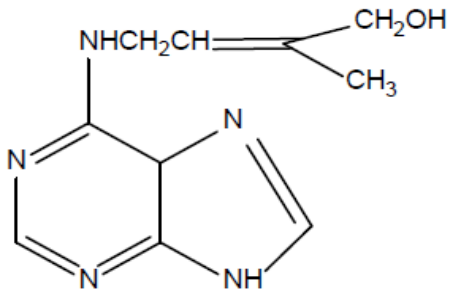
1 Cytokinin

Cytokinins are a class of **phytohormones** with a structure resembling **adenine** which promote **cell division** and have other similar functions to kinetin. This hormone is termed as "cytokinin" because they stimulate cell division (**cytokinesis**). Cytokinins promote cell division or cytokinesis, in plant **roots** and **shoots**. They are involved primarily in **cell growth** and **differentiation**, but also affect **axillary bud growth**, **apical dominance** and **leaf senescence**.

Kinetin was the first cytokinin to be discovered and it is so named because of the compounds' ability to promote cytokinesis (cell division). Though it is a **natural compound**, it is **not made in plants** and therefore it is usually considered a "**synthetic**" **cytokinin**.

The most common form of **natural**ly occurring **cytokinin** in plants today is called **zeatin** which was isolated from **corn** (*Zea mays*). Cambium and other actively dividing tissues also synthesize cytokinins. Approximately **40** different **structures** of cytokinin are known. Other naturally occurring cytokinins include **dihydrozeatin** (DHZ) and **isopentenyladenosine** (IPA).

Cytokinins have been found in almost all **higher plants** as well as **mosses**, **fungi**, **bacteria** and also in **tRNA** of many **prokaryotic** and **eukaryotic** organisms. Today there are more than **200 natural** and **synthetic** cytokinins combined.



1.1 Chemical nature of cytokinin:

Chemical nature of cytokinins is based on two types:

1. **Adenine**-type cytokinins: These cytokinins are represented by **kinetin**, **zeatin** and **6-benzylaminopurine**. Majority of the adenine-type cytokinins are **synthesized** in the **roots**. Cytokinin **biosynthesis** also takes place in the **cambium** and other **actively dividing tissues**.

2. **Phenylurea**-type cytokinins: These cytokinins are represented by **diphenylurea** and **thidiazuron** (TDZ). Till now there is **no evidence** that the phenylurea cytokinins occur naturally in plant tissues.

1.2 Discovery of Cytokinin:

Kinetin was discovered by **Skoog and Miller** (**1950**) from the **tobacco pith callus** and the chemical substance was identified as **6-furfuryl aminopurine**. Some of the very important and commonly known naturally occurring cytokinins are **Coconut milk** factor and **Zeatin**. It was also identified that cytokinin as a **constituent of t-RNA**.

