PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science

Department of Biochemistry

On the Hunt for Zeatin *cis-trans* Isomerase

DIPLOMA THESIS

Author:	**Bc. Tomáš HLUSKA**
Study program:	N1406 Biochemistry
Study branch:	Biochemistry
Study mode:	Full-time
Advisor:	**Petr Galuszka, Ph.D.**
Submitted:	3. 5. 2010

I hereby declare that this thesis has been written solely by myself and that all the sources used in this thesis are cited and included in the References part.

In Olomouc 2th May 2010 .....

Tomáš HLUSKA

Acknowledgments

I'm thankful to my advisor Dr. Petr Galuszka for his vulnerable advices and lot of time he spent when helping me.

I wished to thank to Mgr. Kateřina Václavíková for her help with UPLC/MS analysis, to proteomic group, namely Jana Klásková, Mgr. Lucie Švehlová and Dr. René Lenobel, for the proteomic analysis of zeatin *cis-trans* isomerase samples and to prof. Šebela for help with polyamine oxidase purification, its identification on MALDI-TOF and for his vulnerable advices.

My thanks also belong to Olga Ryparová for her help with zeatin *cis-trans* isomerase purification and HPLC/UV-VIS analysis.

Bibliografická identifikace:

Jméno a příjmení autora	Tomáš HLUSKA
Název práce	Identifikace hypotetické zeatin *cis-trans* isomerasy
Typ práce	Diplomová
Pracoviště	Katedra biochemie
Vedoucí práce	doc. Mgr. Petr Galuszka, Ph.D.
Rok obhajoby práce	2010
Abstrakt	Cytokininy jsou rostlinné hormony ovlivňující řadu fyziologických procesů. Jejich metabolismus a signální dráha byly v posledních letech intenzivně studovány, ale stále existují neobjasněné procesy zasluhující pozornost. Jedním z nich je existence zeatin *cis-trans* isomerasy (ZI). Ta byla sice dříve purifikována, ale protein nebyl blíže identifikován. Použili jsme dříve popsaný systém pro detekci aktivity ZI a lokalizovali ji v různých pletivech kukuřice, fazole a ovsa. Za použití několika chromatografických kolon jsme purifikovali ZI více než 1000-krát a detekovali přítomné proteiny pomocí LC-MS. Dále jsme se pokusili detekovat další aktivity vzájemně přeměňující cytokininy zeatinového typu.
Klíčová slova	Cytokininy, biosyntéza, *cis-trans* isomerasa, zeatin reduktasa, dihydrozeatin oxidasa, kukuřice, purifikace proteinů, LC-MS analýza
Počet stran	81
Počet příloh	0
Jazyk	Anglický

Bibliographical identification:

Author's first name and surname	Tomáš HLUSKA
Title	On the Hunt for Zeatin *cis-trans* Isomerase
Type of thesis	Diploma
Department	Department of Biochemistry
Supervisor	Dr. Petr Galuszka
The year of presentation	2010
Abstract	Cytokinins are plant hormones involved in many physiological processes. Their metabolism and signaling is just being uncovered in the last decade, but there are still some secrets concerning cytokinin metabolism. One of them is the existence of the zeatin *cis-trans* isomerase (ZI). In the past, it has been purified to near homogeneity, but the protein has not been further characterized. We have employed previously described system for detection of ZI to localize the activity in several tissues of maize, bean and oat plants. Using several chromatographic columns, we have purified the ZI more than 1000-fold and detected proteins by LC-MS in fractions with the highest activity. Other activities interconverting zeatin-type cytokinins have been examined in maize tissues too.
Keywords	cytokinins, biosynthesis, zeatin *cis-trans* isomerase, zeatin reductase, dihydrozeatin oxidase, maize, protein purification, LC-MS analysis
Number of pages	81
Number of appendices	0
Language	English

Aim of work

⇒ review on cytokinins and their metabolism with focus on *cis*-zeatin and its significance and metabolism in plants

⇒ detection of zeatin *cis-trans* isomerase in various plant tissues

⇒ purification and characterization of zeatin *cis-trans* isomerase from maize seedlings

⇒ detection of other enzyme activities interconverting zeatin-type cytokinins

Theoretical part

Plant Hormones

The concept of plant hormones as substances regulating plant growth in small concentrations has been worked out since the end of 19th century (Darwin and Darwin 1880; von Sachs 1887). The first proof of existence of low-molecular substances regulating plant growth was famous Fritz Went's experiment in which he put coleoptile on agar block for a period of time and afterwards put this block on decapitated oat plants, what promoted growth, eventually asymmetrical, if the block was put aside (Went 1928). Many substances from diverse sources have been examined for plant growth-promoting activity in the same manner. The original substance was finally identified as indolyl-3-acetic acid, although it was first isolated from human urine (Kögl and Kostermans 1934). It has been successfully isolated from higher plants more than decade later (Haagen-Smith and others 1946). Thus, the plant hormones are defined as naturally occurring substances operating at low concentrations, which are able to translocate within the plant body. This definition collides with the term plant growth regulators, which can be also synthetic substances with phytohormone activity. There is no clear border between these two terms as some substances have debatable classification and their occurrence in plant tissues are presumable but not confirmed yet. However, based on chemical structure, the basic phytohormone groups are specified as auxins, cytokinins, gibberellins, ethylene, abscisic acid, polyamines, brassinosteroids, jasmonates, salicylic acid and newly identified strigolactones.

Cytokinins

In 1955, new compound was isolated from old or autoclaved (but not fresh) DNA from herring sperm and calf thymus (Miller and others 1955). Name kinetin was chosen, because this substance could promote cell division – *cytokinesis*. This ability also gave name to the brand new class of hormones – cytokinins. Cytokinins (CKs) are defined as "substances, which promote cell division and exert other growth regulatory functions in the same manner as kinetin" (Skoog and Armstrong 1970). However, nowadays this term describes all molecules with similar structure, no matter, how active they are. Chemically, the cytokinins are N^6-derivatives of adenine, but the cytokinin activity embody also some phenylurea derivatives.

Besides the cell division, cytokinins regulate shaping of plant; growth of aerial part and roots, differentiation of vascular bundles and release of buds from apical dominance. The levels of cytokinins further positively correlate with amount of chloroplasts and with onset of senescence. They also function as signal of nutrient and light availability and stress conditions and are involved in infection by plant-pathogenic bacteria. Most of these functions are tuned by cooperation with the other plant hormones, especially with auxins (Galuszka and others 2008).

Structural diversity of cytokinins

Naturally occurring cytokinins are adenines, which possess either isoprenoid or aromatic side chain at N^6-position (Fig. 1).

The terminal carbon of the isoprenoid side chain of N^6-(Δ^2-isopentenyl)adenine (iP) can be hydroxylated in either *cis*- or *trans*-position forming zeatin named by the first occurrence in maize (*Zea mays* L.; Letham 1963). The double bond of zeatin is reduced in dihydrozeatin, but reduced equivalent of iP was not detected *in planta* to date.

The first cytokinin identified, kinetin, is N^6-furfuryladenine. The other aromatic

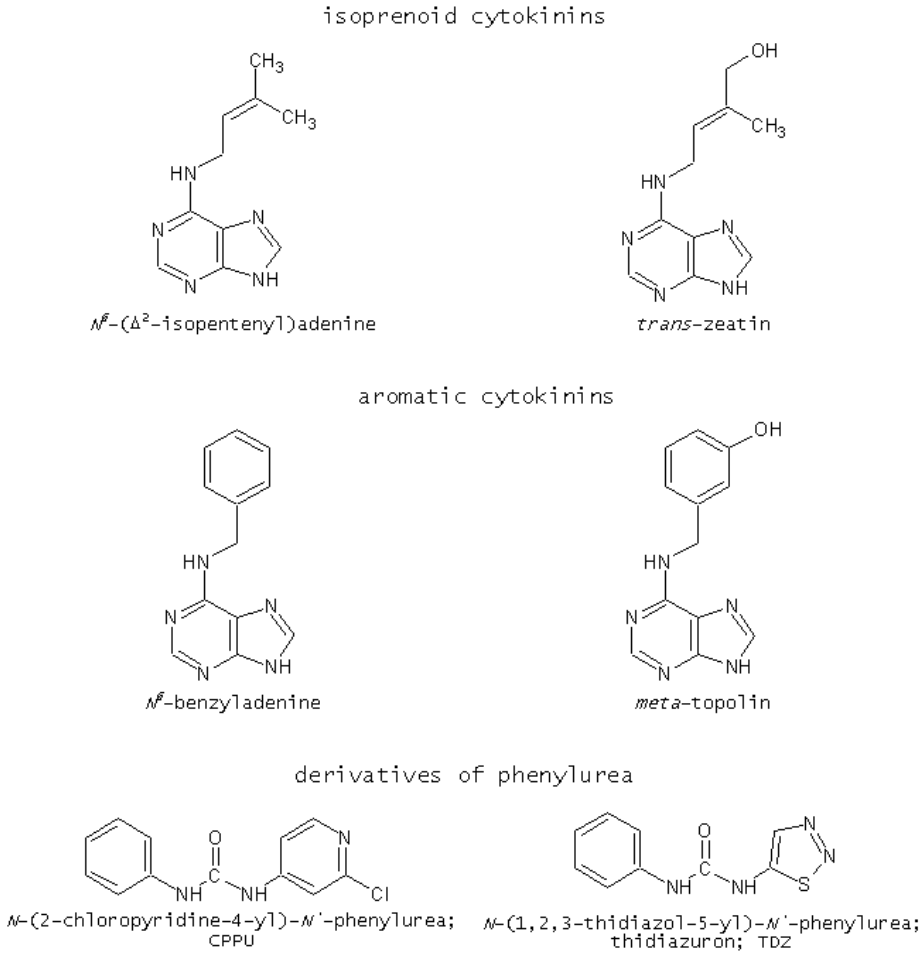

Figure 1: Structures of selected substances with cytokinin activity

cytokinins are derivatives of N^6-benzyladenine (benzylaminopurine; BAP). In plants there are the benzyladenine itself, its derivatives hydroxylated in any position of the phenyl ring and the corresponding methoxy derivatives.

