This article should be cited as follows:

Schmülling, T. (2004) Cytokinin.
In Encyclopedia of Biological Chemistry (Eds. Lennarz, W., Lane, M.D.)
Academic Press/Elsevier Science.

Cytokinin

Thomas Schmülling

Free University of Berlin

Cytokinins are plant-specific chemical messengers (hormones) that play a central role in the regulation of the plant cell cycle and numerous developmental processes. Cytokinins were discovered by F. Skoog, C. Miller and co-workers during the 1950s as factors that promote cell division (cytokinesis). The first cytokinin discovered was an adenine (aminopurine) derivative named kinetin (6-furfuryl-aminopurine; Fig. 1), which was isolated as a DNA degradation product. The first common natural cytokinin identified was purified from immature maize kernels and named zeatin (chemical name: 6-(4-hydroxy-3-methylbut-2-enylamino)purine; Fig. 1). Several other cytokinins with related structures are known today. Cytokinins are present in all plant tissues. They are abundant in the root tip, the shoot apex and immature seeds. Their endogenous concentration is in the low nM range. Typically, several types of cytokinins and their modified forms are present in a given tissue. Cytokinins can act over long distances or in the direct vicinity of the cytokinin producing cells (paracrine signalling). Cytokinins may act also on the cell that produced them (autocrine signalling). Cytokinins are also produced by cyanobacteria, some plant pathogenic bacteria (e.g. *Agrobacterium tumefaciens*, *Pseudomonas savastanoi*, *Rhodococcus fascians*) and the slime-mold *Dictyostelium discoideum.*

I. Cytokinin Structures

Naturally occuring cytokinins are adenine derivatives with a side chain at the N^6-position (Fig. 1). The structure and conformation of the N^6-attached side chain can markedly influence the biological activity of the cytokinin. Depending on the structure of the N^6-substituent, cytokinins are classified as isoprenoid or aromatic cytokinins. The biological activities of both classes are qualitatively similar but they may differ quantitatively in different processes. Isoprenoid cytokinins are the most abundant class. They are either isopentenyl (iP)-type cytokinins, having an isopentenyl N^6-side chain, or zeatin-type cytokinins, having a hydroxylated isopentenyl N^6-side chain. The side chain of a zeatin-type cytokinin occurs in either *cis* or *trans* configuration, depending on which of the two methyl groups is hydroxylated. The *cis* form is usually much less active. Reduction of the double bond in the side chain leads to dihydrozeatin. Aromatic cytokinins have an aromatic benzyl group at N^6. They occur more rarely and much less is known about them. Because of their greater stability aromatic cytokinins are often used in tissue culture, an example is benzyladenine. In addition,

there are the structurally unrelated phenylurea-type cytokinins (e.g. diphenylurea, thidiazuron), a class of synthetic cytokinins. These cytokinins are highly active but do not occur naturally.

