Cytokinins – Their Chemical Structure, Occurrence, Metabolism and Biological Activity

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Abstract

Cytokinins are a group of plant hormones which play an important role in plant growth and development. They especially influence seed germination, de-etiolation, chloroplast differentiation, apical dominance, flower and fruit development and leaf senescence. Molecular and genetic analysis provided valuable insights into the molecular mechanisms of cytokinins action. This review focuses on the cytokinins occurrence, chemical structure and primarily on their metabolism, signaling and biological activity. The analysis of all those factors is necessary to understand the impact that they have on plant physiology and their role as developmental signals.

Keywords: Cytokinins; Kinetin; Zeatin; Adenine; Urea; Fatty Acids; Molecular Mechanism; Biological Activity

Abbreviations

CK: Cytokinin;
K: Kinetin;
CKs: Cytokinins;
Z: Zeatin;
DZ: Dihydrozeatin;
2iP: N6-D2-isopentenyladenine;
IPA: 9-β-ribofuranosyl-2iP;
BA: N6-benzyladenine;
DPU: N,N'-diphenylurea;
TDZ: Thidiazuron;
CPPU: N-phenyl-N-2-chloro-4-pirydylurea;
iP: Isopentenyldadenine;
IPT: Adenosine phosphate-isopentenyltransferase;
DMAPP: Dimethylallyl diposphate;
HMBDP: 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate

Table 1. Chemical structure of cytokinins adenine derivatives in the form of free bases (according to 2).

<table>
<thead>
<tr>
<th>R substituent</th>
<th>Cytokinin [abbreviation]</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-zeatin, zeatin</td>
<td>N°-(∆²-hydroxyisopentenyl)adenine</td>
</tr>
<tr>
<td>cis-zeatin</td>
<td>N°-(∆²-hydroxyisopentenyl)adenine</td>
</tr>
<tr>
<td>ortho-topolin</td>
<td>N°-(2-hydroxybenzyl)adenine</td>
</tr>
<tr>
<td>meta-topolin</td>
<td>N°-(3-hydroxybenzyl)adenine</td>
</tr>
<tr>
<td>[BA]</td>
<td>N°-benzyladenine</td>
</tr>
<tr>
<td>[FA]</td>
<td>N°-furfuryladenine</td>
</tr>
<tr>
<td>[K]</td>
<td>kinetin</td>
</tr>
</tbody>
</table>

Introduction

More than a half of a century has passed since the discovery of the first cytokinin (CK). In 1955, after heat-treated DNA fractionation, a small molecule was identified by Skoog and Miller [1]. It was an adenine derivative, 6-furfurylaminopurine, later named kinetin (K). Its cell division promoting activity was described in relation to tobacco parenchyma tissue, where in the presence of auxins, kinetin stimulated proliferation in culture. In general cytokinins (CKs) play an important role in cell proliferation and differentiation in plants. They also influence plant growth and development, delay the senescence, control shoot/root balance and increase crop productivity. Molecular mechanism of CKs biosynthesis and signal transduction hasn’t been completely clarified yet, but the progress has been made since the identification of genes encoding key enzymes and proteins. Genetic analysis of mutants provided the most valuable insights into molecular mechanisms of CKs action in plants. This review focuses on the relation between CKs chemical structure and metabolism, with special regards on their molecular mechanism of action.

Cytokinins chemical structure

In terms of chemical structure CKs can be divided into several groups of compounds. Naturally occur in plants purine base adenine derivatives, wherein pentose isoprene aliphatic chain with different chemical modification is attached to an amino group located at C-6 position (Table 1). Examples of such CKs are zeatin (Z), dihydrozeatin (DZ), N°-D²-isopentenyladenine (2iP), 9-β-ribofuranosyl-2iP (IPA) and their various modifications with hydroxyl or methyl substituents. Other hormones with an aromatic, e.g. benzyl substituent, are: N°-benzyladenine (BA) and its hydroxyl derivatives: N°-ortho-hydroxybenzyladenine and N°-meta-hydroxybenzyladenine called o- and m-topolins because they were first isolated from poplar (Populus robusta), and subsequently from anise (Pimpinella anisum). Synthetic chemical analogues of cytokinins are also known, e.g., farnesyl-N°-adenine, N°-geranyladenine and most biologically active N°-furfuryladenine otherwise called kinetin [3,4].