The above mentioned free bases exist in plants also in form of nucleosides and nucleotides. The cytokinin bases can be further conjugated to glucose at *N3*, *N7* and *N9* and also at the hydroxyl of the side chain. The hydroxyl group at the side chain can be also conjugated to xylose (e.g. Turner and others 1987). The *N9* can be modified with L-alanine (Entsch and others 1983) and *C2* with 2-methylthio group (Fig. 2; Persson and others 1994; Pertry and others 2009).

Cytokinins combining two above mentioned modifications can occur in plants, also CKs with two sugar moieties attached to each other have been found in Monterey pine (*Pinus* radiata; Taylor and others 1984; Zhang and others 2001). The compound called "gazer", isolated from coconut milk, has been identified as 14-O-(3-O-[β-D-galactopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-α-L-arabinofuranosyl]-4-O-(α-L-arabinofuranosyl)-β-D-galacto-pyranosyl)-trans-zeatin riboside (G3A2-ZR; Kobayashi and others 1995).

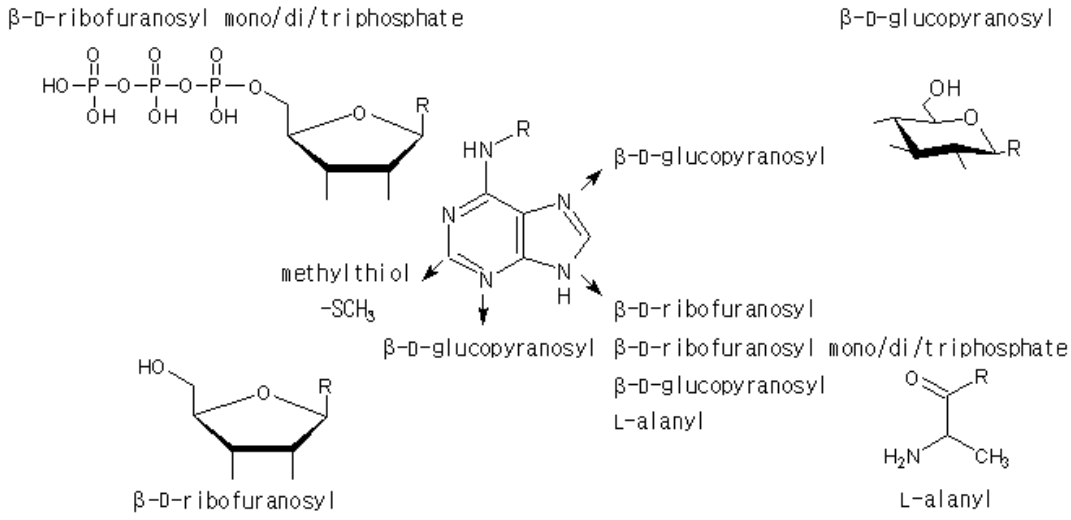

Figure 2: Possible modifications on adenine moiety.

Occurrence in plants

The occurrence of respective CK species can largely vary between plant species (Emery and others 2009; Gajdošová and others, to be published), vascular tissues (Hirose and others 2008), developmental stage (Emery and others 1998; Quesnelle and Emery 2007; Rasmussen and others 2009; Gajdošová and others, to be published) and environmental conditions (e.g. Takei and others 2001b, Vyroubalová and others 2009).

The isoprenoid cytokinins are widespread in the nature; contrary, the aromatic CKs were though for long time to be solely synthetic artifacts.

Trans-zeatin (tZ) and iP-type cytokinins were though to be the predominant cytokinins and the *cis*-isomer being present only in minor quantities with low or no activity (Schmitz and Skoog 1972; Mok and others 1978). This was reflected by ignorance of *cis*-isomer in CK analysis and by designing presumably *trans*-isomer only as zeatin. However, there is increasing number of reports of cZ being the dominant CK species in various plants as potatoes (Mauk and Langille 1978; Suttle and Banowetz 2000), unfertilized hop cones (Watanabe and others 1982), rice (Takagi and others 1985), maize (Veach and others 2003; Václavíková and others 2009; Vyroubalová and others 2009) and in several species of legumes (Emery and others 1998; 2000; 2009; Quesnelle and Emery 2007). Recently, large scale investigation in more than 150 species of *cis/trans* isomers representation was performed (Gajdošová and others, to be published), showing that species with exceeding amounts of cZ-type CKs can be found across all land plant species. *Arabidopsis* was considered to be solely tZ-dominant, but *Arabidopsis* seeds were shown to contain almost 80% of cZ-type CKs and the level of cZ-type CKs increased also in senescing plants (Gajdošová and others, to be published).

Dihydrozeatin occurs predominantly in dormant seeds (Václavíková and others 2009) and apical buds (Rasmussen and others 2009) and it seems to be produced in liquid endosperm of bean (Martin and others 1989; Mok and others 1990). These are all storage organs, suggesting specialized function of reduced cytokinin. Since, it is resistant to degradation by CKX (Galuszka and others 2007), it might serve as a source of active cytokinins before the *de novo* biosynthesis acceleration after germination (Václavíková and others 2009).

Low levels of *trans*-zeatin can be found in tRNA too, but, in about 40-times lower amount than *cis*-zeatin (Vreman and others 1978). However, tRNA isolated from green pea shoots (*Pisum sativum* L. var. Alaska) contains both isomers in amounts of the same order based on UV detection on thin layer chromatography (Vreman and others 1972).

Although the first cytokinin identified was kinetin, it took more than 40 years until it was found in nature. It was detected in commercially available calf thymus DNA and fresh human cells (Barciszewski and others 1996) and in human urine (Barciszewski and others 2000). In plants, it was found in commercially available coconut chunks (Barciszewski and others 1996) as well as fresh coconut endosperm (Ge and others 2005) and also in root nodules of Australian Pine (*Casuarina equisetifolia*) infected by bacteria *Frankia* (Raman and Elumalai 1996). On the other hand, derivatives of benzyladenine (benzylaminopurine; BAP) have been found in nature already in mid 70s (Horgan and others 1973; 1975). The presence in *Populus* species gave them eventually

name topolins from the Czech word "topol" for poplar. Since that time, they were discovered in rising number of species (e.g. in tomato crown gall tumours, Nandi and others 1989; *Arabidopsis*, Tarkowská and others 2003; pea, Gaudinová and others 2005).

Biosynthesis

The biosynthesis of isoprenoid CKs starts with transfer of the isoprenoid moiety to adenine either in its nucleotide forms or bound in RNA. The isoprenoid side chain donors found to date are dimethylallyl pyrophosphate (DMAPP) and (*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBDP; Krall and others 2002; Sakakibara and others 2005). In the case of DMAPP attachment and formation of iP-type cytokinin, the side chain can be further hydroxylated by cytochrome P450 monooxygenase (CYP). The evidence for all reaction steps will be discussed below. The nucleotides (eventually after release from tRNA) are then hydrolyzed up to free bases as discussed in appropriate section.

Isopentenyl transferases – the first step in cytokinin biosynthesis

There are two types of isopentenyl transferases (IPT) performing proposed rate-limiting step in cytokinin biosynthesis in accordance to used side chain acceptor.

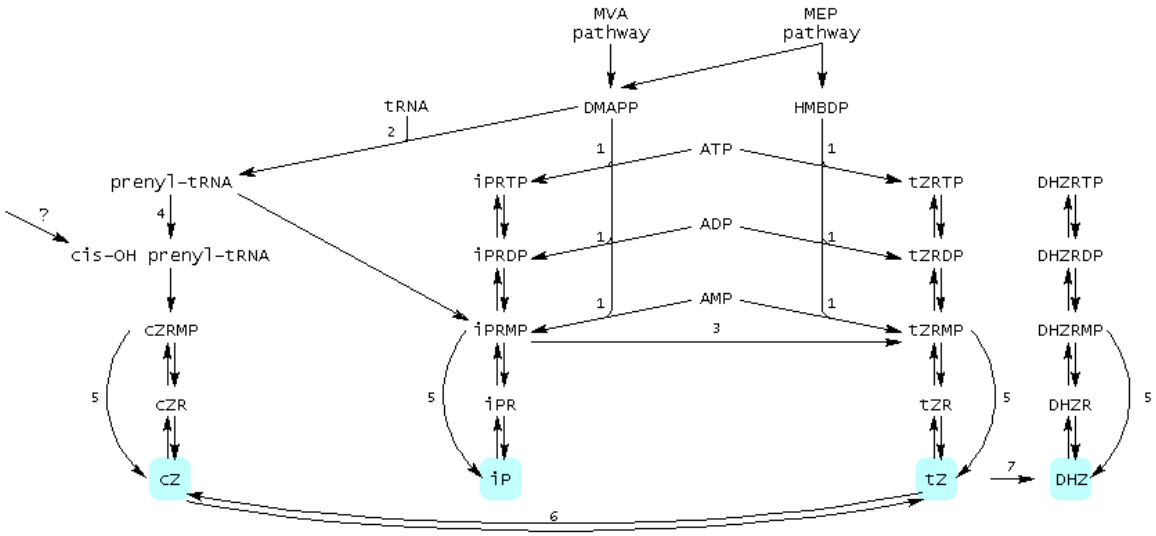

Figure 3: Scheme of cytokinin biosynthesis. Precursors are provided by mevalonate (MVA) or methylerythritol phosphate (MEP) pathway (see Fig. 14). The isoprenoid moiety is attached to side chain acceptor by adenylate IPT (1) or tRNA IPT (2). The free iPRMP can be hydroxylated by cytochrome P450 (CYP735A; 3), the iP moiety in tRNA can be hydroxylated in bacteria by nonheme diiron monooxygenase miaE and in plants by yet unidentified hydroxylase (4). Individual nucleotides are probably interconverted by unspecific phosphatases and kinases. The nucleotide monophosphates can be activated in one-step by LOG (5). The free bases and nucleosides of cZ and tZ are interconvertible by zeatin cis-trans isomerase (6). Free base of tZ can be reduced by zeatin reductase (7). Free bases, the active forms of CKs, are shown in cyan. For more details see the text.