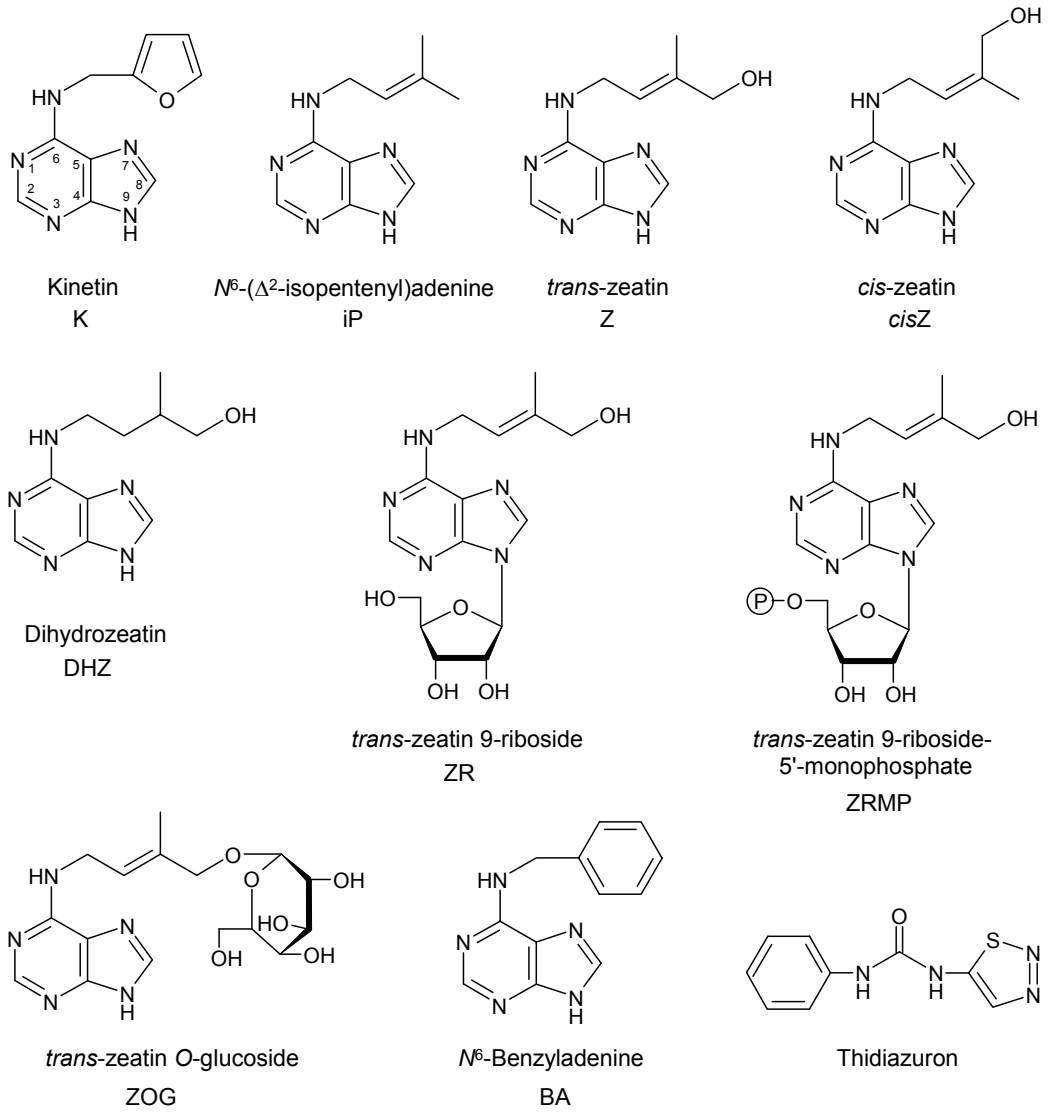

Figure 1. Chemical structures of some naturally occurring and synthetic cytokinins. Common names and abbreviations are indicated below the structures. The numbering of purine ring atoms is shown in for kinetin.

II. Cytokinin Biosynthesis and Metabolism

The rate of *de novo* synthesis, metabolic interconversion and breakdown are, together with transport processes, relevant to the regulation of cytokinin homeostasis in cells. Cytokinin metabolism includes mainly conversions among cytokinin bases, ribosides, ribotides, side chain modification, conjugation and conjugate-hydrolysing reactions and cytokinin degradation.

A. Biosynthesis

The initial and rate-limiting step of biosynthesis of isoprenoid-type cytokinins is the transfer of the isopentenyl moiety from dimethylallyl pyrophosphate

