# Topic: CYTOKININS

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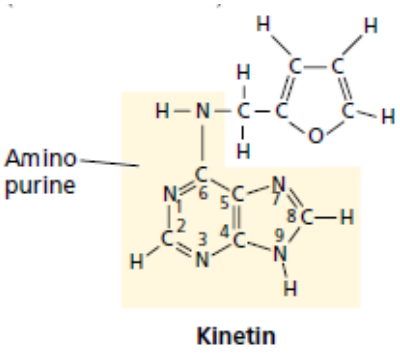
Cytokinins are derivatives of the nitrogenous base adenine and are known for their capacity to encourage cell division in tissue culture. This plant hormone also influence a number of other developmental responses, including shoot and root differentiation in tissue culture, the growth of lateral buds and leaf expansion, chloroplast development, and delay of senescence etc.

## DISCOVERY AND PROPERTIES OF CYTOKININS

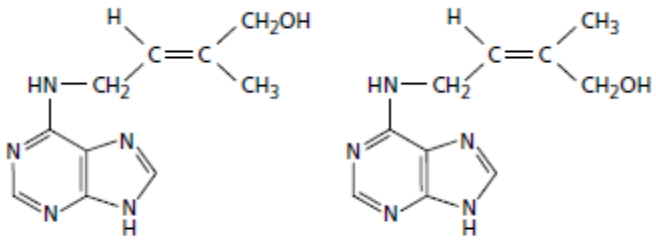
In 1913, G. Haberlandt found that phloem exudates from plant contains a water-soluble substance or substances that has the ability to stimulate cell division in wounded potato tuber tissue (Habelandt, 1913). “The effort to determine the nature of this factor (or factors) eventually led to the discovery of the cytokinins in the 1950s. A great many substances were tested in an effort to initiate and sustain the proliferation of normal stem tissues in culture. Materials ranging from yeast extract to tomato juice were found to have a positive effect, at least with some tissues. However, culture growth was stimulated most dramatically when the liquid endosperm of coconut, also known as coconut milk, was added to the culture medium. Philip White’s nutrient medium, supplemented with an auxin and 10 to 20% coconut milk, supported the continued cell division of mature, differentiated cells from a wide variety of tissues and species, leading to the formation of callus tissue (Caplin and Steward 1948). This finding indicated that coconut milk contains a substance or substances that stimulate mature cells to enter and remain in the cell division cycle. Eventually coconut milk was shown to contain the cytokinin *zeatin* (most abundant natural cytokinin), but this finding was not obtained until several years after the discovery of the cytokinins (Letham 1974). The first cytokinin to be discovered was the synthetic analog kinetin (as a breakdown product of DNA).”




**Kinetin**

[adenine (or aminopurine) derivative, 6-furfurylaminopurine]

"Several years after the discovery of kinetin, extracts of the immature endosperm of corn (Zea mays) were found to contain a substance that has the same biological effect as kinetin. This substance stimulated mature plant cells to divide when added to a culture medium along with an auxin. Letham (1973) isolated the molecule responsible for this activity and identified it as trans-6-(4-hydroxy-3-methylbut-2-enylamino) purine, which he called **zeatin**. The molecular structure of zeatin is similar to that of kinetin. Both molecules are adenine or aminopurine derivatives. In higher plants, zeatin occurs in both the *cis* and the *trans* configurations, and these forms can be interconverted by an enzyme known as zeatin isomerase. Although the *trans* form of zeatin is much more active in biological assays, the *cis* form may also play important roles, as suggested by the fact that it has been found in high levels in a number of plant species and particular tissues."[1]




***trans*-Zeatin** ***cis*-Zeatin**

**6-(4-Hydroxy-3-methylbut-2-enylamino)purine**

"Occurs in both free (hormonally active) and bound forms. Some bacteria and fungi are intimately associated with higher plants. Many of these microorganisms produce and secrete substantial amounts of cytokinins and/or cause the plant cells to synthesize plant hormones, including cytokinins, which include *trans*-zeatin, *cis*-zeatin, and their ribosides. Infection of plant tissues with these microorganisms can induce the tissues to divide and, in some cases, to form special structures, such as mycorrhizae, in which the microorganism can reside in a mutualistic relationship with the plant. In addition to the crown gall bacterium, *Agrobacterium tumefaciens*, other pathogenic bacteria may stimulate plant cells to divide. For example, *Corynebacterium fascians* is a major cause of the growth abnormality known as witches'-broom. The shoots of plants infected by *C. fascians* resemble an old-fashioned straw broom

[1] Taiz and Zeiger (2002)

because the lateral buds, which normally remain dormant, are stimulated by the bacterial cytokinin to grow (Hamilton and Lowe 1972)."[2]