CKs stimulating activity depends mainly on the chemical nature of the substituent at N° position. If it is an aliphatic substituent, the stimulatory activity of the hormone is determined by the chain length, the number and arrangement of the double bonds and further functional hydroxyl or methyl groups. The highest promoting activity have adenine derivatives with the isoprene chain containing double bond in the middle of the molecule and a hydroxyl group bonded to the fourth carbon atom. An example of such active CK is zeatin, which forms two isomeric structures cis and trans. Trans structure is characterized by greater biological activity [5,6].

An additional substituents introduction to the amino group or purine ring adversely affects their stimulating effect. CKs that contain an aromatic (e.g. BA) or heterocyclic (e.g. K) substituent have the highest biological activity if they have only one one-carbon, alkyl bridge adjacent to the N6 nitrogen of adenine amino group. Chemical modifications in this group of compounds, involving the introduction to the amino group,
N-glucosides are usually formed with β-D-ribose, less frequently with β-D-glucose, and occasionally with β-D-xylose with the nitrogen at N6 and N7 position. CKs form ribosides by connecting to ribose, and they are subsequently converted into mononucleotide (called ribotide) after attaching an ortho-phosphoric acid.

These nucleotides are localized close to anticodon and RNA loop side, mRNA poliadenyl chain and rRNA, where they meet the regulatory role as stimulants in the process of translation.

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>The nomenclature of cytokinins and their conjugates</th>
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<tbody>
<tr>
<td>-</td>
<td>H</td>
<td>glucosyl</td>
<td>( N^6-(\Delta^2\text{-isopentenyl})\text{adenine (iP)} )</td>
</tr>
<tr>
<td>-</td>
<td>ribosyl</td>
<td>-</td>
<td>( N^6-(\Delta^2\text{-isopentenyl})\text{adenine (iPR) 9-riboside} )</td>
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<tr>
<td>-</td>
<td>riboside</td>
<td>-</td>
<td>( N^6-(\Delta^2\text{-isopentenyl})\text{adenine (iPRMP) 9-riboside-5'-monophosphate} )</td>
</tr>
<tr>
<td>glucosyl</td>
<td>-</td>
<td>-</td>
<td>( N^6-(\Delta^2\text{-isopentenyl})\text{adenine (iP7G) 7-glucoside} )</td>
</tr>
<tr>
<td>-</td>
<td>H</td>
<td>glcosyl</td>
<td>( \text{trans-zeatin (Z)} )</td>
</tr>
<tr>
<td>-</td>
<td>ribosyl</td>
<td>-</td>
<td>( \text{trans-zeatine 9-riboside (ZR)} )</td>
</tr>
<tr>
<td>-</td>
<td>glucosyl</td>
<td>-</td>
<td>( \text{trans-zeatin 9-glucoside (Z9G)} )</td>
</tr>
<tr>
<td>glucosyl</td>
<td>-</td>
<td>-</td>
<td>( 7 \text{trans-zeatin -glucoside (Z7G)} )</td>
</tr>
<tr>
<td>-</td>
<td>alanyl</td>
<td>-</td>
<td>( 9\text{-alanylo-trans-zeatin – lupinic acid (Z9Ala)} )</td>
</tr>
<tr>
<td>-</td>
<td>ribotide</td>
<td>-</td>
<td>( \text{trans-zeatin 9-riboside-5’-monophosphate (ZRMP)} )</td>
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<tr>
<td>-</td>
<td>H</td>
<td>-</td>
<td>( \text{trans-zeatin O-glucoside (ZOG)} )</td>
</tr>
<tr>
<td>-</td>
<td>ribosyl</td>
<td>-</td>
<td>( \text{trans-zeatin O-glucoside 9-ryboside (ZORG)} )</td>
</tr>
<tr>
<td>-</td>
<td>H</td>
<td>-</td>
<td>( \text{dihydrozeatin (DHZ)} )</td>
</tr>
<tr>
<td>-</td>
<td>ribosyl</td>
<td>-</td>
<td>( \text{dihydrozeatin 9-ryboside (DHRZ)} )</td>
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<tr>
<td>-</td>
<td>glucosyl</td>
<td>-</td>
<td>( \text{dihydrozeatin 9-glucoside (DHZ9G)} )</td>
</tr>
</tbody>
</table>
Cytokinins