(DMAPP) to AMP, ADP or ATP. The reaction is catalyzed by DMAPP::AMP/ADP/ATP isopentenyltransferases (IPT). ADP and ATP are the preferred substrates of most of the known plant IPT enzymes, while bacterial enzymes prefer AMP. The reaction leads to the formation of isopentenyl-AMP, -ADP and -ATP, which are the precursor molecules of biologically active cytokinins. The isopentenyl side chain is subsequently hydroxylated to form zeatin-type cytokinins (Fig. 1). An alternative pathway, in which an already hydroxylated side chain is directly added to the N^6-position of the adenine moiety may exist. IPT enzymes are encoded in *Arabidopsis* by a small gene family with seven members (*AtIPT1*, *AtIPT3*-*AtIPT8*). *AtIPT* genes are expressed in specific tissues of the root and shoot (e.g. vasculature), indicating that cytokinin synthesis occurs in all major organs. Another possible source of cytokinins is tRNA, since tRNAs of most organism contain isopentenylated adenine and other structural derivatives with cytokinin activity. However, it is generally assumed that tRNAs play only a minor role, if any, as a cytokinin source.

B. Interconversion

A characteristic feature of cytokinin metabolism is the rapid metabolic interconversion of base, ribosides and ribotides. The biologically most active form of cytokinins is the base. Attachment of ribose or ribose-5′-phosphate to the N^9 atom of the adenine ring leads to the formation of ribosides and ribotides, respectively, which do have lower activities (Fig. 1). Interconversion of cytokinins is presumably an important mechanism to regulate the concentration of active compounds. Cytokinin ribosides are probably relevant as a transport form. The interconversions may be catalyzed by the same enzymes that metabolize adenine, adenosine and AMP. The conversion between the *cis*- and *trans*-isomers of zeatin is catalyzed by the enzyme *cis-trans* zeatin isomerase. Zeatin is converted to dihydrozeatin by a NADPH-dependent zeatin reductase.

C. Conjugation

Cytokinins can be stably or transiently inactivated by glycosylation of the purine ring or of the side chain. The purine ring can be glycosylated at the N^3-, N^7- and N^9-position. In addition, the N^6-side chain group can form *O*-glycosyl conjugates if it bears a hydroxyl-group. Most often, glucose is the conjugated sugar molecule, more rarely xylose is attached. N^7- and N^9-conjugates are biologically inactive and extremely stable. Thus they are irreversibly inactivated cytokinins. N^3-and *O*-conjugates are biologically inactive but can be readily hydrolysed. They are believed to be transient storage forms of cytokinins. Glycosyl conjugation is considered to be important in the regulation of cytokinin activity levels, at least in some tissues and species. Several genes coding for cytokinin glycosyltransferases and glycosidases have been identified. Some conjugates of cytokinins and amino acids (alanine) have been described as well.

D. Catabolism

Cytokinins are irreversibly degraded in a single enzymatic step by oxidative cleavage of the N^6-side chain. The reaction is catalyzed by cytokinin oxidases/dehydrogenases (CKX), which contain FAD as a cofactor. The reaction products are adenine and an aldehyde. The preferred substrates of CKX are isopentenyladenine, zeatin and their corresponding ribosides. Ribotides, *O*-glucosides, dihydrozeatin and aromatic cytokinins are not degraded by CKX. The *Arabidopsis* genome contains seven *AtCKX* genes, which are preferentially expressed in zones of active cell division and growth. The corresponding enzymes are located in the endoplasmatic reticulum, in the apoplast and in the vacuole.

III. Cytokinin transport

Cytokinins are transported from roots to shoots in the xylem, and in the opposite direction in the phloem. Transported cytokinins may have a role in coordinating root and shoot development, for example by carrying information about nutrient availability. Multiple cellular importers and exporters are required to allow efficient mobilization and targeted translocation of cytokinins, but very little is known about cytokinin transporters. Transport studies indicate that a common H^+-coupled high-affinity purine transport system transports cytokinins.

IV. Cytokinin Signalling

The mechanism of cytokinin signalling is just beginning to emerge. The cytokinin signal is perceived and transduced by a multi-step phosphorelay system through a complex form of the two-component system (TCS) pathway. The TCS is common among prokaryotes and lower eukaryotes, among the higher eukaryotes it is unique to plants. In this signalling system, a membrane-located receptor kinase with an extracellular ligand-recognition domain (sensor) dimerizes upon binding a ligand and autophosphorylates a histidine within its cytoplasmic transmitter domain. The phosphoryl group is first transferred to an aspartate residue within the receiver domain at the C terminus of the receptor and from there to a His-containing phosphotransmitter (Hpt), which ultimately phosphorylates and thus activates a response regulator (RR) at a central Asp residue.