The substrates can either be AMP, ADP or ATP, but not adenosine nor adenine, for adenylate isopentenyl transferase (AIPT; EC 2.5.1.27; Blackwell and Horgan 1993; Kakimoto 2001; Takei and others 2001a). Interestingly, IPT from mulberry (*Morus alba*; MaIPT) can also accept dATP, dADP, CDP and GDP. In the case of GDP, the isopentenyl moiety was transferred to the exocyclic N^2-group, rather than endocyclic *N1*, which is closer to *C6* (Abe and others 2007). Although it was originally described, that the IPT from hop (*Humulus lupulus*; HlIPT) does not accept GMP, IMP, CMP nor UMP (Sakano and others 2004), the recent report, concerning crystal structure of HlIPT, describes binding affinity for nucleotides in order ATP > dATP ~ ADP > GTP > CTP > UTP (Chu and others 2010). The other type, tRNA isopentenyl transferase (EC 2.5.1.8), incorporates prenyl group to adenine in tRNA. It has been proposed (Xie and others 2007) and later shown (Zhou and Huang 2010), that the modified adenine is flipped out similarly as with other nucleic acid modifying enzymes (Klimasauskas and others 1994; Slupphaug and others 1996; Xie and others 2003). This type of IPT is present almost in all living organisms including bacteria (Caillet and Droogmans 1988), animals (Golovko and others 2000), yeast (Yevdakova and others 2007) and of course plants (Golovko and others 2002; Miyawaki and others 2006). However, they are not found in Archeabacteria.

The isopentenylation of adenine in tRNA influences translational efficiency and fidelity (reviewed by Persson and others 1994). It was shown, that they improve

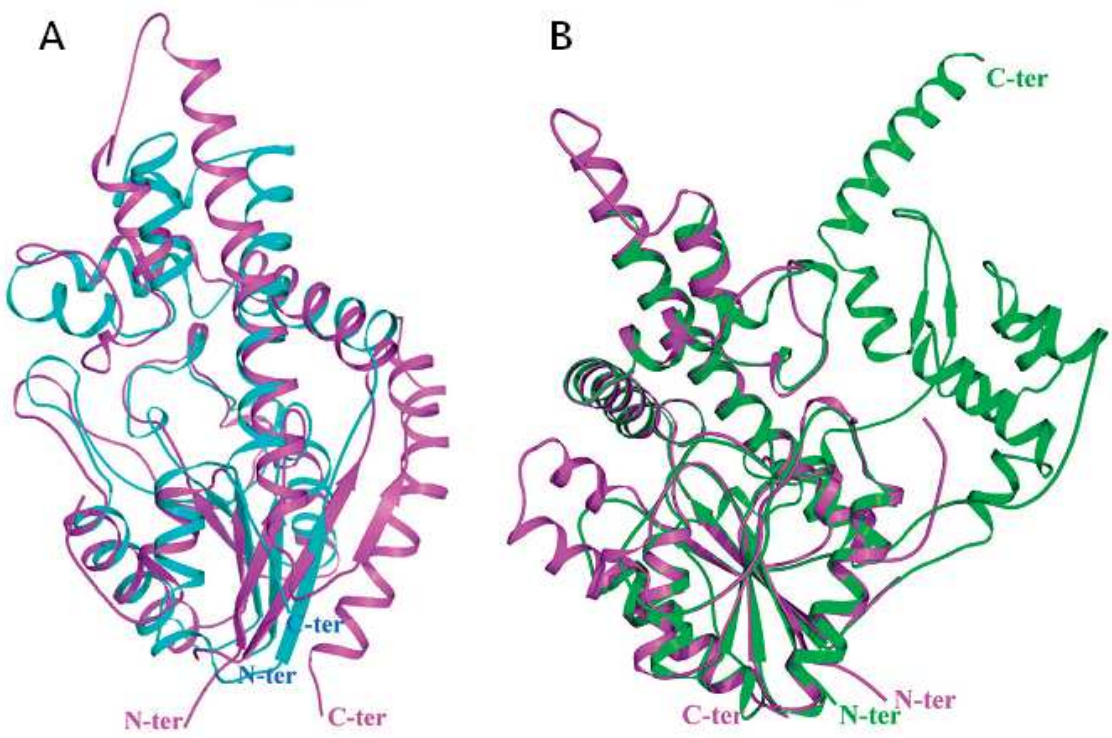

Figure 4: Overall structure of HlIPT. The HlIPT is compared with the structure of *Agrobacterium* AIPT (A) and with *S. cerevisiae* tRNA IPT (B). The tRNA IPT contains an extra domain, which is involved in tRNA anticodon stem binding. (taken from Chu and others 2010)

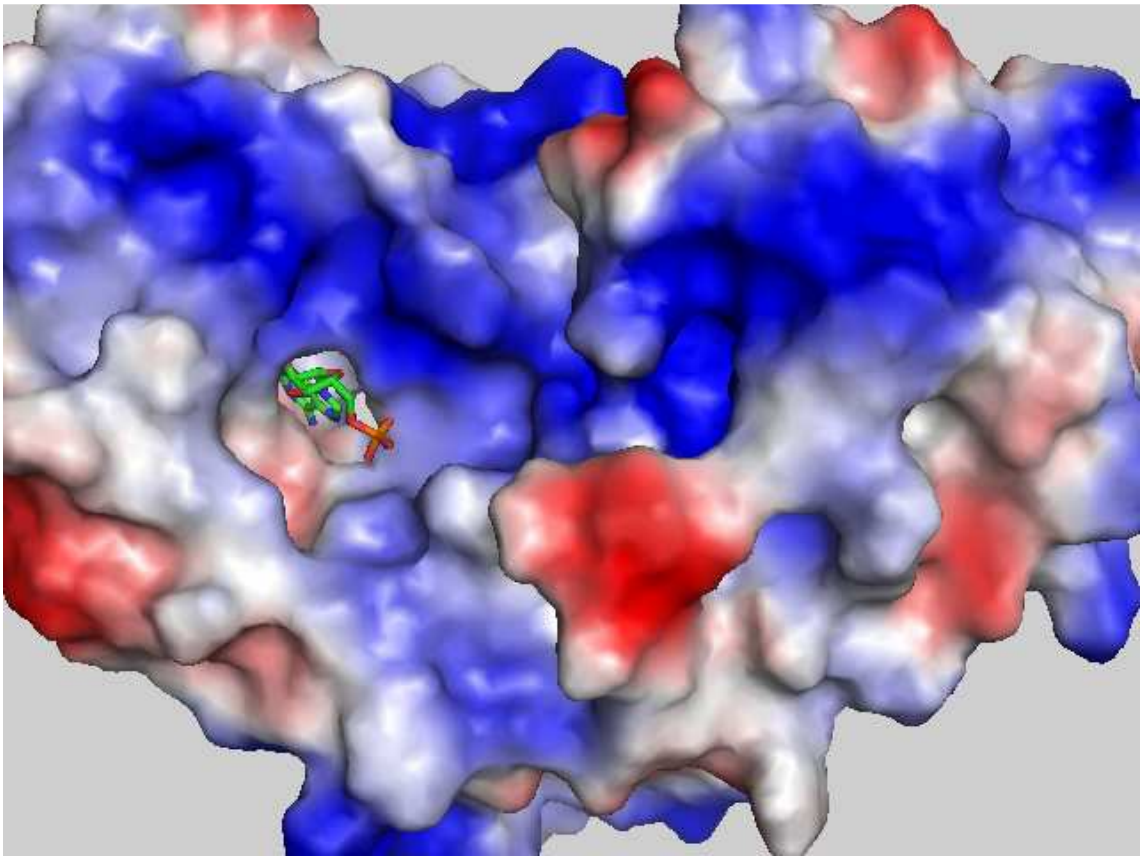

Figure 5: The model of Staphylococcus epidermidis IPT shown as surface coloured according to residue charge (in blue positively charged residues; in red negatively charged residues). As substrate was chosen AMP, here shown as sticks (taken from 2ZE6 structure). There is visible band of positively charged residues, involved in tRNA binding.

translational proof-reading by decreasing misreading at the first position of codon. The cytokinins were found also in rRNA (Taller and others 1987 and references therein), but their function in this type of RNA is unclear. The tRNA was originally proposed to be source of cytokinins, however, the calculations showed, that they could account no more than 40% of cytokinins due to long tRNA half-life (Klämbt 1992).

All three important model plants with sequenced genomes, *Arabidopsis*, maize and rice, contain two putative tRNA IPT (AtIPT2, AtIPT9, ZmIPT1, ZmIPT10, OsIPT9 and OsIPT10). The tRNA IPTs share homology with AIPTs (approximately 35% vs. homology of AIPTs ranging in 40 – 60%) as well as the overall folding structure, although they contain additional domain (Fig. 4; Takei and others 2001a). This C-terminal extension forms one side of U shape and is involved in tRNA binding. As has been shown on model of *Staphylococcus epidermis* IPT, there is wide band of positively charged residues (Fig. 5), proposed in binding negatively charged sugar-phosphate backbone (Hluska 2008). Zhou and Huang (2008) have shown 69-nt long L-shaped tRNA docked into the U-shaped IPT confirming the function of this band in binding the tRNA (Fig. 6). AtIPT2, ZmIPT1, OsIPT9 and MOD5 from *Saccharomyces cerevisiae* contain additional conserved Zn-finger-like motif CxxCx{12-18}HxxxxxH. This zinc finger is sitting on the top