Cytokinins are found in procaryote and eucaryote organisms. They were detected in bacteria, vascular plant pathogens and symbionts, animal and plant pathogenic fungi, angiosperm and gymnosperm vascular plants, and also in lower plants: mosses, ferns, horsetails and algae. Among the species of symbiotic bacteria in which CKs are found, *Rhizobium japonicum* that synthesizes zeatin (Z) and *Paenibacillus polymyxa* that

<table>
<thead>
<tr>
<th>Glucosyl</th>
<th>Alanyl</th>
<th>9-Alanylo-Dihydrozeatin (Dihydrolupinic Acid) (DHZ9Ala)</th>
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<tr>
<td>Ribotide</td>
<td></td>
<td>Dihydrozeatin 9-Riboside-5'-Monophosphate (DHZRMP)</td>
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<td></td>
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<td></td>
<td>H</td>
<td>Dihydrozeatin O-Glucoside (DHZOG)</td>
</tr>
<tr>
<td></td>
<td>Ribosyl</td>
<td>Dihydrozeatin O-Glucoside 9-Riboside (DHZROG)</td>
</tr>
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<tr>
<td></td>
<td></td>
<td>N6-Benzyladenine (BA)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>N6-Benzyladenine 9-Riboside (BA9R)</td>
</tr>
<tr>
<td></td>
<td>Ribosyl</td>
<td>N6-Benzyladenine 9-Glucoside (BA9G)</td>
</tr>
<tr>
<td>Glucosyl</td>
<td></td>
<td>N6-Benzyladenine 7-Glucoside (BA7G)</td>
</tr>
</tbody>
</table>

Table 2. Chemical structure and nomenclature of glycosidic conjugates of naturally occurring adenine derivatives of cytokinins, containing two sugar groups: β-D-glucopyranosyl and β-D-ribofuranosyl (according to 10).

Cytokinins in the form of free bases and ribosides conjugates have the highest biological activity. O-Glucosides with glucose or xylose, and phosphoric or acetic acid esters with an aliphatic chain hydroxyl group localized at the N6 nitrogen of zeatin and dihydrozeatin amino group are very scarce. The alanine conjugate with N9 nitrogen in CK structure was also described. It was named lupinic acid, because it was detected in the young stems and roots of lupine (*Lupinus sp.*) and it does not exhibit any stimulatory activity [7,11-13].

The next group of compounds with CK properties are urea derivatives with phenyl or unsaturated, heterocyclic substituents, eg.: piridine, thiadiazole and their chemical modifications with hydroxyl, chloride, methyl, methoxyl and methylenedioxy group (Table 3).

One of the first, urea-derived CKs with established stimulating activity was N,N'-diphenylurea (DPU). Several compounds from this group are currently known, eg.: thidiazuron (TDZ), N-phenyl-N-2-chloro-4-pirydylurea (CPPU), N-2,3- or N-3,4-di-methoxyphenyl-N'-phenylurea and N-2,3- or N-3,4-methylenedioxyphenyl-N'-phenylurea [7,14].

Urea- and adenine-derived CKs have in their structure similar domain, which determines their biological activity. In purine type cytokinins this is a part of the adenine ring: –N=CH-NH-domain, corresponding to the position 7, 8 and 9, whereas in urea type cytokinins this is –HN-CO-NH-domain [15,16].

CK-like properties have also unsaturated fatty acid derivatives, such as traumatic acid (trans-2-dodecenedioic acid), its aldehyde derivative – traumatin (2-dodeceno-1-al-10-carboxylic acid), and allantoin, which is purine partial degradation product (Figure 1) [4,17]. Considerably weaker activity exhibit other purine bases derivatives e.g. guanine and hypoxanthine with the hydroxyl group substituents in the C-6 position. Similar level of activity show pyrimidine-, especially cytosine – derivatives with the amino group or the hydroxyl group substituents that are localized spatially at the same carbon atom like in purine bases [4].