A. Signal Perception

Cytokinin receptors are histidine kinases consisting of an extracellular sensing domain, a cytoplasmic histidine kinase transmitter and receiver domains. Three cytokinin receptors (CRE1/WOL/AHK4, AHK2, AHK3) have been identified in *Arabidopsis*. They all share a ~270 amino acid long extracellular cyclases/histidine kinases associated sensing extracellular (CHASE) domain, which presumably recognizes cytokinin. This domain might have been acquired by plants through lateral gene transfer from cyanobacteria. Loss-of-function mutants of CRE1/WOL/AHK4 lack the phloem in their primary roots, indicating a role for cytokinins in embryo development.

B. Signal Transduction

Current knowledge suggests that downstream signalling components of the cytokinin signa-transduction pathway in *Arabidopsis* consist of five Hpt and 22 response regulators of the A- or B-type. Hpts transmit the signal from the receptor, which is presumably localized in the plasma membrane, to type-B RRs, which are in the nucleus. B-type RRs consist of an N-terminal receiver domain and a C-terminal output domain, containing a DNA recognition motif called GARP, which is distantly related to the Myb repeat. The DNA motif optimal for binding is 5´-(A/G)GAT(T/C)-3´. Activated type B ARRs transcribe primary response genes of cytokinins. Some of the known primary response genes, which are rapidly and specifically upregulated by cytokinins, code for type A response regulators. Type A ARRs resemble type B ARRs but lack the C-terminal DNA binding and activation domain. Type A ARRs fulfil at least two different functions. On the one hand they exert a negative feedback regulation of the cytokinin signalling pathway through protein-protein interaction. On the other hand they mediate the cytokinin-dependent modulation of other pathways, e.g. light signalling. Type-A response regulators can be positive or negative regulators, depending on the individual response regulator and the output reaction analysed.