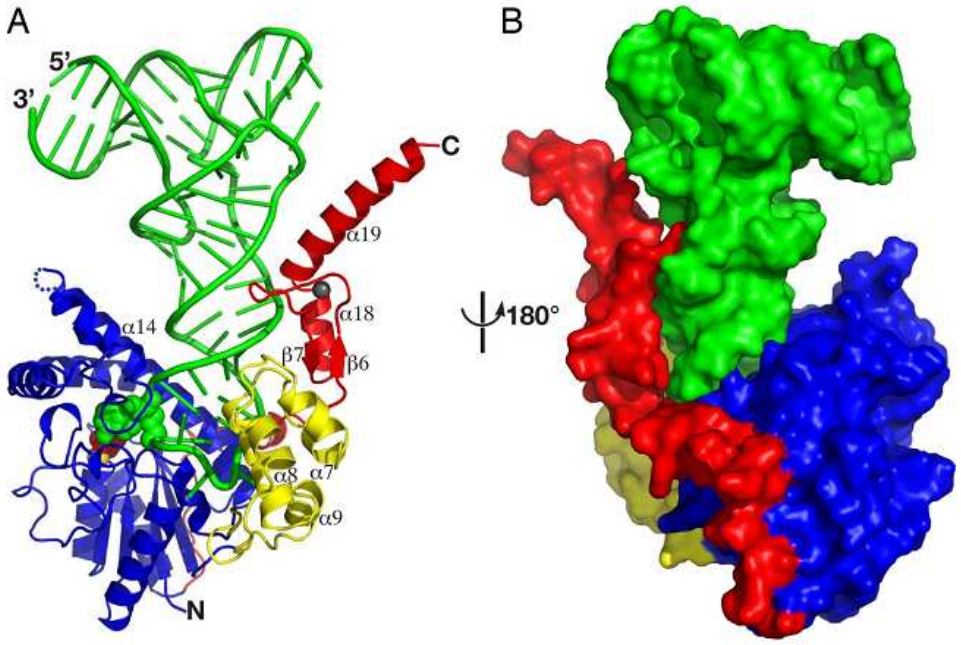

Figure 6: Structure of tRNA from Saccharomyces cerevisiae in complex with tRNA and pyrophosphate. (A) Ribbon diagram. Green, tRNA; blue, IPT core domain; yellow, IPT insertion domain; red, IPT C-terminal extension. The components in the reaction channel of the IPT, including the targeted adenine37, the pyrophosphate and the magnesium ion are highlighted in spheres and colored green, red and gold, respectively. The zinc ion of the C-terminal zinc finger is depicted as a small sphere in dark grey. Surface representation of the structure viewed from the opposite side. (taken from Zhou and Huang 2008)

of the insertion domain (Fig. 6) and provides additional interactions with tRNA. Zhou and Huang (2008) proposed two possible functions:

- it can reinforce the stability of the insertion domain, which may be susceptible to protease degradation in eukaryotes
- additional interactions may enhance the substrate specificity

Although isopentenyl transferases share only few conserved amino acids (Takei and others 2001a), the N-terminal part is an exception. There is the highly conserved consensus sequence Gx**T**xxGK[ST]x{5}[VLI]x{7}[VLI][VLI]xx**D**xxQx{57-60}[VLI][VLI]xGG[ST]* (Fig. 7; Kakimoto 2001). The p-loop GxTxxGK[ST] in the N-terminal part is common to NTP binding proteins like kinases. However, the first threonine is missing in these kinases but it is crucial in IPTs for the side chain donor binding and its activation. The oxygen bound to DMAPP's *C1* is additionally stabilized by arginine (Arg138 in Tzs; Table 1; Fig. 7). This arginine is flipped away after the reaction (Fig. 9D).

* Symbols in square brackets mean one of amino acids on place, x mean any amino acid and numbers in braces are number of amino acids

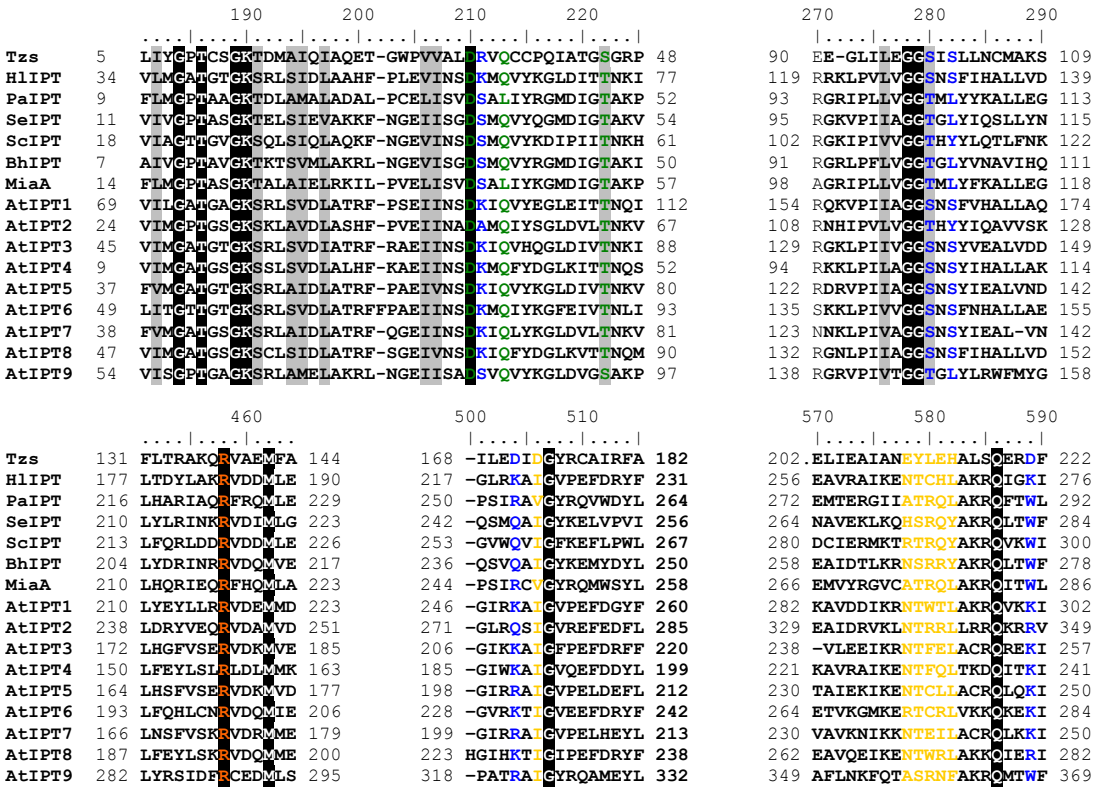

Figure 7: Alignment of isopentenyl transferases with resolved structure (see Table 1) and of AtIPTs. The alignment was done by T-COFFEE algorithm based on 3D structures called Expresso (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi). Identical and similar residues are highlighted in black and grey, respectively. Residues discussed in text are shown as follows (numbering in Tzs): binding DMAPP's oxygen in orange (R138); residues in proximity to methyl groups of isoprenyl side chain donor in gold (D173, E210-H214); residues binding N6 group of adenine shown in green (D33, Q36, S45) and residues at the entrance shown in blue (R34, S99, S101, D171, D221).

Close to proposed *trans*-side of DMAPP in Tzs is hydrophilic region (Glu 210 to His214 and Asp173). After mutagenesis of each of them, the preference for HMBDP[**] has decreased; in the case of His214 and Asp173 as much as 505- and 90-fold, respectively (Sugawara and others 2008). These residues are in AtIPTs substituted by hydrophobic residues (Fig. 7). However, mutagenesis of corresponding residues in AtIPT4 to residues present in Tzs did generally decrease activity with DMAPP and only the mutation L233H did slightly increase affinity for HMBDP (the activity with DMAPP was <10% of activity of WT enzyme), the other mutations did even lower the preference (Sugawara and others 2008). On the *cis* side there is Tyr211 in Tzs, whereas in HlIPT is in this place Thr265 opening larger cavity (Fig. 9C; as shown previously for MaIPT (Hluska 2008), the closest homologue of HlIPT; Fig. 10), which enables to accommodate longer isoprenoid precursors as well as to form N^2-isopentenylguanosine diphosphate (Abe and others 2007). However, this tyrosine is substituted by serine or threonine in all other examined IPTs (Fig. 7).

[**] Expressed by specificity ratio as k_{cat}/K_M for DMAPP divided by k_{cat}/K_M for HMBDP

Table 1: Summarization of structures of isopentenyl transferases published to date. Besides the organism and type of IPT are shown abbreviations used in the text and primary references.

organism	type	abbr.	reference
Pseudomonas aeruginosa	tRNA IPT	PaIPT	Xie and others (2007)
Agrobacterium tumefaciens	adenylate IPT	Tzs	Sugawara and others (2008)
Saccharomyces cerevisiae	tRNA IPT	ScIPT	Zhou and Huang (2008)
Escherichia coli	tRNA IPT	MiaA	Chimnaronk and others (2009)
Escherichia coli	tRNA IPT	MiaA	Seif and Hallberg (2009)
Humulus lupulus	adenylate IPT	HlIPT	Chu and others (2010)
Staphylococcus epidermidis	tRNA IPT	SeIPT	Fourouhar and others (to be published a)
Bacillus halodurans	tRNA IPT	BhIPT	Fourouhar and others (to be published b)

Another crucial residue is the aspartate shown in bold above. It is involved in binding N^6-amino group of the adenine. This hydrogen binding is stabilized by glutamine from the conserved pattern (Asp46 and Gln49 in ScIPT; Fig. 8). However, this glutamine is substituted by leucine in some IPTs. Yet another binding moiety is threonine located nine amino acids downstream from the above mentioned glutamine (Thr58 in ScIPT; Thr74 in HlIPT). Although it is not included in the above mentioned consensus, it is highly conserved with only minor exceptions (e.g. in Tzs), where it is replaced with highly homological serine. This implies its importance (Fig. 8). There are several conserved residues at the entrance of active site cavity (Ser129 and Ser131 in HlIPT), whereas others are variable. Thus in the HlIPT, there are several basic residues (Fig. 9A). Lys63 is exchanged by Arg34 in Tzs binding β- and γ-phosphate groups or α-phosphate group of nucleotide substrate, respectively. On the other hand, Lys220 and Lys275 of HlIPT are both substituted by aspartates in Tzs (Fig. 7; Fig. 9B), proposing explanation for different affinities to different adenosine phosphates of bacterial vs. plant AIPTs. Remarkably, Lys63 and Lys 275 are substituted mostly with serine and tryptophan, respectively, in the tRNA IPTs (Fig. 7).

