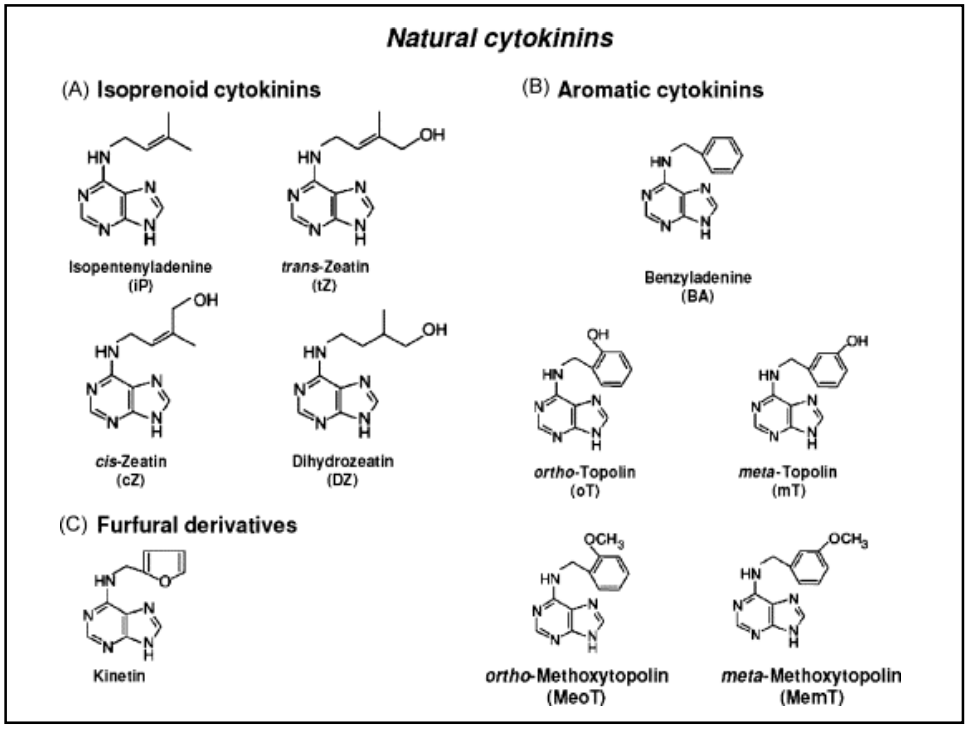


Kinetin

Zeatin is the most abundant natural cytokinin

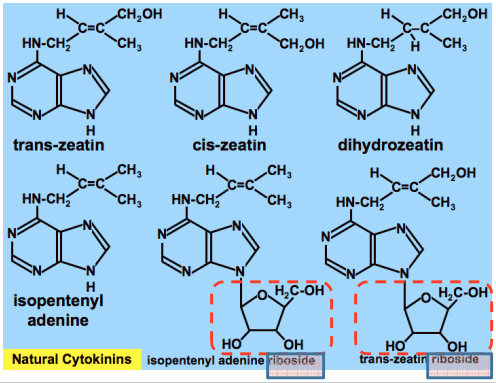
- extracts of the immature endosperm of corn (*Zea mays)* contains subtance stimulates mature plant cells to divide when added to a culture medium along with an auxin : *trans-6-(4-hydroxy-3*-methylbut-2-enylamino)purine, which called **zeatin:**
- **Kinetin = zeatin : adenine or aminopurine derivatives.** The side chain attached to 6 nitrogen of aminopurin
- Double bound → *trans* (more active) and *cis*
- A gene encoding a glucosyl transferase enzyme specific to *cis-zeatin*
- In a plant : riboside, ribotide, glucoside