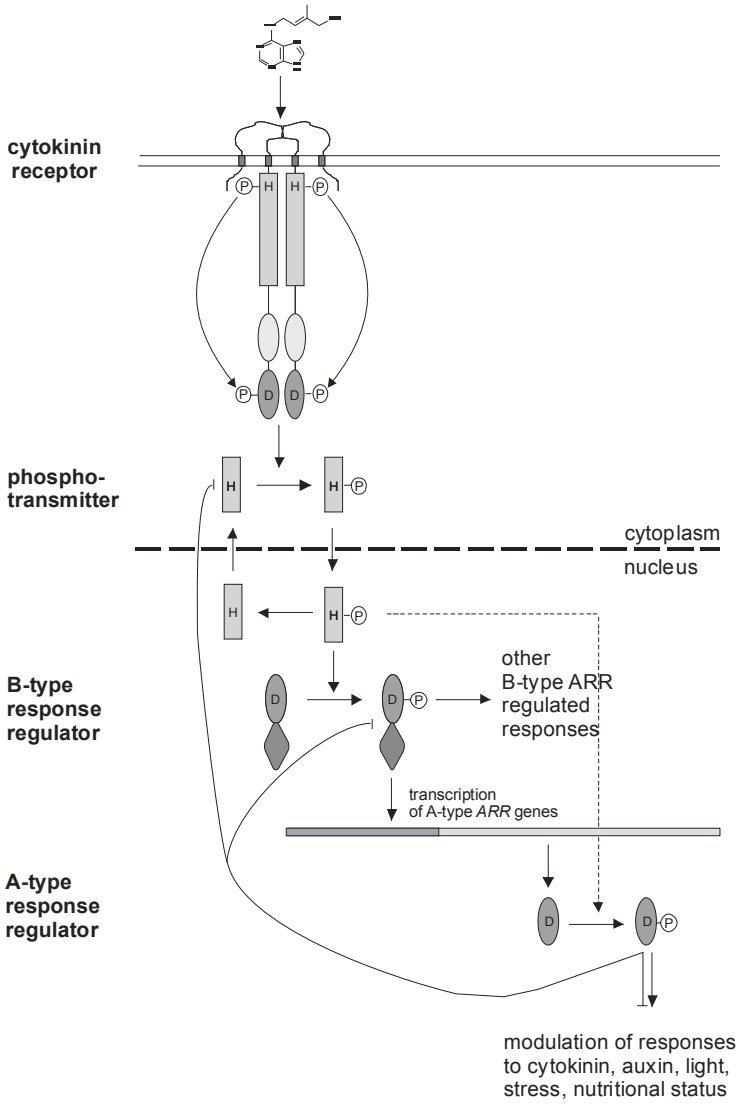

Figure 2. A model for cytokinin signal transduction via a His-to-Asp phosphorelay. The structure of CRE1/WOL/AHK4 is shown as an example. Ligand binding induces receptor dimerization and autophosphorylation. Transfer of the phosphoryl group by activated receptors activates histidine phosphotransmitter proteins (Hpts) which transport the signal from the cytoplasm to type-B ARRs in the nucleus. Type-B response regulators transcribe target genes, among them type-A *ARR* genes. Type-A response regulators may downregulate the primary cytokinin signal response via a negative feedback loop, modulate downstream activities of cytokinins in a positive or negative fashion or modulate other signalling pathways through protein-protein interaction. A more complex regulation than shown in the model may exist. Abbreviations: D, aspartate residue, H, histidine residue, P, phosphoryl group.

V. Cytokinin Functions

A. Cell cycle

Cytokinins are required for cell division during embryogenesis, in the shoot apical meristem, young leaves, the cambium and cultured plant cells. In contrast, they have a negative regulatory role in the root meristem. Cytokinin restricts root growth as it controls the exit of dividing cells from the meristem. Changes in cytokinin levels occur during the cell cycle of cultured cells, the level being highest during the late S and during the M phase. Cytokinins have been functionally linked to all stages of the cell cycle but their mechanism of action has only been partially elucidated. Cytokinin up-regulates expression of the D-type cyclin gene *CycD3*, which is

important in regulating the G1/S-transition of the cell cycle. Cytokinin increases the number of replication origins during S-phase and it may also play a role in regulating G2/M transition.

B. Plant Development and Growth

Cytokinin participates in regulating numerous aspects of plant development throughout the life cycle. These include seed germination, cotyledon expansion, chloroplast differentiation, de-etiolation, differentiation of vascular tissue, apical dominance (shoot branching), root elongation and branching, nutritional signalling, regulation of sink strength, the transition from the vegetative to the reproductive growth phase, flower and fruit development, leaf senescence, and plant-pathogen interactions. A role for the hormone in vascular morphogenesis during embryonic development is firmly established. During post-embryonic development cytokinins are required to maintain meristem activity and leaf development in the plant shoot. Local exogenous cytokinin application to the shoot leads to premature growth of lateral buds, retarded leaf senescence, partial photomorphogenesis in the dark, increased sink strength and an altered vasculature. In contrast to their stimulatory activities in the shoot, cytokinins have a negative regulatory role in the control of root elongation and branching. Additionally, cytokinin regulates important physiological parameters that determine biomass formation and distribution via central genes of primary metabolite pathways, including invertases, hexose transporters and key genes of phosphate and nitrogen metabolism and signalling (e.g. nitrate reductase). Changes in cytokinin levels are generally positively correlated with levels of mineral nutrients, especially nitrogenous nutrients. Cytokinin levels are decreased by water stress. In vitro, the ratio of cytokinin to auxin determines the differentiation of cultured plant tissues to either shoots or roots. A high cytokinin to auxin ratio promotes shoot formation, a low ratio: root formation. Owing to their stimulatory effect on plant regeneration cytokinins are widely used in plant tissue culture.