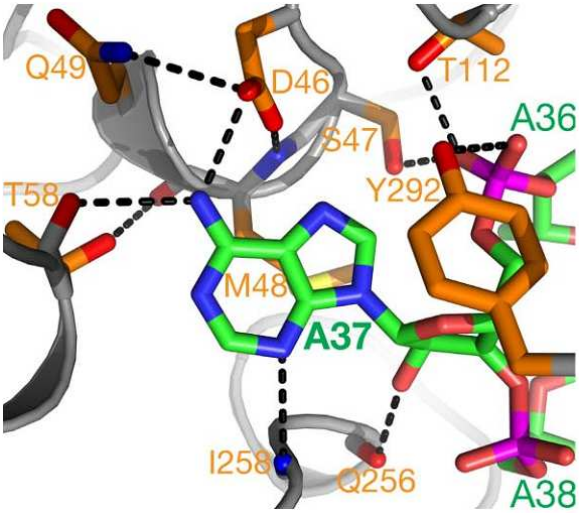

Figure 8: Molecular recognition of the substrate adenine in Saccharomyces cerevisiae tRNA IPT. The main chain of IPT is represented as grey ribbons. The side chains or IPT and tRNA are depicted as sticks. Hydrogen bonds are depicted as dotted lines in black. The carbon atoms of IPT and tRNA are colored in orange and green, respectively. Hetero-atoms are colored individually, with nitrogen in blue, oxygen in red, phosphorus in magenta and sulfur in yellow. (taken from Zhou and Huang 2008)

The properties of adenylate isopentenyl transferases have been extensively reviewed earlier (Hluska 2008).

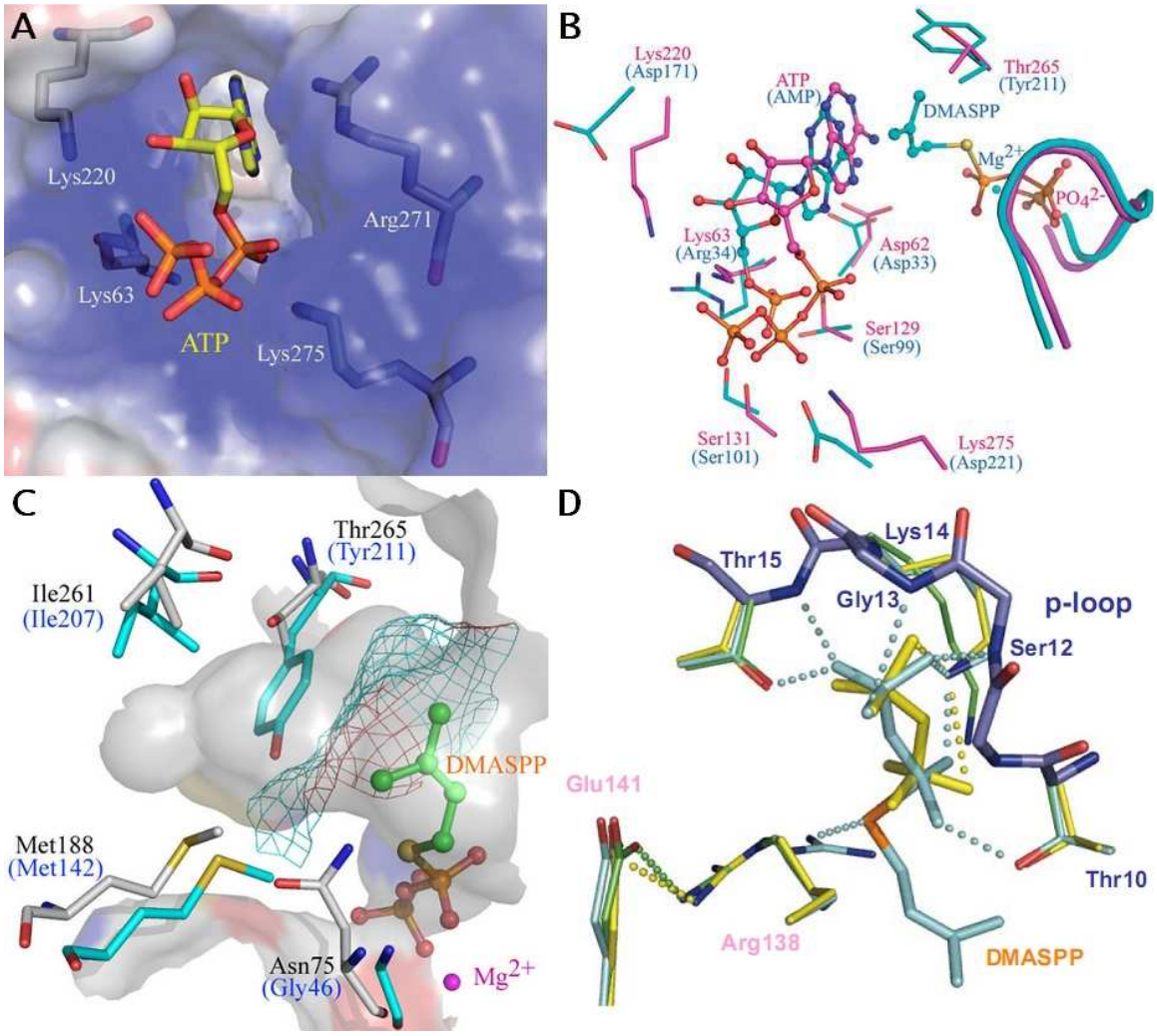

Figure 9: (A) The entrance into the active site in HIIPT. The surrounding basic residues are shown in sticks. (B) Superposition of adenylate and DMAPP binding sites of HIIPT (magenta) and Tzs (cyan). The p-loop binding the pyrophosphate moiety is shown as well. (C) Comparison of binding site surrounding the hydrophobic end of the isopentenyl group. Equivalent residues of HIIPT and Tzs are shown in grey and cyan, respectively. The surfaces of the isopentenyl group binding cavity are shown as mesh in magenta and cyan, respectively. (D) Superimposition of the reaction centers of Tzs-AMP (green), Tzs-AMP/DMASPP (cyan) and Tzs-PP_i (yellow). Notice the position of Arg138. (A, B and C taken from Chu and others 2010; D taken from Sugawara and others 2008)

Here, only the most important and relevant data will be discussed.

The first cytokinin biosynthetic activity was detected in slime mold *Dictyostelium discoideum* (Taya and others 1978), where is the activity important for production of discadenine, a spore germination inhibitor. Interestingly, discadenine shows cytokinin activity in assays of tobacco callus growth (Nomura and others 1977).

The first cytokinin biosynthetic gene was identified in plant pathogenic bacteria *Agrobacterium tumefaciens*. The gene *Tmr* located in the T-DNA (transfer) region on T_i-plasmid (tumor-inducing) was shown to be able to induce tumorigenesis independently of other T-DNA located genes (Lichtenstein and others 1984). The activity of adenylate isopentenyl transferase was confirmed in 1984 (Barry and others 1984; Akiyoshi and

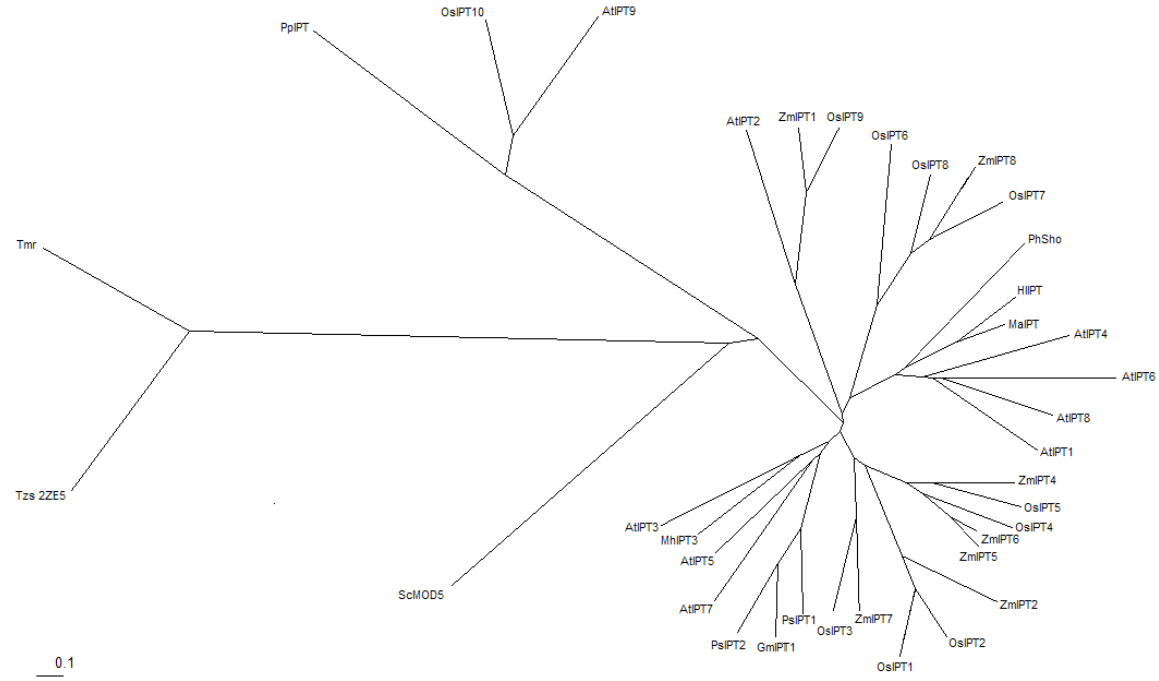

Figure 10: Phylogenetic unrooted tree of isopentenyl transferase proteins from different species.

others 1984). The gene was later cloned and expressed in *E. coli* (Blackwell and Horgan 1991; 1993). The K_M values for both AMP and DMAPP were low (85.7 nM and 8.28 μM, respectively), but the k_{cat}/K_M ratio was also low (4.7×10^5 s^{-1} M^{-1}). However, even lower enzyme efficiency was reported for all AIPTs studied to date (e.g. Takei and others 2001a; Kakimoto 2001; Sakano and others 2004; Abe and others 2007). The cytokinin biosynthetic genes have been identified in many other plant pathogenic bacteria (for review see Kakimoto 2003a).