trans-Zeatin
6-(4-Hydroxy-3-methylbut-2-enylamino)purine
cis-Zeatin



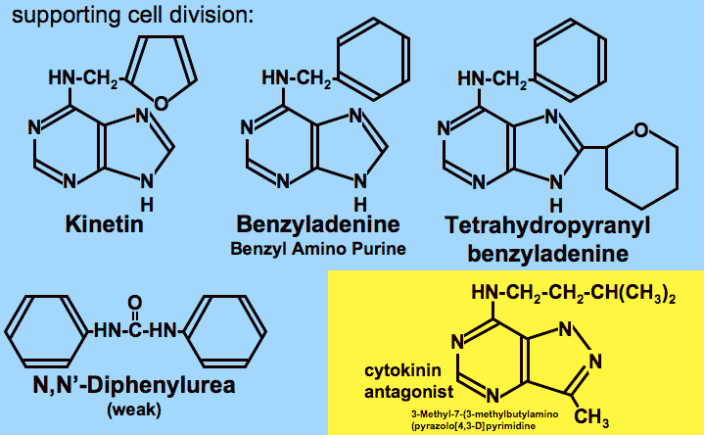
In the plant as a:

- **Riboside** : a ribose sugar is attached to the 9 nitrogen of the purine ring
- **Ribotide** : the ribose sugar moiety contains a **phosphate** group
- **Glycoside** : a sugar molecule is attached to the 3, 7, or 9 nitrogen of the purine ring, or to the oxygen of the zeatin or dihydrozeatin side chain)



Some Synthetic Compounds Can Mimic or Antagonize Cytokinin Action

Tissue culture of cells initially required the use of coconut milk in the medium, but the reason for this was unknown. These synthetic chemicals were found to replace coconut milk in supporting cell division:



Cytokinins Occur in Both Free and Bound Forms

- as free molecules (not covalently attached to any macromolecule) in plants and certain bacteria
- in a wide spectrum of angiosperms, algae, diatoms, mosses, ferns, and conifers.
- zeatin is the most abundant naturally occurring free cytokinin, but *dihydrozeatin (DZ) and isopentenyl adenine (iP) also are commonly found in higher* plants and bacteria.

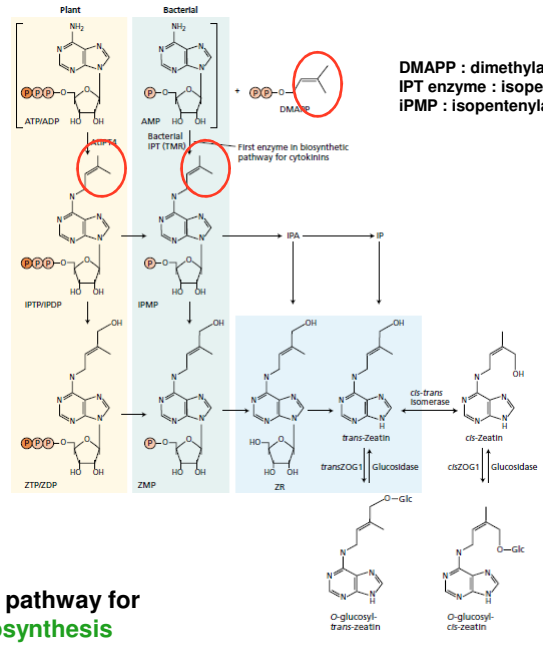
The Hormonally Active Cytokinin Is the Free Base

- the free-base form of *trans-zeatin,* but not its riboside or ribotide derivatives, binds directly to cytokinin receptor CRE1
- some other compounds have cytokinin activity, either because they are readily converted to zeatin, dihydrozeatin, or isopentenyl adenine, or because they release these compounds from other molecules, such as cytokinin glucosides.
- excised radish cotyledons grow in a solution containing the cytokinin base benzyladenine (BA, an N6-substituted aminopurine cytokinin) → The cultured cotyledons readily take up the hormone and convert it to various BA glucosides, BA ribonucleoside, and BA ribonucleotide → When the cotyledons are transferred back to a medium lacking a cytokinin, their growth rate declines, as do the concentrations of BA, BA ribonucleoside, and BA ribonucleotide in the tissues. However, the level of the BA glucosides remains constant.
- the glucosides cannot be the active form of the hormone

BIOSYNTHESIS, METABOLISM AND TRANSPORT OF CYTOKININS

The **side chains** of naturally occurring cytokinins are constructed from **isoprene** units

The **precursor**(s) for the isoprene formation are either **mevalonic acid** or **pyruvate** plus 3-phosphoglycerate, depending on which pathway is involved