Fig.1 Witches' broom on balsam fir (*Abies balsamea*)

## BIOSYNTHESIS

"A major site of cytokinin biosynthesis in higher plants is the root. High cytokinin levels have been found in roots, especially the mitotically active root tip, and in the xylem sap of roots from a variety of sources. It is generally seen that roots are a principal source of cytokinins in most plants and that they are **transported** to the aerial portion of the plant through the xylem. Immature seeds and developing fruits also contain high levels of cytokinins; the first naturally

[2] Taiz and Zeiger (2002)

occurring cytokinins were isolated from milky endosperm of maize and developing plum fruits."[3]

"The side chains of naturally occurring cytokinins are chemically related to rubber, carotenoid pigments, the plant hormones gibberellin and abscisic acid, and some of the plant defense compounds known as phytoalexins. All of these compounds are constructed, at least in part, from isoprene units. These cytokinin side chains are synthesized from an isoprene derivative. Large molecules of rubber and the carotenoids are constructed by the polymerization of many isoprene units; cytokinins contain just one of these units. The precursor(s) for the formation of these isoprene structures are either mevalonic acid or pyruvate plus 3-phosphoglycerate, depending on which pathway is involved. These precursors are converted to the biological isoprene unit dimethylallyl diphosphate (DMAP)."[2]

"Here, the key reaction is the addition of a dimethylallyl diphosphate (DMAP) group to the nitrogen at the 6-position of adenosine−5'-monophosphate (AMP). DMAP, is also known as $\Delta^2$-isopentenyl diphosphate or $\Delta^2$-iPP. This addition reaction is catalyzed by the enzyme adenosine phosphate-isopentenyl transferase (IPT). The product is N6-($\Delta^2$--isopentenyl)-adenosine−5_-monophosphate or iPRMP. The IPT catalyzed reaction is also the rate limiting reaction in cytokinin biosynthesis. In the next two steps, the phosphate group and the ribose group removed from [9R−5'P]iP to form the active cytokinin N6-($\Delta^2$-isopentenyl)-adenine (iP). Alternatively, the isopentenyl side chain of [9R − 5'P]iP may be hydroxylated before the phosphate and ribose groups are removed to form zeatin (Z). Zeatin and iP are thought to be the most biologically active cytokinins in most plants. Reduction of the double bond in the side chain of zeatin would give the dihydrozeatin derivative, which is particularly active in some species of legumes.

[3] Hopkins and Huner (2009)