Agrobacterium contains additional IPT gene. The *Tzs* (*trans*-zeatin synthesizing) gene is not, contrary to *Tmr*, transferred to the plant genome, but rather is expressed in the bacteria *in loco* (Morris and others 1993). Tmr and Tzs are very homologous proteins (Fig. 10); the amino acids crucial for substrate recognition are the same, but differ from plant AIPTs

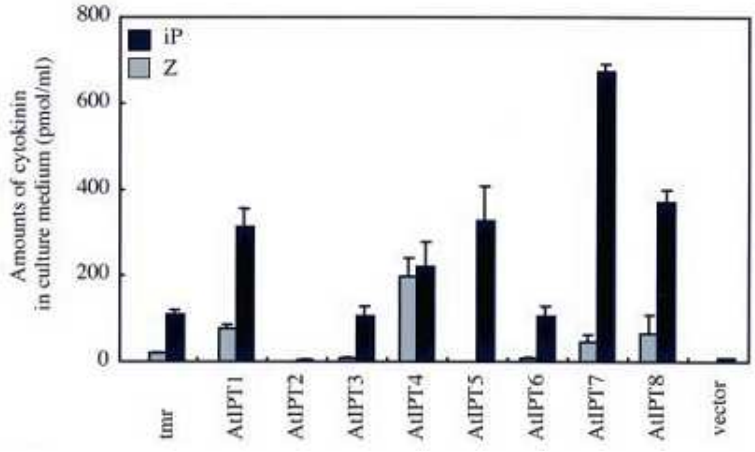

Figure 11: Content of cytokinins in media from cultures of E. coli harbouring AtIPTs genes with Tmr from Agrobacterium tumefaciens as control. Cells were grown in the presence of 1 mM IPTG in M9 minimal media for 4 h. The culture media were collected, CK fraction purified and molecular species determined by ELISA. Values shown are the means of three independent replicates. (taken from Takei and others 2001a)

(Kamada-Nobusada and Sakakibara 2009; Chu and others 2010 and see above), revealing preference of both enzymes for HMBDP.

Identification of the first plant IPTs was enabled as far as the first sequencing of first plant genome of the thale cress (*Arabidopsis thaliana* L.). The AtIPTs were identified by BLAST search against bacterial IPT genes concluding with 9 genes (Kakimoto 2001; Takei and others 2001). Two of them are putative tRNA IPTs (AtIPT2 and AtIPT9). The activity of AtIPT9 has not been detected yet, but AtIPT2 has been confirmed as tRNA IPT (Golovko and others 2002). The supernatants of bacteria expressing all other AtIPTs did secrete cytokinins (Fig. 11) indicating for their cytokinin biosynthetic activity.

The expression pattern of individual *AtIPT* genes is differential (Fig. 12; Takei and others 2004b; Miyawaki and others 2004). *AtIPT3, 5* and *7* are expressed in all examined tissues with *AtIPT3* being the most abundant one. *AtIPT6* and *AtIPT1* were expressed in siliques and *AtIPT1* additionally in flowers. However, *AtIPT6* is a pseudogene in *Arabidopsis* ecotype Wassilewskija and no expression is detected in there (Kakimoto 2001).

The expression of AIPTs was shown to respond to phytohormones content; *AtIPT3, 5* and *7* are upregulated by auxin in roots, but not if cytokinins are present

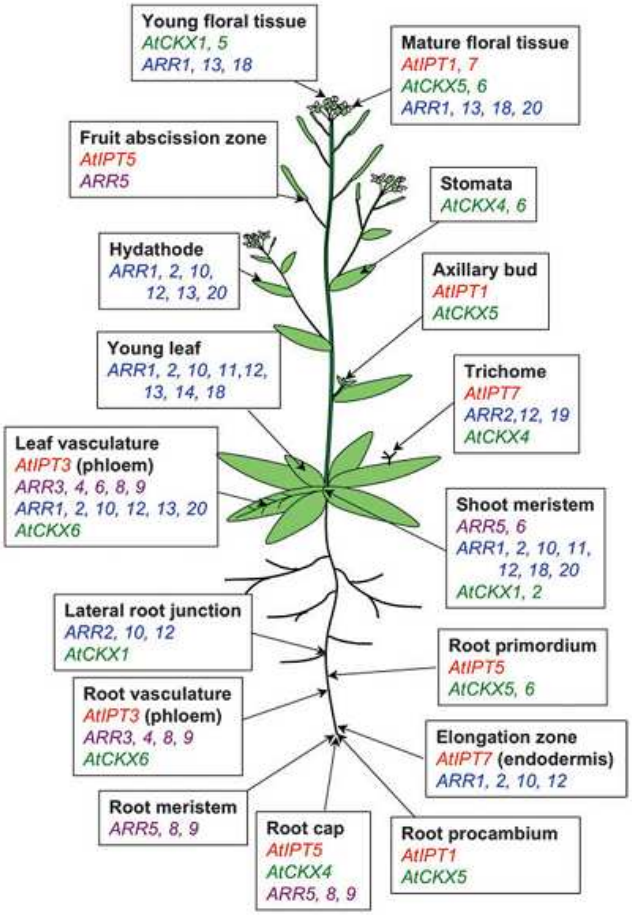

Figure 12: Spatial distribution of cytokinin-related gene expression in *Arabidopsis*. (taken from Hirose and others 2008)

(Miyawaki and others 2004), but in decapitated pea shoots is the expression of *PsIPT1* and *PsIPT2* upregulated in absence of auxin (Tanaka and others 2006); in soybean (*Glycine max* L.) was expression of *GmIPT1* only slightly upregulated after treatment with auxin (Ye and others 2006), but treatment with gibberellin resulted in 2.5-fold increase of expression.

The *Arabidopsis AIPTs* were shown to respond to nutrient status as well (Fig. 13; Takei and others 2001b; 2004b; Miyawaki and others 2004; Hirose and others 2008). Expression of *AtIPT5* in roots was positively correlated with ammonia and nitrate levels in media, whereas in shoots, it was decreased as well as the transcript levels of *AtIPT1* and *AtIPT7* (Takei and others 2004b). *AtIPT3* levels decreased in roots, when grown on ammonia, whereas in nitrogen-limited *Arabidopsis AtIPT3* transcript quickly increased after nitrate supply in both roots and shoots (Miyawaki and others 2004; Takei and others 2004b). Level of *AtIPT3* is also dependent on availability of other nutrients as sulphate, phosphate and iron (Fig. 13; Hirose and others 2008; Séguéla and others 2008).

The levels of *ZmIPT* transcripts in response to salinity and/or osmotic stress were

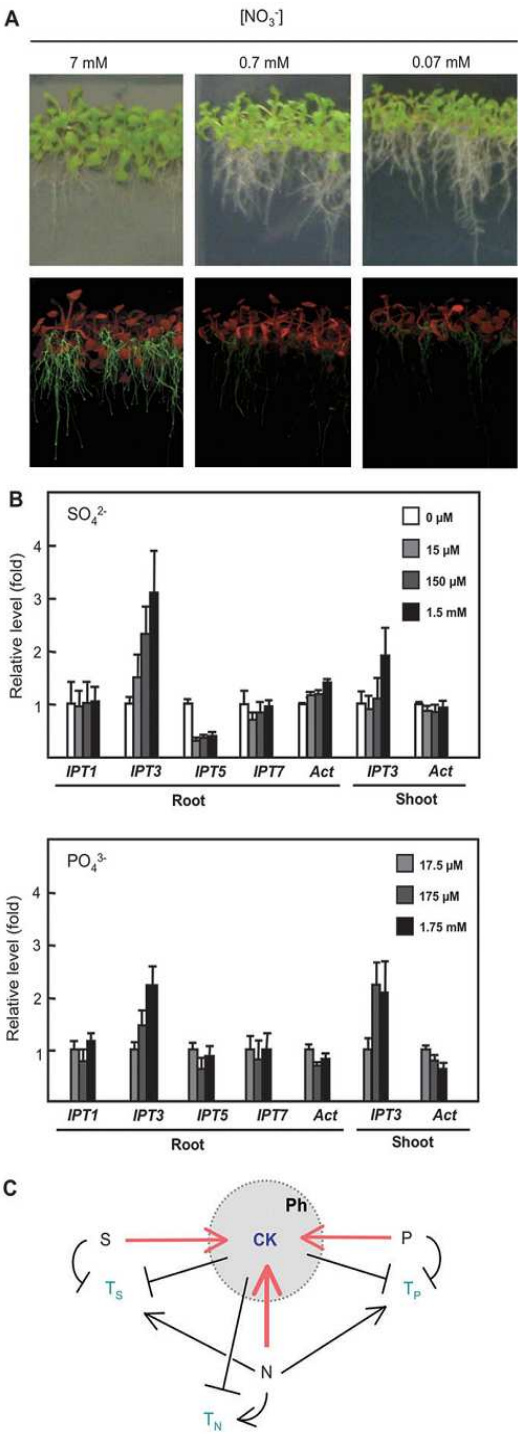

Figure 13: Effect of macronutrients in IPT expression. Nitrate concentration dependent fluorescence in IPT3 promoter:GFP transgenic Arabidopsis (A). Effects of sulphate and phoshphate in growth medium on AtIPT expression (B). Schematic representation of the regulatory relationship among nutrients, cytokinin biosynthesis and nutrient transporters (C) N – nitrate; S – sulphate; P – phosphate; T_N – nitrate transporter gene; T_S – sulphate transporter gene; T_P – phosphate transporter gene; Ph – phloem. Arrowhead bar indicates positive regulation and flat bar indicates negative regulation. (taken from Hirose and others 2008)

determined as part of large-scale transcriptome analysis in stressed maize seedlings (Vyroubalová and others 2009). In leaves, levels of ZmIPTs increased 72 hours after the stress treatment. Although *tRNA* IPTs are generally assumed to be expressed constitutively, without being affected by plant hormones or nutrient status (Miyawaki and others 2004), the expression of maize ZmIPT10 is raised after longer exposition to stress environment predominately in leaves (Vyroubalová and others 2009). The transcript level of soybean *GmIPT1* has increased 1.6 and 1.9-times after 5 hour treatment with cold and salt (Ye and others 2006).