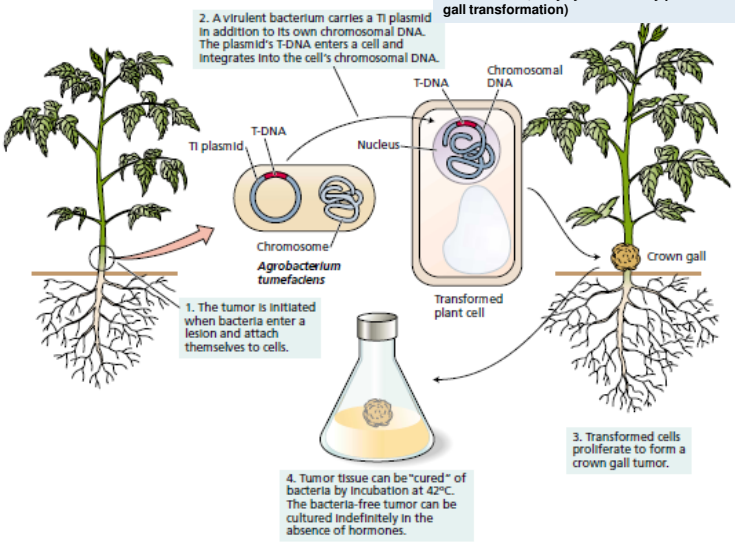


DMAPP : dimethylallyl diphosphate
IPT enzyme : isopentenyl transferase
iPMP : isopentenyladenosine-5'-monophospahte)

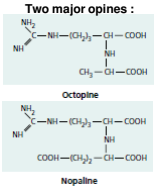
Biosynthetic pathway for cytokinin biosynthesis

Crown Gall Cells Have a Gene for Cytokinin Synthesis

T-DNA carries genes necessary for the biosynthesis of *trans-zeatin (ipt* gene) *and* auxin (2 auxin biosynthetic genes), as well as a member of a class of unusual nitrogen containing compounds called *opines* (N source for bacterium, only synthesized by plants after crown gall transformation)

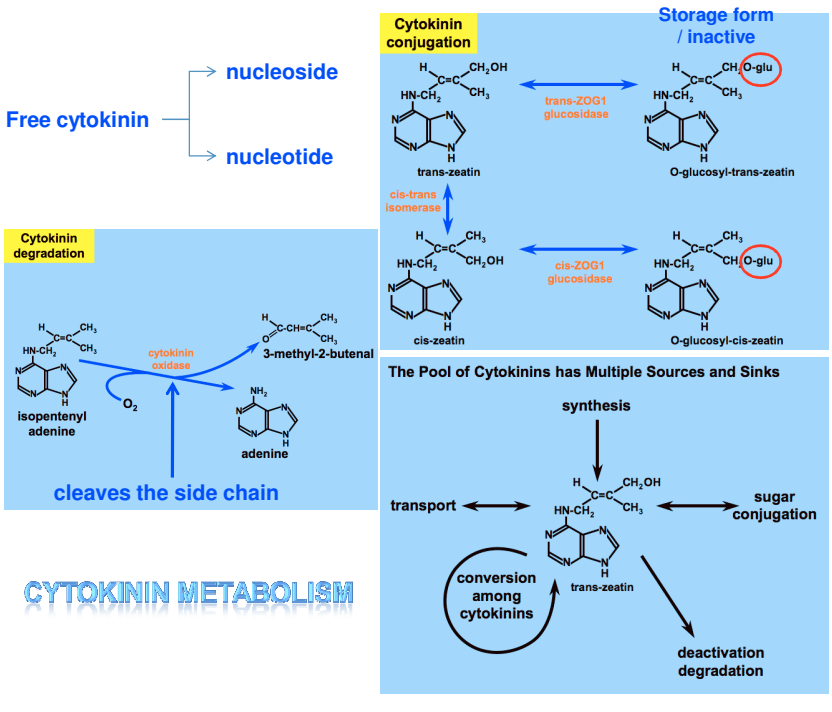


Two major opines :



Cytokinins from Root Are Transported to Shoot via Xylem

- Root apical meristems are **major sites of synthesis** of the free cytokinins in whole plants. BUT, young maize embryos synthesize cytokinins, as do young developing leaves, young fruits, and many other tissues.
- The cytokinins synthesized in roots to move acropetally through the **xylem** into the shoot, along with the water and minerals taken up by the roots.
- The cytokinins in the xylem are mainly in the form of **zeatin ribosides** → a **signal** from the shoot regulate cytokinin transport (grafting experiment)
- environmental factors that interfere with root function, such as **water stress**, reduce the cytokinin content of the xylem exudate
- **resupply of nitrate** to N-starved maize roots → elevation of cytokinins concentration in the xylem sap → induction of cytokinin-regulated gene expression in the shoots (Takei *et al*. 2001).