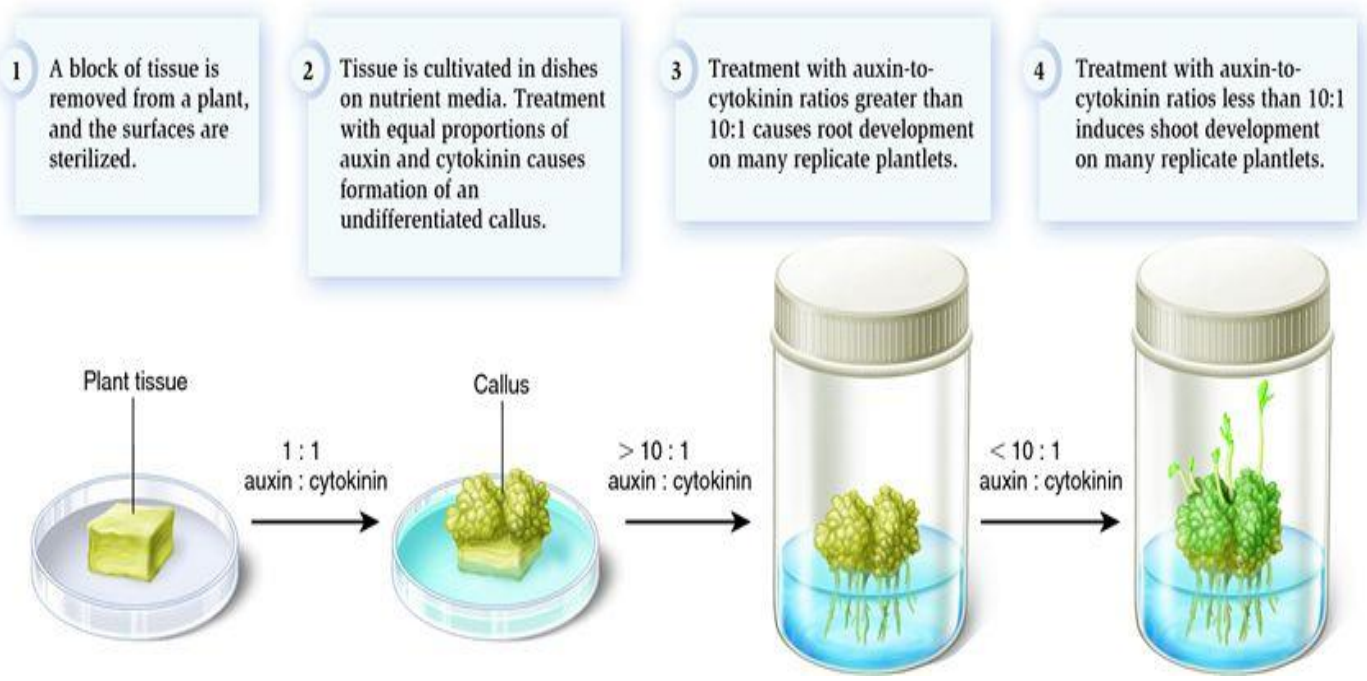
Cytokinin was discovered during experiments designed to define the conditions needed for **culturing plant tissues**. In the tissue culture procedure, **pieces of stem**, **leaf**, or **root** are removed from a plant. They are briefly **sterilized** on their surface to remove any microbes and then are placed in an enclosed **flask** contain a **nutrient medium**.

The medium contains a **carbon** source such as **sucrose** or **glucose**, **nutrient minerals**, and certain **vitamins**. To induce the cells to **divide** and ***grow hormones*** *must also be added*.

Auxin is important, but most plant cells placed in a medium containing **only auxin** as a hormone **enlarge without dividing**. Early studies showed that a second hormone, found in solutions of **boiled DNA** and in **coconut milk** (a liquid endosperm), is also necessary. The active ingredient turned out to be **cytokinin**, a modified form of adenine, which is a component of DNA and RNA. In a medium containing nutrient components plus **auxin and cytokinin**, plant cells could **divide and grow rapidly**.

Further experiments showed that **auxin** and **cytokinin** influence the development, as well as the growth, of plant tissue cultures.

1. **Equal** concentration of auxin and cytokinin lead to the growth of **undifferentiated growth.** The amorphous mass of cells, often loose and fluffy, is called a **callus**.
2. When the **auxin** concentration in the medium is **further increased** about **10 times more auxin** than **cytokinin** added to the cell culture the **callus produces roots**.
3. But when the **cytokinin** concentration is **increased**, the *callus becomes green and compact and* ***produces shoots***.