The occurrence of cytokinins

Cytokinins are found in procaryote and eucaryote organisms. They were detected in bacteria, vascular plant pathogens and symbionts, animal and plant pathogenic fungi, angiosperm and gymnosperm vascular plants, and also in lower plants: mosses, ferns, horsetails and algae. Among the species of symbiotic bacteria in which CKs are found, *Rhizobium japonicum* that synthesizes zeatin (Z) and *Paenibacillus polymyxa* that
Table 3. Chemical structure of cytokinin urea derivatives (according to 2).

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Cytokinin nomenclature</th>
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<tbody>
<tr>
<td>Urea</td>
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<tr>
<td>N,N'-diphenylurea</td>
<td>Thidiazuron (TDZ)</td>
</tr>
<tr>
<td>N-phenyl-N'-1,2,3-thiazol-5-yl-urea</td>
<td>CPPU</td>
</tr>
<tr>
<td>N-(2,3-methylenedioxyphenyl)-N'-urea</td>
<td>N-(3,4-methylenedioxyphenyl)-N'-urea</td>
</tr>
<tr>
<td>N-(2,3-dimethoxyphenyl)-N'-urea</td>
<td>N-(3,4-dimethoxyphenyl)-N'-urea</td>
</tr>
</tbody>
</table>

Figure 1. The chemical structure of a) traumatic acid and b) traumatin

*bacterium tumefaciens, Pseudomonas savastanol* [22]. CKs production in the soil occurs in the rhizosphere area and in places where exist a large amount of substrates and microorganisms. High levels of CKs were observed in the soil where grow plants which are in symbiosis with microorganisms. Herbicides have also an impact on the metabolism of plant hormones, because they affect the entire plant - microorganism interaction system. The best-known species of fungi from which hormones from the group of CKs have been isolated, include: *Amanita rubescens, Boletus edulis, Dictyostelium discoideum, Exobasidium myrtilli E. uva-ursi, Plasmodiophora brassicae, Taphrina amen torum, T. betulina, T. cerasi and T. deformans* [23].

CKs are also present in the yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, especially prenylated adenine derivatives [24]. They occur in many fungi species: vascular plant pathogens and animal pathogens. They can penetrate into the plant from the pathogenic organisms and cause an excessive thickening of the tissue and galls formation [25,26]. They have been identified in all species of vascular plants – both gymnosperms and angiosperms. First of all, they are detected in places where the most intensive biosynthesis occurs, i.e. in meristem of roots and aboveground shoots. They are also present in xylem and phloem because these are plant transport pathways. Since they intensively stimulate cell division, they are also present in large quantities in the flower buds, stamens, pistils, germinating seeds, pollen, intensively growing fruits, tubers, rhizomes, bulbs, young leaves and buds, and root apical meristem [27].

In higher plants Z and its derivatives are very often detected, whereas BA and its derivatives are less common. Zeatin occurs as two isomers: cis- and *trans*- zeatin. Cis –zeatin is considered to be less biologically active, whereas trans-zeatin is rarer in plant kingdom [28]. Kinetin, initially regarded as only synthetic CK, was identified in coconut fruit extracts [29]. Beyond naturally occurring cytokinins which are adenine derivatives, similar properties have also urea derivatives and compounds of a completely different chemical structure such as traumatic acid [30]. Traumatic acid, which is synthesized from unsatu-
Cytokinins biosynthesis and metabolism

CKs biosynthesis occurs primarily in meristem, especially in the apical meristems of the root system, callus tissue and intensively growing, young fruit and seeds. According to current data, there are two CK biosynthesis pathways: de novo biosynthesis and tRNA degradation. The second pathway involves mainly releasing of the adenine-type CKs during ribonucleic acids degradation, primarily tRNA, which contains the highest amount of such compounds. In this manner synthesized cis-zeatin could be converted into the active form - trans-zeatin under the influence of the enzyme cis-trans-isomerase. However, tRNA turnover is too slow to provide an adequate amount of CKs in the cell through ribonucleic acids degradation. This pathway does not have sufficient experimental evidence to prove its existence [2,7]. Nevertheless, since tRNA-degradation is a source of cis-zeatin type CKs, it should not be completely neglected because some of plant species such as maize or rice contain substantial amounts of cis-zeatin type CKs [34].