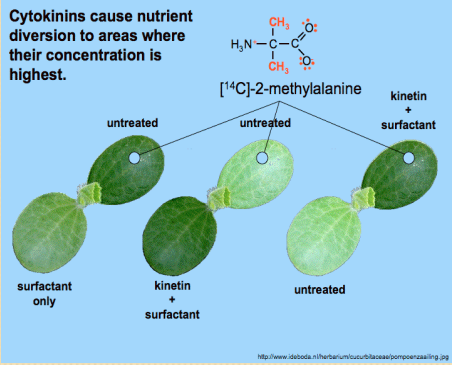
Cytokinins Are Rapidly Metabolized by Plant Tissues

- Free cytokinins are readily converted to **nucleoside** and **nucleotide** forms involve enzymes common to purine metabolism
- Many plant tissues contain the enzyme **cytokinin oxidase** (encoded by multigene)**,** which cleaves the side chain from zeatin (both *cis* and *trans), zeatin riboside, iP, and their N-glucosides, but* not their *O-glucoside derivatives*
- Cytokinin levels can also be regulated by **conjugation** of the hormone at various positions. The nitrogens at the 3, 7, and 9 positions of the adenine ring of cytokinins can be conjugated to glucose residues. Alanine can also be conjugated to the nitrogen at the 9 positon, forming lupinic acid.
- The conjugations at the side chain can be removed **by glucosidase enzymes** to yield free cytokinins, which are the active forms. Thus, **cytokinin glucosides** may be a storage form, or metabolically inactive state, of these compounds.

BIOLOGICAL ROLE OF CYTOKININS

Regulate Cell Division in Shoots and Roots
Regulate Specific Components of the Cell Cycle
The Auxin: Cytokinin Ratio Regulates Morphogenesis in Cultured Tissues
Modify Apical Dominance and Promote Lateral Bud Growth
Induce Bud Formation in a Moss,
Overproduction Has Been Implicated in Genetic Tumors
Delay Leaf Senescence
Promote Movement of Nutrients
Promote Chloroplast Development
Promote Cell Expansion in Leaves and Cotyledons
Regulate Growth of Stems and Roots
Cytokinin-Regulated Processes Are Revealed in Plants That Overproduce Cytokinin

CYTOKININS PROMOTE MOVEMENT OF NUTRIENTS



- *cytokinin-induced nutrient mobilization*
- nutrients (sugars, amino acids, and so on) radiolabeled with ^{14}C or ^{3}H are fed to plants after one leaf or part of a leaf is treated with a cytokinin
- the hormone causes nutrient mobilization by creating a new source–sink relationship

When the leaf become senescent :

- The content of chlorophyll, proteins, RNA : ↓ ; → → : rate of synthesis ↓ or rate of degradation ↑; both may occur simultaneously
- Followed by translocation of minerals, amino acids, sugars & other metabolites from the leaf to the other part
- Then, an increase in the permeability of cell membrane & degeneration of cell organelle like nucleus and chloroplasts → → leaf become yellow
- Cytokinin delaying senescence : zeatin riboside and dihydrozeatin riboside be transported into the leaves from the roots through the xylem, along with the transpiration stream

Etioplasts

- Dark-grown seedlings : to be **etiolated**
- The hypocotyl and internodes : more elongated,
- cotyledons and leaves do not expand, and
- chloroplasts do not mature.
- do not synthesize chlorophyll or most of the enzymes and structural proteins required for the formation of the chloroplast thylakoid system and photosynthesis machinery.