1.3 Biosynthesis

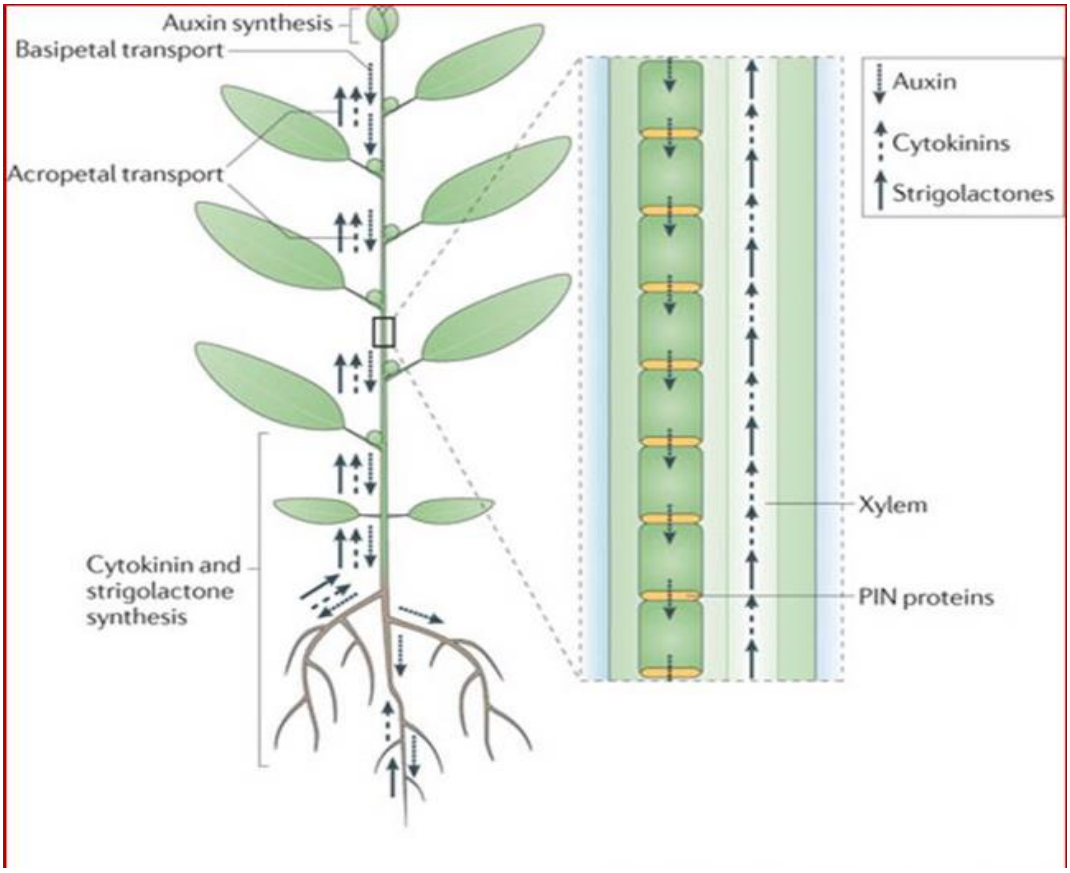
Almost ***all naturally*** *occurring cytokinins* are the **products** of **purine nucleotide** derivatives. The presence of **iospentenyl adenine** in some **tRNAs** has misled people to believe that the source of cytokinins is tRNAs but it is not the case. Nevertheless, the site of synthesis of cytokinins in young and developing plants is restricted to **meristematic regions** of the root tips; *where certain* ***enzymes*** *utilize* ***purine*** *and* ***convert*** *them to* ***cytokinins***.

The active principle responsible for **inducing cell division** was isolated first from the extracts of **yeasts**. Such a substance was called **kinetin**, later the name was changed and called as **cytokinin**; kinetin terminology was ***misleading*** *for another class of compounds called* ***kinins*** which were already known to be found is **animal** systems.

However, the term ***cytokinin*** *has been given to all those compounds that are capable of inducing* ***cell division*** *in the presence of optimal* ***concentration of auxin*** *in plants.* Now it is known that a good source for cytokinin is **coconut liquid** endosperm and **milky endosperm** of sweet corns.

1.4 Transport of Cytokinins:

Most of the cytokinins required for the plant body are **synthesized** in the **root tip meristems**, and then they are translocated to different regions particularly to meristematic region and expanding tissues; transportation is through xylem stream. This observation has been supported by many studies. Concentration cytokinin is ~**0.1 to 10ppm/gm fresh weight**.



1.5 Plant Responses to Cytokinins:

Cytokinins play a prominent role in all the phases of plant development from cell division and enlargement to the formation of flowers and fruits. They increase resistance to aging and to adverse environment.

1. **Cell Division:**

For continued **in vitro growth** and **cell division** of **tissue** accompanied by **DNA synthesis**, cytokinin is necessary along with auxin. While **auxin** and **gibberellin** are also able to stimulate **DNA synthesis** and **mitosis**, **cytokinin** alone can **stimulate cytokinesis**. Quite opposite to the pro-motive effect of auxin and gibberellin, **cytokinin inhibits elongation of stem sections**. Root growth is generally inhibited by cytokinins.