Biosynthesis and metabolic processes are particularly well known in the case of isoprenoid CKs, since they are widely distributed among higher plants and are detected more often than aromatic CKs (Figure 2). Aromatic CKs biosynthesis has not been completely clarified yet despite the fact that they are found in higher plants, mosses and unicellular algae [35]. Among natural, isoprenoid CKs dominate 4 basic molecules: N6-(Δ2-isopentenyl)adenine (iP), trans-zeatin (tZ), cis-zeatin (cZ) and dihydrozeatin (DZ). These CKs differ in side chain structure, mainly presence or absence of hydroxyl group at the end of isoprenoid chain. However at present there is no confirmed explanation for physiological significance of these various structural options, differences in their activity and stability in vivo were detected. The first step in isoprenoid CKs biosynthesis is catalyzed by the adenosine phosphate-isopentenyltransferase (IPT; EC 2.5.1.27), which catalyzes reaction of adenosine 5’-phosphate (AMP, ADP or ATP) with dimethylallyl diphosphate (DMAPP) or with 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBDP) at the N6 terminal of the mononucleotide amino group. IPTs were identified in many plant species, in higher plants and in some phytopathogenic organisms such as Agrobacterium tumefaciens and Rhodococcus fascians [36,37]. As a result of the reaction iP nucleotides arise, especially iP 5’triphosphate in the form of ribose (iPRTP) and iP 5’-diphosphate riboside (iPRDP), and as an acceptor - ATP and ADP, less frequently AMP, are preferred [36,38]. DMAPP – a donor of aliphatic chain in IPT catalyzed reaction, may derived from a methylerythritol phosphate (MEP) transformations that occur in plastids or from mevalonate (MVA) transformation that occur in cytosol. tZ and iP aliphatic chains derive primarily from MEP metabolism, and that fact suggests a significant role of plastids in the first step in CKs biosynthesis. However exist confirmed data considering a considerable participation of MVA in CKs biosynthesis [39-41].

The other pathway of CKs biosynthesis is tRNA prenylation, catalyzed by tRNA-isopentenyltransferase (tRNA-IPT; EC 2.5.1.8). Prenylation involves covalent binding of prenyl groups, namely linear isoprene derivatives which are mevalonate pathway products. Certain tRNA radicals undergo prenylation at the adenine that is localized at the 3’-end of anticodon. Prenylated tRNA has cis-hydroxyl group, therefore its degradation generates cZ. tRNA-IPT is widespread among many organisms: bacteria, plants and animals [35]. iP nucleotides that are produced in plant cells under the influence of IPT undergo hydroxylation in prenylated part of the side chain, what leads to the formation of tZ nucleotides. In Arabidopsis this reaction is catalyzed by two P450 cytochrome monoxygenases - CYP735A1 and CYP735A2 [42]. Both CY-
P735A1 and CYP735A2 preferentially catalyze iPRMP and iP-RDP reaction, rather than iPRTP reaction [35].

To become biologically active compounds, CKs in the form of nucleotides have to be converted into the form of free bases. The enzyme was described that activates CKs through their conversion into free bases. LOG – phosphoribohydrolase was identified in rice [43]. It has phosphoribohydrolase activity and releases ribose-5'-monophosphate moiety from cytokinin nucleosides that are 5'-monophosphorylated. All of the four 5'-monophosphate nucleosides: iPRMP, tZRMP, dZRMP and cZRMP undergo reaction catalyzed by LOG, but di- and tri-phosphates, AMP and nucleosides aren’t appropriate LOG substrates. Enzyme name comes from the mutant phenotype, in which shoot meristem is defective and flowers often contain only one rod and no pillars, therefore name comes from words: lonely guy – LOG. This phenotype clearly indicates the importance of LOG dependent CK activation pathway in rice shoot apical meristems. Genes sequence similar to LOG were detected in many species, even in organisms that do not produce CKs, however their function is still not known [43]. Conversion of CK nucleotides into free bases involves two-step enzymatic reaction catalyzed by nucleotidase and nucleosidase. Enzymes with such activity have been identified in wheat germ, but genes encoding these enzymes haven’t been isolated so far. On the other hand, CKs in the form of nucleosides are presently detected in plants. Study made with isotopes revealed that exogenously administered CKs in the form of bases are converted into nucleosides and nucleotides, what indicates that nucleosides may come from CK bases, but this is still not confirmed [35,44].