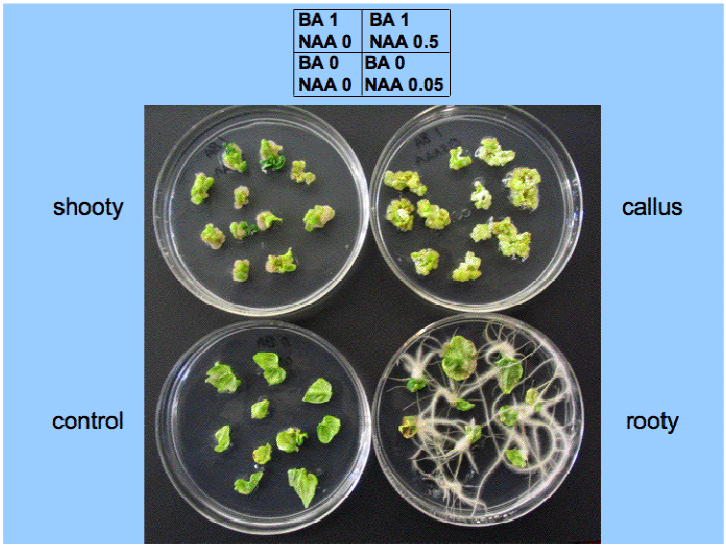
- Instead of maturing as chloroplasts, the proplastids of dark-grown seedlings develop into **etioplasts**

Cytokinins Promote Chloroplast Development:

- Cytokinin treatment form chloroplasts with more extensive grana, chlorophyll and photosynthetic enzymes are synthesized at a greater rate upon illumination.
- Cytokinins — along with other factors, such as light, nutrition, and development — regulate the synthesis of photosynthetic pigments and proteins.

Apical Dominance

- Cytokinin reduces apical dominance
- Cytokinin appears to favor the differentiation of vasculature of lateral buds, which becomes connected with the main stem.
- This facilitates the flow of water and nutrients from the stem to lateral buds

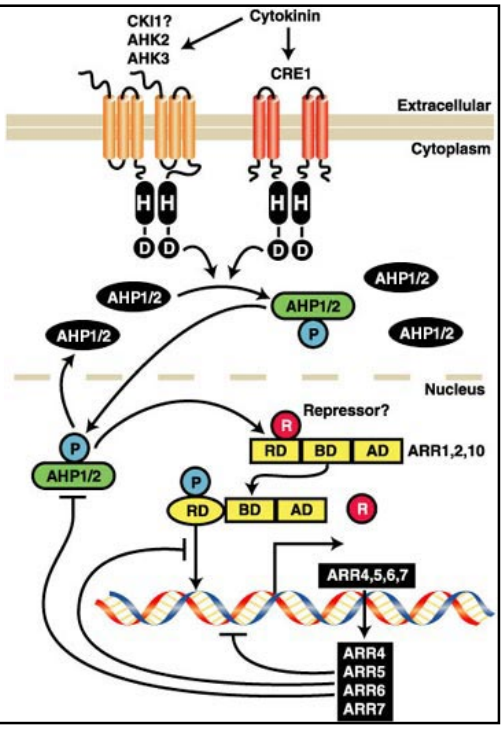


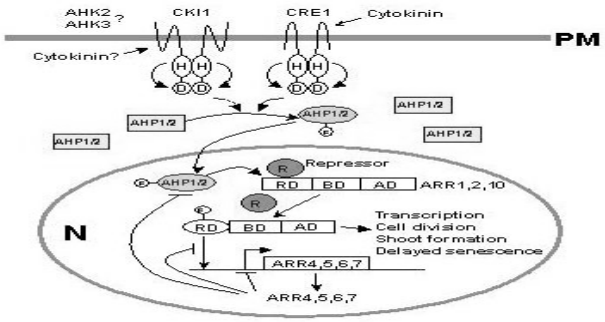
The steps in cytokinin signaling:

- A cytokinin, like zeatin, binds to a receptor protein embedded in the plasma membrane of the cell.
- The internal portion of the receptor then attaches a phosphate group to a protein in the cytosol.
- This protein moves into the nucleus
- it activates one or more nuclear transcription factors.
- These bind to the promoters of genes.
- Transcription of these genes produces mRNAs that move out into the cytosol.
- Translation of these mRNAs produces the proteins that enable the cell to carry out its cytokine-induced function.