The ChloroP 1.1 program (Emanuelsson and others 1999) predicted for AtIPT1, 3, 5 and 8 to be localized in plastids. This localization was confirmed for AtIPT1, 3 and 5 in leaf cells by fusion with green fluorescent protein (GFP). AtIPT8 exhibited the same pattern as AtIPT1 and AtIPT3 in root cells (Kasahara and others 2004). Recently, it was found, that AtIPT3 contains CaaX motif in the C-terminus and is farnesylated. This farnesylation drives translocation to nucleus in onion (*Allium cepa* L.) epidermal cells (Galichet and others 2008), where it could serve as local source of cytokinins for cell cycle progression. This could seem contradictory to previous report, however, Kasahara and others (2004) used GFP fused to C-terminus, which would block farnesylation and thus the translocation to the nucleus. Nevertheless, attempts to confirm the nuclear localization were unsuccessful (Sakakibara, personal communication). AtIPT4 and tRNA AtIPT2 were localized in cytosol and AtIPT7 signal peptide driven fluorescence was found in mitochondria (Fig. 14; Kasahara and others 2004). Noticeably, the *Agrobacterium tumefaciens* Tmr protein is localized into plastids too, although it is missing the amino-terminal extension (Sakakibara and others 2005).

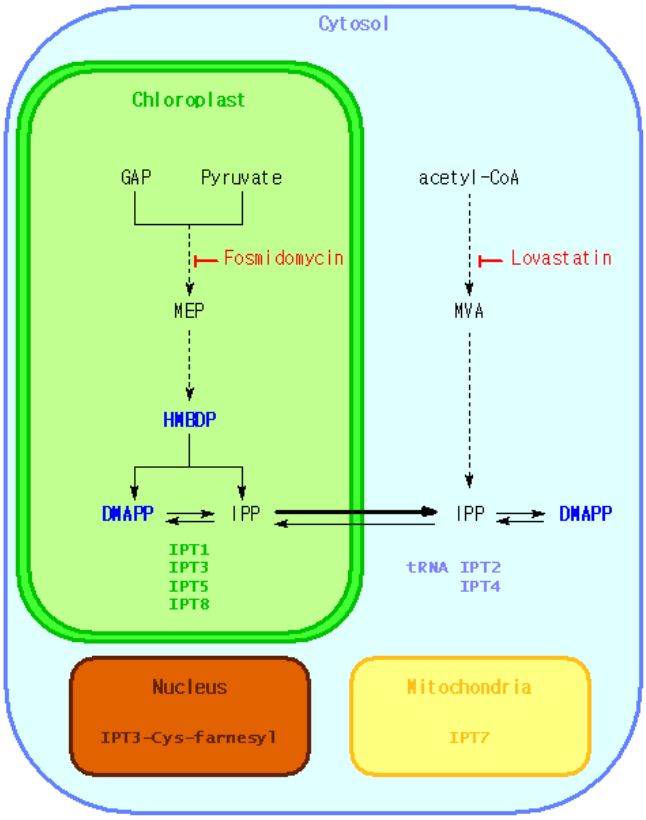

Figure 14: Schematic representation of isoprenoid biosynthesis and its compartmentation in plants. In bold blue are shown precursors fro CK biosynthesis; in red are shown inhibitors of both pathways. Localization of AtIPTs is shown based on Kasahara and others 2004 and Galichet and others 2008.

Origin of zeatin

There are proposed two possible biosynthetic pathways for tZ-type cytokinins:
iP-dependent pathway, where iP nucleotide is produced first and it is later hydroxylated by cytochrome P450 monooxygenases (Takei and others 2004a).
iP-independent pathway, where tZ-type CK is produced directly by transferring hydroxylated side chain from proposed precursor (*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBDP) to adenine ring (Åstot and others 2000; Sakakibara and others 2005).

The question, which pathway is more relevant is connected to the origin of the side chain donor. In plants, there are two pathways for production of isoprenoids. The first one, mevalonate (MVA) pathway, which works also in animals, fungi, Archaea, and a few Eubacteria, is located in cytosol and mitochondria and produces precursors for biosynthesis of sterols, certain sesquiterpens and the side chain of ubiquinone. The second one, methylerythritol phosphate (MEP) pathway localized in plastids was discovered in 1993 and is responsible for production of monoterpenes, certain sesquiterpens, diterpens, carotenoids and the side chains of chlorophylls and plastoquinone (Fig. 14; Rohmer and others 1993; Lichtenthaler 1999; Laule and others 2003). The most important steps of both pathways from CK biosynthesis point of view are:

The mevalonate (MVA) pathway starts with condensation of three acetyl-CoA molecules and subsequent reduction to mevalonate. This reduction is directly controlled by isoprenoid levels and is inhibited by statins (e.g. lovastatin or mevastatin). The mevalonate is then twice phosphorylated to produce mevalonate 5-diphosphate and another phosphorylation with subsequent decarboxylation-driven dephosphorylation yields IPP, which can be isomerased to DMAPP serving as a precursor for cytokinins.

The methylerythritol phosphate (MEP) pathway starts with condensation of pyruvate and glyceraldehyde 3-phosphate producing 1-deoxy-D-xylulose-5-phosphate. It is afterward converted to methylerythritol phosphate. This step is performed by reductoisomerase, which is inhibited by fosfidomycin (Proteau 2004). The MEP is conjugated with cytidylphosphate moiety into CDP-ME, which follows another phosphorylation, release of CMP and cyclization. Reduction yields (*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBDP), which can be further reduced to IPP and to lesser extent to DMAPP. Both HMBDP and DMAPP can work as cytokinin precursors, but the level of HMBDP in plastids is about 5-fold higher (Sakakibara and others 2005).

Although both pathways produce IPP/DMAPP, their localization and purpose is different and exchange between IPP/DMAPP pools can occur, at least to some extent (Laule and others 2003).

It has been shown, that Tzs is capable to synthesize directly ZMP from AMP and HMBDP (Krall and others 2002); Z-type cytokinin levels increased in *Arabidopsis* plants overexpressing Tmr, while iP-type CK levels remained low (Åstot and others 2000). On the other hand, *E. coli* expressing Tmr secreted predominantly iP-type CKs (Fig. 11; Takei and others 2001a). Since K_M values for both HMBDP and DMAPP are similar (Sakakibara and others 2005), the preference for either precursor may be given by their availability. Indeed, the concentration of HMBDP in plastids is 5-fold higher compared to DMAPP (Sakakibara and others 2005). The Z-type CKs in *Arabidopsis* overexpressing Tmr levels increased even after addition of metyrapone, the inhibitor of cytochromes P450 (Åstot and others 2000). The direct biosynthesis was also confirmed by labeling experiments (Åstot and others 2000; Sakakibara and others 2005). This implies major involvement of plastidial methylerythritol phosphate pathway (MEP) to Z-type CK pool in agreement with Tmr localization into plastids (Sakakibara and others 2005).

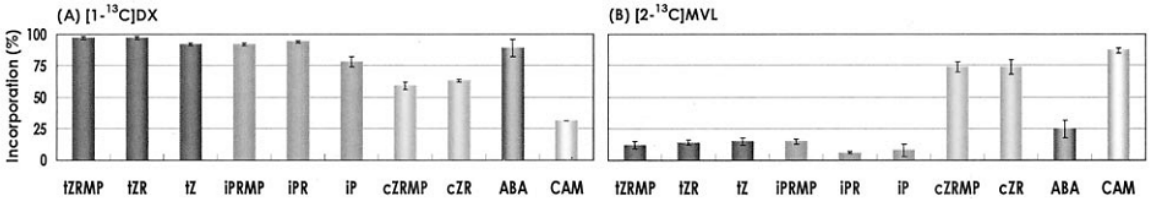

Figure 15: Relative levels of 13C incorporation into CKs, ABA and CAM after addition of labeled intermediate of MEP (A) or MVA (B) pathways. The 13C incorporation was calculated using the molecular ion cluster after substraction of natural 13C abundance. (taken from Kasahara and others 2004)

The involvement of MVA pathway in CK synthesis has been traditionally considered to be low or any (Piaggesi and others 1997 and references therein). Dual labeling study of incorporation from both pathways into CKs has revealed almost exclusive incorporation from MEP pathway into tZ and iP type CKs (Fig. 15; Kasahara and others 2004). Only minor incorporation from MVA pathway was observed, what could be explained either by exchange between pools of both pathways as described before (e. g. Laule and others 2003) or by actual use of MVA pathway precursors, probably by cytosolic AtIPT4 and AtIPT7 (Kasahara and others 2004). Both theories can be evaluated by comparison with incorporation into abscisic acid (ABA), which was previously described to be synthesized by the MEP pathway (Hirai and others 2000). The incorporation into CKs should be approx. the same or higher, respectively, but it was actually even lower (Kasahara and others 2004). Although the influence of the cytosolic and mitochondrial AtIPTs should not be neglected, their expression is not insignificant and probably not relevant for designed experiment.

On the other hand, the incorporation into cZ-type CKs was ambiguous. The incorporation into cZ-type CKs after addition of MEP and MVA pathway labeled

precursors were approx. 60 and 75%, whereas for ABA and campesterol (CAM; proposed to be synthesized by MVA pathway) it was about 90 and 25% vs. 30 and 90%, respectively (Kasahara and others 2004). However, the levels of cZ-type CKs were lower, so in absolute numbers, the exchange of all CKs may be similar.

Contrary, use of mevastatin, the inhibitor of cytosolical mevalonate pathway (MVA), decreased the deuterium incorporation into ZMP (Åstot and others 2000). Also, the application of lovastatin decreased CK level in tobacco BY-2 cells (Laureys and others 1998; 1999).

Auxin has been shown to modulate Z levels both by decrease expression of the CYP735As (Takei and others 2004a) as well as by inhibition of the iP-independent pathway (Nordström and others 2004).