CKs within plant cells undergo various transformations and biochemical modifications. As a result of this transformations biologically inactive forms of CKs with transport or backup function may be produced, mainly in order to increase their pool. A backup pool can be used to adjust the various phases of growth and development of the whole plants, individual tissues and even single cells. Metabolic changes in this group of compounds usually concern conversion between forms of bases, and nucleotide and nucleoside conjugates, but they may also relate to purine structure and its aliphatic chain [2,7]. The example of such transformation may be an enzymatic conversion mainly dephosphorylation of CK ribotides into ribosides, which become free CK bases after ribose detachment (Figure 3). The excess of biologically active forms CKs in the cell is inactivated in two ways: by degradation or conjugation to equal, low molecular weight metabolites, such as sugars or amino acids.

Currently, the only plant enzyme known as a capable of catalyzing the CKs degradation to inactive products without N6 side chain is cytokinin oxidase (OC). The preferred substrates for this enzyme are N6-(Δ2-isopentenyl)-adenosine, and zeatin.

However enzymes from different sources show higher specificity for N6-(Δ2-isopentenyl)-adenosine as compared to zeatin. The presence and localization of double bond in isoprenoid chain seems to be important for enzyme activity. Therefore dihydrozeatin is resistant for OC activity in vitro and it is a main CK that is detected in plant tissues in which high OC activity is observed. Side chain O-glucosylation also protects CKs from oxydases attack in vitro. On the other hand N-glucosylation or other ring substitution may cause a decrease in OC affinity to CKs, but it need not completely eliminate the substrate activity [45]. Nucleotides cannot be substrates for OC. Plant OC have little or no activity against N6-benzyladenine or kinetin in vitro and these CKs are degraded very slowly in vivo [46]. OC activity can be induced by a transient increase in concentration of exogenous CKs added to culture medium. In vivo CKs degradation is also under the influence of other hormones eg: abscisic acid suppresses the kinetin degradation (in lettuce seedlings) and zeatin to dihydrozeatin conversion [47]. The available data indicate that CKs degradation varies in different tissues, and even within the same tissue during development, which may mean that OC probably plays a significant role in the control of the CKs content during plant development [48].

The second manner of phytohormones deactivation is conjugation. A common feature of the plant hormones is that they are often found in cells as conjugates with various compounds mainly from the group of sugars and amino acids. Such conjugates have usually poor physiological activity, which often correlates with the degree of their hydrolysis in plant tissues.
Conjugation is a common way to remove free, active hormones from the tissue. However this process is reversible. Even though conjugates can be accumulated in excess relatively to free phytohormones, the conjugates pool must be considered as a source of free hormones and may represent the form in which they are stored and transported. CKs are detected as N-glucosides and O-glucosides [49,50]. Except for glucosylated forms of CKs, also conjugates with amino acids were identified [51].

The biological activity of cytokinins and their molecular mechanism of action in plant cells

CKs are group of compounds that stimulate anabolic, biochemical and physiological processes. In plants they influence mainly metabolic processes connected with nucleic acids and proteins transformations and their activity is significantly higher that other hormones such as auxins and giberelins. They enhance the intensity of cell division of bacteria, fungi, algae and higher plants and delay the processes of aging. CKs change the expression of genes associated with plant growth and development, especially with the cyto-, histo-, morpho- and organogenesis. They activate the processes such as: seed germination, weakness of apical dominance in main shoots, flower buds induction, stimulation of growth and regeneration of parenchymal tissue, vascular tissue and cuticula. They also play an important role in the extension of the vegetation period, the increase of the number of micropores and improve the quality of the active transport across the membranes. The activity of the enzymes connected with membrane transport is also higher. Due to an easy transport, the number of organic metabolites and mineral components in the cell increases. These compounds meet the building functions or are involved in metabolism. Cell membranes biopotential is increased [2,57-59].

Identification of the CKs mechanism of action in plant cells took a long time due to their diverse biochemical and metabolic responses and the interactions with other phytohormones [9,60].