C. Pathogenicity

Cytokinins are produced by several plant pathogenic bacteria and play a role in pathogenicity. One such pathogen is *Agrobacterium tumefaciens*, the causative agent of the crown gall disease. During the infection process, *A. tumefaciens* transfers a small stretch of DNA, the T-DNA, to the host plant, where it becomes integrated in the nuclear genome. The T-DNA harbors an *IPT* gene, which is expressed in the host cell and causes cytokinin overproduction. This leads, together with an enhanced auxin content, to tumorous cell proliferation. Other cytokinin-synthesizing pathogens are *Pseudomonas syringae*, which induces gall formation and *Rhodococcus fascians*, which causes fasciation and a growth abnormality called witches´ broom disease. The root-nodule forming and nitrogen-fixing plant symbiont *Rhizobium spec.* is also known to produce cytokinin.

D. Biotechnology

Practical use of cytokinin in agricultural is currently limited. Modulation of the endogenous cytokinin content of plants or interfering with cytokinin signalling has a high potential for biotechnological applications in agriculture. Plants with increased cytokinin content are more branched and senesce later. Moreover, cytokinins alter sink-source relations, a promising approach to improve yield attributes. Plants with reduced cytokinin content develop a larger root system. An improved root system means improved acquisition of minerals and water, factors which are often limiting for plant growth.

Glossary

cytokinin conjugate
compound formed by the union of a cytokinin and a sugar moiety
cell cycle
the sequence of events between mitotic divisions, divided into G1, (G standing for gap), S (synthesis phase), G2 and M (mitosis)
meristem
the growing tip of roots and shoots
senescence
programmed aging leading to organ or plant death
two-component system
signal transduction system of bacteria, lower eukaryotes and plants; involves autophosphorylation of a histidine kinase that transmits the signal via phosphorelay to response regulator proteins

Further Reading

Davies, P.J. (1995) Plant Hormones. Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht.

Heyl, A., Schmülling, T. (2003) Cytokinin Signal Perception and Transduction. *Curr Op Plant Biol*, in press.

Hoykaas, P.J.J., Hall, M.A., Libbenga, K.R. (1999) Biochemistry and Molecular Biology of Plant Hormones. Elsevier, Amsterdam.

Kakimoto, T. (2003) Biosynthesis of Cytokinins. *J Plant Res* **116**, 233-239.

Kakimoto, T. (2003) Perception and Signal Transduction of Cytokinins. *Ann Rev Plant Biol* **54**, 605-627.

Kieber, J.J. (2002) Cytokinins. *In* The Arabidopsis Book. Edited by Somerville C, Meyerowitz E, Rockville, MD, USA, American Society of Plant Biologists. URL: http://www.aspb.org/publications/arabidopsis.

Mok, D.W.S., Mok, MC (1994) Cytokinins. Chemistry, Activity, and Function. CRC Press, Boca Raton.

Mok, D.W.S., Mok, M.C. (2001) Cytokinin Metabolism and Action. *Annu Rev Plant Physiol Plant Mol Biol* **52**, 89-118.

Schmülling, T., Werner, T., Riefler, M., Krupková, E., Bartrina y Manns, I. (2003) Structure and Function of Cytokinin Oxidase/Dehydrogenase Genes of Maize, Rice, *Arabidopsis* and Other Species. *J Plant Res* **116**, 241-252.

Taiz, L., Zeiger, E. (2002) Plant Physiology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Biographical Statement

Thomas Schmülling is a Professor of Genetics and chair of Developmental Biology of Plants at the Free University of Berlin. His principal research field is the biology of cytokinins. He studied biology at the University of Cologne, accomplished his doctoral thesis at the Max Planck-Institute for Plant Breeding and has been Assistant Professor at the University of Tübingen. He has made contributions to the understanding of *Agrobacterium rhizogenes* T-DNA genes and cytokinin metabolism and signalling. His laboratory was the first to generate cytokinin-deficient plants by means of genetic engineering.