2. **Cell Enlargement:**

Cytokinins may stimulate **radial growth** of stem tissue by swelling rather than by longitudinal extension. In fact, cytokinin appears to **promote overall enlargement** of cells

and not simply elongation. Cytokinin effect on cell enlargement may be due to an influence on **micro fibril** orientation from longitudinal to radial direction.

3. **Tissue Differentiation:**

Organs in tissue culture show a spectacular response to cytokinin. With a low cytokinin supply, the tissue remains as an amorphous undifferentiated callus.

Bud formation and shoot initiation depend on higher concentrations of cytokinin **by changing cytokinin auxin ratios.** An interesting observation on morphogenesis in tobacco callus cultures is that a high cytokinin auxin ratio results in the production of shoots but no roots, but a low ratio leads to an opposite effect producing roots only.

4. **Chloroplast formation**

Cytokinins also play a regulatory role in **chloroplast formation**. When cytokinin is absent, **plastids** are formed but remain **undifferentiated**. Presence of both light and cytokinin is necessary for grana development and conversion of pro-plastids into chloroplasts.

5. **Delay of senescence (Richmond-Lang effect):**

The retardation of senescence by cytokinin is a well-known phenomenon. Richmond and Lang first discovered that when **leaf discs** are kept in **water**, **senescence** appears within a few days as evident by the loss of chlorophyll and protein. But when cytokinin is added to the leaf discs, senescence is delayed through the maintenance of chlorophyll and protein.

This senescence-retarding property of cytokinin as mediated through the retention of chlorophyll is known as **Richmond-Lang effect.**

6. **Mobilization of Nutrients:**

Mothes observed that when a **particular area of leaf is treated with cytokinin** that treated area remains green showing delay of senescence, while the untreated area loses its green color and becomes yellowish showing symptoms of senescence. Here the nutrients are drawn or mobilized from other parts of leaf so that the treated area remains green at the expense of the untreated area.

7. **Release of Dormancy of Seeds and Buds:**

Applications of cytokinins can stimulate germination and **break dormancy**. One of the remarkable characteristics of cytokinins is their ***ability to modify the effects of other hormones without any marked effects by themselves***.

When dormancy is imposed either by high temperature (thermo dormancy) or by an accumulation of inhibitor like ABA (**inhibitor dormancy**), then GA alone is not capable to **overcome dormancy**. Addition of cytokinin opposes the action of inhibitor and permits germination.

Thus cytokinin has been documented as a **permissive agent** in germination by antagonizing the inhibitor action — a case of cytokinin-inhibitor **antagonism**. In bud growth also inhibitor (preventive) and cytokinin (permissive) show opposite effects. Thus **inhibitor-induced** bud dormancy can be **overcome** by cytokinin.

8. Apical Dominance:

Cytokinin applied on **lateral buds** is able to **release** them from apical dominance whether it is due to the presence of **terminal bud** or due to applied **auxin**. This has been interpreted as an increase in **IAA** transport and mobilization of metabolites from the **apical region** to the point of application of cytokinin which is supported by the striking influence of cytokinin on phloem transport.

9. Resistance to Adverse Factors:

Cytokinins increase the **resistance** of plants to **adverse factors** such as **high** and **low temperatures** and certain **disease**. The nature of the action of cytokinins in bringing about these effects is still unknown.

10. Stomatal Movement:

Cytokinin has a distinct action on the **mechanism of stomatal movement**. Although the stomatal aperture in the isolated epidermal systems is not much influenced by cytokinins, treatment of whole leaf with cytokinin has been reported to **increase the stomatal aperture** and thereby transpiration.

11. Other Developmental Effects:

The development of **inflorescence** is **influenced** by cytokinin treatment by **increasing** both the **number** and **size**. Cytokinin has been shown to cause a **male-flowering** plant to **produce hermaphrodite flowers**. Enhancement of fruit set and fruit size in grape varieties and induction of parthenocarpy in fig has also been reported.