Study on the transformed Arabidopsis plants allowed for the isolation of a protein CKI1 (Cytokinin Independent 1). Mutants with CKI1 overexpression, despite the low level of endogenous CKs, undergo rapid cell divisions, produce sprouts, are green and have inhibited root growth, which means that they show a response typical for CKs [61-63]. CKI1 protein has a molecular weight 125 kDa, and is a polipeptide chain that consists of 1122 amino acids and its amino acids sequence resembles a two-component model of signal transduction that occurs in bacteria. It consists of a receptor and a response regulator. Receptor domain is N-terminal domain which is a fragment consists of 300 amino acids and 11 potential N-glycosylation sites located outside the CKI1 protein membrane loop between two transmembrane domains. Response regulator domain and histidine - aspartate kinase domain (C-terminal part) are localized in the intracellular fragment of the molecule. After CK molecule attachment to the receptor domain, receptor dimerization and autophosphorylation occur. Phosphate group is transferred to the histidine residues of the regulatory protein (phosphorylation site His 450), followed by its activation. The next step is phosphate group transfer to aspartate residues in response regulator domain (Asp 1050), which after phosphorylation activates probably MAP kinase cascade or other signal transduction proteins. The last stage is induction of specific genes expression. Study confirmed that CKI1 protein induces ARR family genes expression. These are genes of early CK response [60,64-66].

Inoue H et al, and Suzuki T et al identified in Arabidopsis a
single mutant line cre1 (cytokinin resistant) that has less sensitivity to exogenous CK addition. CRE1 gene encodes hybrid histidine kinase CRE1, which shows similarity to CKI1 protein. In the structure of CRE1 protein two C-terminal response regulator domains, two catalytic histidine kinase domains, and two transmembrane domains that flanking the extracellular N-terminal domain were identified. Study results revealed that CRE1 may serve as a signal transduction receptor and generator. Signal appears in the cell after the perception of cytokinin [67-69]. CRE1 protein function was described due to the study on the Arabidopsis thaliana wol mutants. Wol mutants (wol-wooden leg) have phenotype with the vascular tissue defect in roots and lower parts of the hypocotyl. The defect is caused by disturbances in the division and differentiation of phloem and xylem progenitor cells. These plants develop into fertile plants through the rescue of the short root phenotype by the development of adventitious roots [70]. According to the literature even CK triple receptor KO mutants develop some real leaves [71]. The negative effect of the wol mutation (it phenocopies the primary root phenotype of CK triple receptor KO, whereas CRE1 Kos do not do that) is due to the constitutively active phosphatase function of wol onto the CK signal transduction cascade [72]. Test results revealed that mutation in WOL gene causes a replacement of one amino acid in the extracellular domain of the CRE1 protein, that is, the probable site of an attachment of the ligand and the CK with receptor binding is impossible. Therefore Arabidopsis wol mutants do not show any reaction for exogenous Cks. However CRE1/WOL gene expression is localized in root and germ, and is limited to the progenitor cells mainly in hypocotyl procambium. While in the aerial parts of the plant CRE1 protein plays an unknown role or even can be superfluous [73,74].

Histidine kinases AHK (Arabidopsis His Kinase) were detected in aerial parts of a plant and in roots. It turned out that they are the predominant receptors in prokaryote, which initiate a signal cascade in which the phosphate groups are transferred between histidine and aspartate residues to activate or inhibit suitably bonded response regulators RRs. Understanding the complete Arabidopsis genome resulted in the identification of all potential components of the signal path. There are eight transmembrane AHK, six phosphor-transfer proteins containing HPTs histidine (Histidine Phosphotransfer Proteins) and more than 20 RRs [75,76]. HPTs proteins constitute a bridge carrying a phosphate group between histidine residues present in the receptor (recipient) hybrid kinase domain and asparagine residues in the domain of the response regulator protein. Their function is therefore signal transduction from the place of its perception through located in the cell membrane receptors into the nucleus, where expression of certain genes occurs [9]. In contrast, response regulator proteins are synthesized and activated by CK binding to the corresponding receptor. RRs proteins consist of 122-690 amino acids and constitute a distinct family of proteins, and the expression of their genes takes place mainly in the area of root and shoot meristem. They contain conservative, characteristic moieties: asparagine and lysine and can be divided into two groups: RRs type A and RRs type B. Type A RRs have receiver domain and short N- and C-terminal regions and are synthetized in plant cells directly and very soon after the introduction of exogenous Cks, which indicates the fact that these are early CK response genes. Type B RRs have also extracellular receiver domain and long C-terminal region structured of 80 amino acids within which: response domain, DNA binding domain and transcriptional activation domain can be distinguished. Encoding genes however are not the early CK response genes as within 10 minutes after exogenous hormones administration increased levels of these proteins in the plant cells could not be detected. Between two RRs protein groups exists close biochemical correlation because type B RRs proteins play a crucial role in type A RRs proteins biosynthesis regulation, which depends on Cks [77-79].

