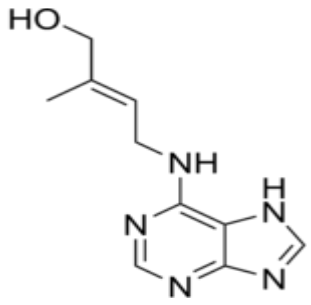
Unit 5.1

Cytokinins- Discovery, Biosynthesis and Physiological Role

Cytokinins are plant growth hormones/ substances which are primarily involved in inducing cell division in parenchymatous cells by stimulating the process of mitosis. Increased mitosis results in plant growth and the formation of shoots and buds, as well as the development of fruits and seeds. Skoog, Strong and Miller (1965) have defined Cytokinins as chemicals which, regardless of their other activities, promote cytokinesis in the cells of various plant organs. Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria and also in tRNA of many prokaryotes and eukaryotes. The most common form of naturally occurring Cytokinins in plants is called Zeatin which was isolated from corn (*Zea mays*). At present, there are more than 200 natural and synthetic Cytokinins.

Cytokinins are synthesized in meristematic regions of roots (root meristems) particularly during the seedling stage. Like the gibberellins, they move upward in the xylem and pass into the young roots, young leaves and developing fruits. Thus these are produced in the regions where cell division occurs; mostly in the roots and shoots. They help in the production of new leaves, lateral shoot growth, chloroplasts in leaves etc.

All Cytokinins are purines (adenine) ring with a side chain at N^6 position (amino substituted adenine). Zeatin is more active than all other known Cytokinins because it contains a highly reactive allylic –OH group in its side chain.



The Cytokinin- Zeatin

Discovery

The discovery of Cytokinins dates back to 1921, when an Austrian Plant Physiologist, G. Haberlandt discovered that vascular tissues of various plants contained an unknown diffusible factor, which stimulated cell division. Johannes Van Overbeek found that milky endosperm of immature coconut also had this factor which stimulated cell division and differentiation in very young Datura embryos.

Jablonski and Skoog (1954) extended the work of Haberlandt and reported that a substance present in the vascular tissue was responsible for causing cell division in the pith cells. Miller and his co-workers (1954) isolated and purified the cell division substance in crystallized form, from autoclaved herring fish sperm DNA This active compound was named as Kinetin because of its ability to promote cell division and was the first Cytokinin to be named. Kinetin was later on identified to be 6-furfuryl-amino purine. Later on the generic name kinin was suggested to include kinetin and other substances having similar properties.

The first naturally occurring cytokinin was isolated and crystallized simultaneously by Miller and D.S. Letham (1963-65) from the milky endosperm of corn (Zea mays) and named as Zeatin. Lethem ((1963) proposed the term **Cytokinins** for such substances.

Biosynthesis

Cytokinin is synthesized in the roots from where they are transported to shoots by xylem tissues. Biosynthesis of purine type of cytokinin is more clearly known than of other types. Various components of purine are derived from several small molecules including formyltetrahydro folate, aspartate, CO_2, glutamine, glycine and methylidyne tetrahydrofolate. The biosynthesis of purine nucleotide (purine base + ribose sugar + phosphate) began with the ribose phosphate and the purine ring is built on it, step by step. The first nucleitide to be synthesized by this process is inosine monophosphate (IMP) .IMP is then converted to either guanosine monophosphate (GMP) or adenosine monophosphate (AMP). Free Cytokinins are synthesized from adenosine monophosphate(AMP) and isopentenyl pyrophosphate by the condensation reaction in the presence of enzyme isopentenyl transferase The product formed is N^6 (Δ^2- isopentenyl)

adenosine 5' monophosphate. The compound is supposed to be the precursor of all other Cytokinins. The $N^6(\Delta^2-$ isopentenyl)-adenosine 5' monophosphate is converted to N^6 $(\Delta^2-$ isopenttenyl)-adenosine by the removal of the phosphate by phosphatase and further converted to N^6 $(\Delta^2-$ isopentenyl)- adenine, which is then hydroxylated form free zeatin. Reduction of the double bond in isopentenyl side chain of zeatin would give rise to dihydrozeatin.

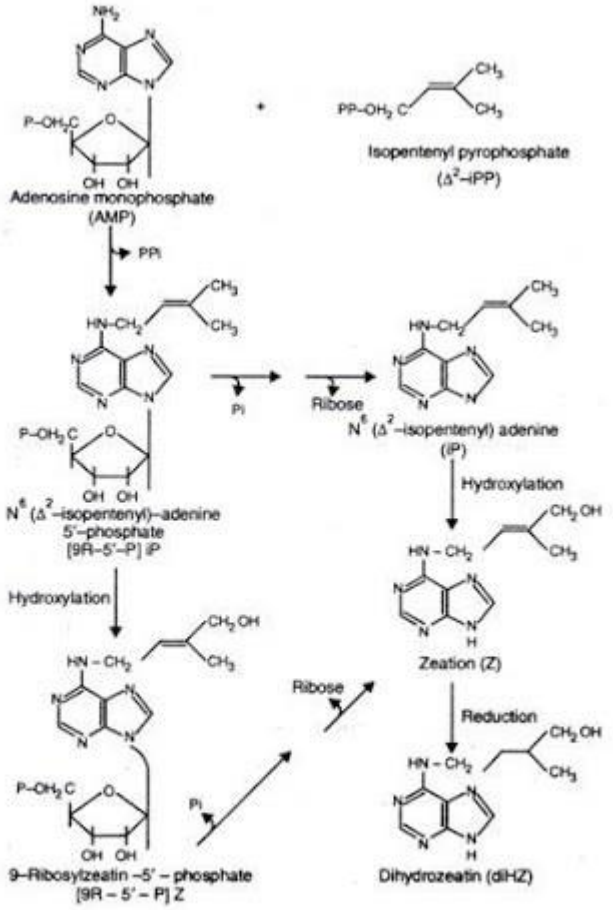


Fig. 1 Biosynthesis of naturally occurring cytokinins iP, zeatin and dihydrozeatin from adenosine monophosphate and isopentenyl pyrophosphate.