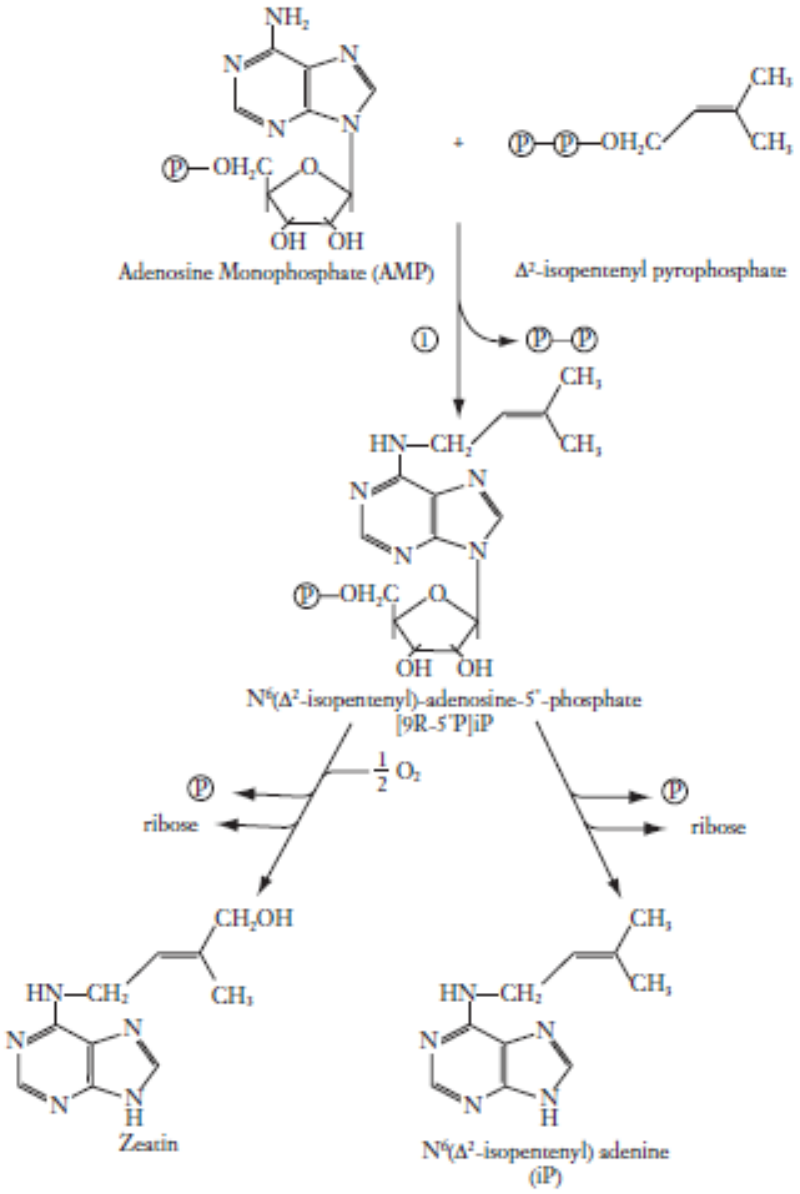



Fig. 2. A general outline for the biosynthesis of isopentenyl adenine (iP) and *trans*-zeatin (tZ) from adenosine monophosphate and isopentenyl pyrophosphate.[4]

[4] Hopkins and Huner (2009)

## ROLE/ EFFECTS

"Although discovered as a cell division factor, cytokinins can stimulate or inhibit a variety of physiological, metabolic, biochemical, and developmental processes when they are applied to higher plants. The discovery of the tumor-inducing Ti plasmid in the plant-pathogenic bacterium *Agrobacterium tumefaciens* provided plant scientists with a powerful new tool for introducing foreign genes into plants, and for studying the role of cytokinin in development. In addition to its role in cell proliferation, cytokinin affects many other processes, including differentiation, apical dominance, and senescence.

Some of their effects include-

1. Cytokinins regulate cell division in shoots and roots. Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle. Zeatin levels were found to peak in synchronized culture tobacco cells at the end of S phase, mitosis, and G1 phase."[5]

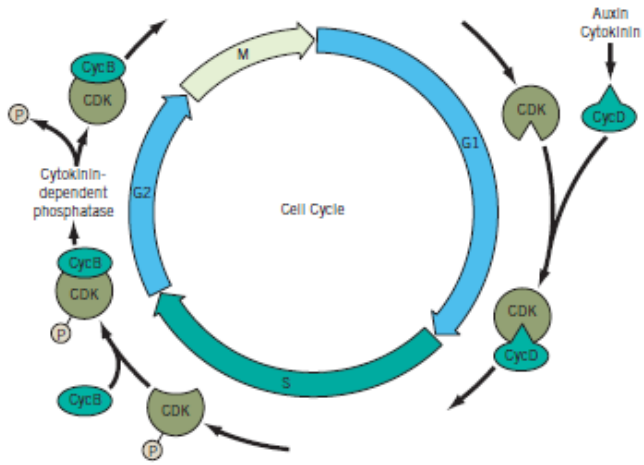



Fig.3. A simplified model for hormonal control of the cell cycle in plants. Cytokinin promotes the onset of mitosis (the G2 to M transition) by activating a phosphatase that removes an inhibitory phosphate group from the cyclin-dependent kinase (CDK)/cyclin B complex. Auxin and cytokinin also promote the accumulation of G1 cyclins (shown here as cyclin D), necessary for the onset of the S (synthesis) phase.[6]

[5] Taiz and Zeiger (2002)
[6] Hopkins and Huner (2009)

2. “Auxin and cytokinins have antagonistic actions with respect to root shoot formation in cultured tobacco tissues. Both auxin and cytokinins are required to maintain callus cultures. However, when auxin is present alone, or if the ratio of auxin to cytokinin is high, cultures will initiate root formation. Conversely, a high cytokinin-to-auxin ratio promotes shoot production and roughly equal amounts of auxin and cytokinin will cause continued proliferation of undifferentiated callus. This phenomenon has been put to practical application in the technique of regenerating large numbers of plants by micropropagation.”[7]

3. Delays senescence (Richmond Lang effect).

4. Cytokinins modify apical dominance and promote lateral bud growth; also induces bud formation in moss.

5. Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as cytokinin-induced nutrient mobilization.

6. Promote chloroplast development, cell expansion in leaves and cotyledons.

“The mechanism of action of cytokinin is yet to be clearly understood. A cytokinin receptor has been identified in *Arabidopsis*. This transmembrane protein is related to the bacterial two-component sensor histidine kinases. Cytokinins increase the abundance of several specific mRNAs. Some of these are primary response genes that are similar to bacterial two-component response regulators. The signal transduction mechanism from CRE1 to transcriptional activation of the type-A *ARR*s involves other homologs of two-component elements.”[8]

[7] Hopkins and Huner (2009)
[8] Taiz and Zeiger (2002)

## *Selected References*

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