As a result of conducted study, a model was created that distinguishes four main stages of CK transduction signal from the cell membrane to the cell nucleus: 1) CK molecule attachment and the signal initiation through AHK receptors, 2) phosphate group transfer to HPTs proteins and their nuclear translocation, 3) phosphotransfer to the nuclear type B RRs, that activate transcription, 4) negative feedback activated by type A RRs which are products of the CK early response genes (Figure 4) [61,80]. Not all of the eight AHK identified in Arabidopsis are CK receptors.

![Figure 4](image_url)  
**Figure 4.** Multistep model of cytokinin signal transduction. H - histidine, D - aspartate, HK - histidine kinase receptor, HPT - phosphotransfer proteins, N - asparagine (according to 80)

For example, two AHK encode ethylene receptors, one AHK is probably the osmosensor, and another has histidine kinase activity with overexpression. Their detailed function with re-
spect to the CK signaling pathway has remained unclear [81]. It appeared also that in addition to the kinases role, CK receptors exhibit phosphatase activity that removes phosphate groups from HP proteins when there is no CK bounded to the receptor. AHK phosphatase activity allows for rapid and effective inactivation of CK pathway in the absence of CKs [72]. One of the six proteins HPTs - AHP6 was identified as "pseudo-HPT" due to a mutation in the conserved histidine residue required for the attachment of a phosphate group derived from the receptors. AHP6 inhibits CK signaling pathway probably through competition with other AHP at the stage of interaction with the active receptor or RRs. CK signal in response suppresses AHP6 transcription. Absence of AHP6 function causes a displacement of CK signal, leading to defects in the vascular tissue. Therefore the presence of AHP6 may limit the number of CK-responsive cells and plays an important role in cell differentiation limitation [72].

In plants, not only the feedback is used to control CK signaling pathway, but also other factors influence the course of the above described processes. WUSCHEL transcription factor required for the function of shoot stem cells directly impairs the transcription of certain type A RRs genes, which typically stimulate the activity of CK signaling pathway in the shoot stem cells and increase their size [82]. Next group of transcription factors involved in CK signal transduction pathway was detected. Research on subgroup of factors of the response to CKs (CRFs Cytokinin responsive factors) revealed their CK dependent nuclear translocation. CRFs and RRs type B affect certain genes associated with the CK pathway, but do not control the most important genes RRs type A. Phenotype of crf mutants seems to have the characteristics of dependence and independence of CKs [83]. Research was conducted for establishing whether there is a common element in these seemingly different responses to CKs. Exogenously administered CKs cause an enhancement in cell proliferation and shoot growth in cell culture. An important mediator in these processes appears to be the regulator of the cell cycle - cyclin D3. Consequently triple mutants in CK receptors genes, and triple mutants in genes encoding type B RRs have inhibited the growth of shoots and roots [76]. Although plants with the partial reduction of the endogenous CKs level or with changes in CK signal transduction show an increase in the size of the root system. Detailed analysis of CKs functions in the root confirmed their role in cell differentiation promoting and proliferation counteracting. The explanation for these contradictory results, which is based on the CKs signaling activity may be the fact that plants with a strong reduction in the CKs perception and CK signal transduction have weakened vascular system, what may limit the potential growth. It is also possible that a different CK concentration threshold is essential for the cell proliferation, differentiation and elongation [76,84,85].

Conclusions

Future studies that establish the relation between CKs biosynthesis, degradation, import and export in plant systems are needed. Our knowledge regards CKs molecular mechanisms of action and early stages of signal transduction has increased to great extent but still some important questions remain unsolved. The regulatory mechanisms of the enzymes that are involved in CKs biosynthesis, conversion and degradation must be also uncovered.

References


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