Physiological role

1. Promotion of cell division: The major physiological role of naturally occurring cytokinins is to promote cell division. It is now wellestablished that these are true cell division factors in a number of lower and higher plants. In the presence of auxins, nearly all cytokinins stimulate cell division and susbsequent callus growth in the parenchymatous cellsof several plants.

2. Cell enlargement: The cytokinins can also induce cell enlargement like auxins and gibberellins. This effect of cell enlargement may be due to an influnce on micro fibril orientation from longitudinal to radial direction and has been observed in tobacco pith cultures, cortical cells of tobacco roots and pumpkin cotyledons. Cytokinins also promote cell expansion (although without proton extrusion) in leafy cotyledons of some plants like mustard, sunflower, cucumber, radish,etc. The expansion of cotyledons is rather more due to cell enlargement than due to cell division

3. Morphogenesis: Cytokinins play a vital role in the morphpgenins in plants. It is now well known that kinetin- auxin interaction controls the morphogenetic differentation of shoot and root meristems. Skoog (1956-57) demonstrated that tobacco pith callus tissue grown in high auxin and low kinetin produced only roots whereas high kinetin and low auxin could promote the formation of shoot buds. The cytokinins also stimulate the production of buds in leaf segments of various plants such as *Bryophyllum*, *Begonia*,etc. The cytokinins also cause other morphogenetic responses like maturation of protoplastids into plastids, differentation of tracheids, induction of parthenocarpy and induction of flowering.

4. Counteraction of apical dominance: External application of Cytokinins promotes the growth of lateral busd even if the apical bud is intact. Sorokin and Thimann (1964) experimentally demonstrated that cytokinins caused the formation of a vascular connection (which was not allowed to be formed by auxin released from the apical bud) which increases water and solute supply for a renewed growth of the lateral buds. Thus, the cytokinins reverse the auxin induced inhibition of lateral buds and counteract the apical dominance.

5. Breaking the dormancy: Cytokinins can stimulate germination and break dormancy. It has the ability to change the effects of other hormones without any marked effects on themselves. Gibberellins alone are not capable to overcome the thermo-dormancy or inhibitor dormancy.

Addition of cytokinin along with GA opposes the action of inhibitor and permits germination. Thus cytokinin has been documented as a permissive agent in germination antagonizing the inhibitor action (cytokinin-inhibitor antagonism). Cytokinins treatment, in dark overcomes the dormancy of certain light sensitive seeds as littuce and tobacco. Dormant buds which remains inactive due to certain adverse factors, can be treated with cytokinins to overcome the dormancy.

6. Delay of senescence (Richmond- Lang effect): Senescence is the phenomenon in which the mature leaves lose their pigment chlorophyll, turn yellow, proteins are degraded and ultimately they shed from the plant. Richmond and Lang (1957) while working on detached leaves of *Xanthium*, observed that application of cytokinins delays the process of senescence for a number of days. The phenomenon of delaying senescence by application of ctyokinins is known as Richmond- Lang effect. This effect is due to the prevention of degradative catabolic processes by the way of repression activity of few hydrolysing enzymes like protease, RNAse,DNAse, etc. This physiological effect of cytokinins has now been observed in many plants and helps them to remain green for much longer. A correlation between the age of leaf and the kinetin has been established. Mature leaves of tobacco respond more vigorously to kinetin treatment in delaying senescence than the young leaves.

7. Promotion of chloroplast development: Exogenously applied cytokinins promote chloroplast development in callus tissues and excised cotyledons. Tabacco callus tissue in dark contains etioplasts devaid of chlorophyll with no grana and lamellae. If it is treated with cytokinin in dark, promotes lamellar deveploment and if light is also applied simultaneouslt (with cytokinin) grana and chlorophyll also appear. Thus, ctyokinin is essential for the transformation of etioplasts to chloroplasts.

8. Anthocyanin synthesis: Anthocynins are flavonoid pigments which are responsible for the red, pink, purple and blue colours in plants. Cytokinin treatment increases anthocynin content in many culture cells and tissues and ih parts of intact plants. For example-in suspension cultured cells of carrot and sunflower, in yhe seedlings of balsam and cauliflower and in the petals of rose, cytokinin application increases anthocynin production.

9. Other roles/ effects: Some other roles or effects of cytokinins are- differentation of interfascicular cambium and lignification, accumulatiom of solutes, stimulation of several enzymes especially those concerned with photosynthesis, etc.

Reference /Syllabus Books (For material & diagrams)

1. A Text Book of Plant Physiology by H. S. Srivastava (Rastogi Publication)

2. A Text Book of Plant Physiology by S. K. Verma (S. Chand & Company Ltd.)

3. A Text Book of Plant Physiology by V. Verma (Emkay Publications).

4. Plant Physiology and Metabolism by Dr. H.N. Srivastava (Pradeep Publications)

5. Plant Physiology and Metabolism by Dr. Kamaljit & co-workers (S. Vinesh & Co.)

6. Plant Physiology and Metabolism by Dr. B.B. Arora (Modern Publishers)