Sistem Reseptor Sitokinin

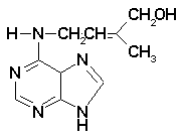
1. **receptor** with a **histidine kinase domain (AHKs)**
2. when activated by binding the hormone, the **kinase transfers a phosphate to an aspartate**, then to a phosphotransfer protein **(AHPs)**
3. the phosphotransfer protein then phosphorylates the **response protein**, which becomes active as a transcription factor (**ARRs**) or may affect some other cellular response to cytokinin
4. there are multiple cytokinin receptors and many different response proteins, implying redundancy of function and/or specialization of response functions



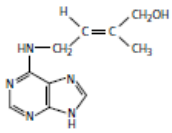


Two-Component Circuitry in Cytokinin Signaling

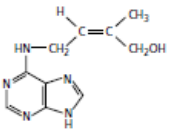
- **cytokinin receptors →AHKs : Arabidopsis histidine protein kinase (AHK4 = CRE1: cytokinin response, AHK 2, AHK3 → They require extracelullar cytokinin for their activation.**
- **cytokinin receptors → CKI1, CKI2 (=AHK5) : activate cytokinin responses in the absence of exogenously added cytokinin. CKI1 is constitutively active**
- **AHPs : histidine phosphotransfer proteins → transmit the signal from AHKs to nuclear response regulators (ARRs) → activate or repress transcription**



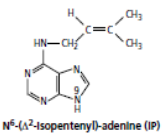
Isoprenoid cytokinins – free bases



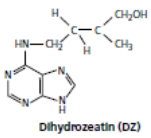
trans-Zeatin
6-(4-Hydroxy-3-methylbut-2-enylamino)purine



cis-Zeatin

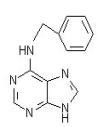


N^6-(Δ^2-Isopentenyl)-adenine (IP)
isopentenyladenine

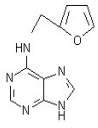


Dihydrozeatin (DZ)
dihydrozeatin

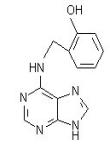
Aromatic cytokinin - free bases



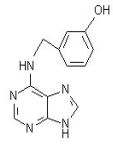
benzyladenine



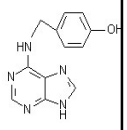
kinetin



(*o*-OH)benzyladenine
o-topolin



(*m*-OH)benzyladenine
m-topolin



(*p*-OH)benzyladenine
p-topolin

In seedling A, the left cotyledon was sprayed with water as a control. The left cotyledon of seedling B, and the right cotyledon of seedling C, were each sprayed with a solution containing 50mM kinetin.

The dark stippling represents the distribution of the radioactive amino acid as revealed by autoradiography.

The results show that the cytokinin-treated cotyledon has become a nutrient sink. However, radioactivity is retained in the cotyledon to which the amino acid was applied when the labeled cotyledon is treated with kinetin (seedling C).

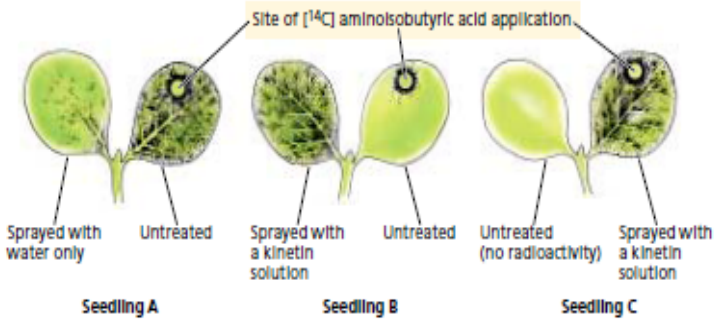


FIGURE 21.19 The effect of cytokinin on the movement of an amino acid in cucumber seedlings. A radioactively labeled amino acid that cannot be metabolized, such as aminoisobutyric acid, was applied as a discrete spot on the right cotyledon of each of these seedlings. (Drawn from data obtained by K. Mothes.)

Natural cytokinins can be more effective than synthetic ones