Both *de novo* biosynthetic pathways are to date relevant only for *trans*-zeatin. Since, to date neither *cis*-hydroxylase has been identified in plants, nor isoprenoid precursor with hydroxyl group in *cis*-position has been found in nature, tRNA degradation is the only widely accepted source of *cis*-zeatin. Interestingly, in the *Arabidopsis* plants, with knock-out of AtIPT2, cZ level in tRNA was decreased, whereas in plants lacking AtIPT9 the levels of iP in tRNA decreased 25-times (Table 2; Miyawaki and others 2006), suggesting, that there could be some *cis*-hydroxylated side chain precursor.

Table 2: Cytokinins released from tRNA isolated from tRNA IPT knockout plants. Measurements are nanograms of riboside equivalent per microgram of tRNA. ND, below detection limits. (taken from Miyawaki and others 2006)

Line	tZR	cZR	iPR
WT	ND	2.13 ± 0.28	0.0740 ± 0.0113
atipt2	ND	0.68 ± 0.10	0.0720 ± 0.0074
atipt9	ND	1.36 ± 0.31	0.0027 ± 0.0006
atipt2 9	ND	ND	ND
atipt1 3 5 7	ND	2.26 ± 0.37	0.0685 ± 0.0073

In *Salmonella typhimurium* the *miaE* gene was identified as tRNA 2-methylthio-N^6-isopentenyladenosine *cis*-hydroxylase (Persson and Björk 1993). It is not present in *E. coli* (Persson and Björk 1993) and the Clusters of Orthologous Groups (COG database) contains only 4 homologous proteins (Kaminska and others 2008) and our BLAST search on PubMed (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and TAIR (http://www.arabidopsis.org/Blast/ index.jsp) did not find any homologues in eukaryotes. Interestingly, the search on MaizeSeq (http://www.maizesequence.org/blast) found few proteins with significant homology (Table 3), some of them spanning whole length of sequence. It was shown, that *miaE* insertion mutant is unable to grow on the citric acid cycle intermediates (Persson and Björk 1993), however, the influence on enzymes of

Table 3: Results of BLAST search using *miaE* from *Salmonella* as query against maize proteome database. Only hits with E-value lower than 1E-05 are shown.

accession number	score	E-value	% identity	length
AC195387.3_FGP002	179	2.60E-08	32.85	137
GRMZM2G331652_P01	179	2.60E-08	32.85	137
AC194630.3_FGP004	155	2.20E-06	30.3	132
AC190732.3_FGP009	155	2.20E-06	30.3	132
GRMZM2G153096_P01	155	2.20E-06	30.3	132
GRMZM2G332049_P01	155	2.20E-06	30.3	132
GRMZM2G104363_P01	153	7.20E-06	30.43	138
AC197071.3_FGP006	152	3.30E-06	29.89	174
AC215195.3_FGP007	152	1.10E-05	29.45	292
GRMZM2G088313_P02	149	1.70E-05	23.98	246
GRMZM2G468404_P01	149	3.70E-05	26.07	303
GRMZM2G047198_P02	148	1.90E-05	30.81	211
GRMZM2G047198_P01	148	1.90E-05	30.81	211
AC211689.4_FGP002	148	2.00E-05	27.11	225
AC211689.3_FGP002	148	2.00E-05	27.11	225
GRMZM2G150934_P01	148	2.40E-05	27.11	225
GRMZM2G048763_P01	147	1.40E-05	28.9	218
AC217433.3_FGP002	147	2.60E-05	28.92	166
AC205399.3_FGP003	147	2.60E-05	28.92	166
AC198651.3_FGP006	142	7.00E-05	24.82	282
AC199207.3_FGP002	142	7.30E-05	27.01	274
GRMZM2G457929_P01	142	7.30E-05	27.01	274

glycolysis, citric acid cycle and respiratory chain has been ruled out and the reason has not been found (Persson and others 1998). Later, it was characterized as a nonheme diiron monooxygenase (Mathevon and others 2007).

Another predicted source of cZ is activity of zeatin *cis-trans* isomerase (ZI). It was first described in and isolated from immature seeds of bean (*Phaseolus vulgaris*; Bassil and others 1993). The enzyme requires flavin and light; as well DTT was needed for the reaction and probably also for inhibition of CK degradation enzymes in measured plant extracts. Similar requirements were described for *cis-trans* isomerase of geraniol and geranyl phosphate (Shine and Loomis 1974). It was desribed, that in the presence of oxygen, product formation was only 25% of that observed in N_2, thus the thiol compounds may be also necessary for establishment of reductive environment. On the other hand, flavin (both FAD and FMN can be used) must be in oxidized form, not reduced, although it can be partially reduced and in that case, the light is even not essential (Bassil and others 1993).

The zeatin *cis-trans* isomerase has been purified using Mono-Q, Superose 12 and Phenyl Superose with ~150-fold purification and 60% recovery. The last step was either polyacrylamide gel electrophoresis (PAGE) or Con A Sepharose. The PAGE

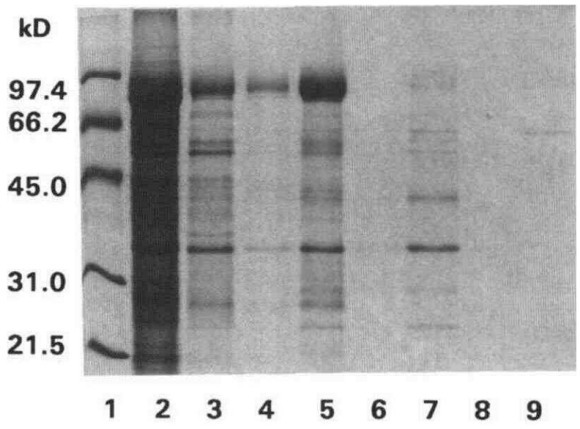

Figure 16: Coomasie brilliant blue stained SDS PAGE gel of pooled active fractions after each purification step. Protein in each line is from 10 µl of endosperm or multiple, if indicated. Lanes: 1 - molecular markers; 2 - 30 to 60% ammonium sulphate precipitation; 3 - anion exchange chromatography; 4 - Superose 12 FPLC; 5 - Superose 12 FPLC (10×); 6 - Phenyl Superose FPLC; 7 - Phenyl Superose FPLC (10×); 8 - PAGE; 9 - PAGE (50×). (taken from Bassil and others 1993)

resulted in further 5-fold purification, though the recovery was only 10%. On the other hand, Con A chromatography yielded in additional 15-fold purification giving total 2271-fold purification with 44% recovery (Fig. 16). However, since that time, no more further characterization has been performed and there are only few reports of zeatin *cis-trans* isomerase activity *in vivo*. For instance, after application of radioactively labeled *cis*-zeatin to potato (*Solanum tuberosum* L.) tubers, about 10% of recovered radioactivity was associated with *trans*-zeatin 4 and 7 days after application (Suttle and Banowetz 2000). Contrary, there are other reports, where no isomerization was detected (Nandi and Palni 1997; Kuroha and others 2002; Yonekura-Sakakibara and others 2004). Furthermore, in the *Arabidopsis* multiple IPT mutants, where either adenylate AtIPT1, 3, 5 and/or 7 or tRNA AtIPT2 and 9 were knock-outed, the levels of tZ or cZ type CKs were markedly reduced, respectively (Miyawaki and others 2006). If there was any zeatin *cis-trans* isomerase activity, the plant should be able to balance ratio between *cis/trans* isomers caused by multiple knockouts. This is in agreement with distinct origin of side chains of cZ and tZ in *Arabidopsis* with only very low exchange between MEP and MVA pathways (Fig. 15; Kasahara and others 2004). However, the isolation of ZI from immature seeds of *Phaseolus* (Bassil and others 1993) and the activity predominantly found only in young roots of maize (this work; see below), suggest that the ZI activity may be restricted to only some developmental stages contrary to other cytokinin metabolizing activities.

The origin of dihydrozeatin (DHZ) is still unclear. The only source found to date is putative zeatin reductase (EC 1.3.1.69). The conversion of zeatin to corresponding derivatives of dihydrozeatin was first described in excised bean axes (*Phaseolus vulgaris*; Sondheimer and Tzou 1971). Later, it was detected in embryos of *Phaseolus coccineus* and *P. vulgaris*, but not of *P. lunatus* (Mok and others 1990) and in leaves of pea (*Pisum sativum*), but not in leaves of bean (*Phaseolus vulgaris*) and soybean (*Glycine max*; Gaudinová and others 2005). The enzyme was purified to near homogeneity and partially characterized (Martin and others 1989). The reaction was

NADPH dependent, but concentration higher than 0.25 mM was inhibitory. NADH did not work as proposed cofactor (Martin and others 1989). The bean enzyme did not require ATP nor cations (Mok and others 1990), but the pea enzyme was inhibited by addition of diethyldithiocarbamate, the chelating agent, suggesting, that a metal cofactor may be needed (Gaudinová and others 2005). The bean seeds contained two isoforms with estimated molecular weights 55 ± 5 and 25 ± 5 kDa (Martin and others 1989). The specific activity of purified enzyme was particularly low suggesting protein instability. The only identified substrate was *trans*-zeatin; cZ, tZR or tZOX were not accepted. The reverse reaction with DHZ and NADP has not been observed (Martin and others 1989).

Activation of cytokinin nucleotides and nucleosides

Cytokinins are *de novo* synthesized as low-active nucleotide mono-, di- or tri-phosphates; the release of CKs from tRNA leads to nucleotide monophosphates. Historically it is considered, that the interconversions of the nucleotides, nucleosides and free bases are performed by enzymes of adenine metabolism. However, a low affinity of these enzymes toward cytokinins may be insufficient due to physiological concentrations of cytokinins in plant tissues that are up to six orders lower than those of adenine derivatives (Galuszka and others 2008).

The cytokinin nucleotide phosphates, which can be *in vivo* dephosphorylated by phosphatases with broad substrate specificity, such as 5'-nucleotidase (EC 3.1.3.5; Chen and Kristopeit 1981) or by any abundant alkaline (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2). Deribosylation of the cytokinin nucleoside can be mediated by enzyme activity of adenosine nucleosidase (EC 3.2.2.7). *Arabidopsis* mutants deficient in this enzyme accumulated more cytokinin nucleotides and nucleosides compared to wild type plants (Auer 1999; Auer 2002). Recently, novel uridine ribohydrolase URH1 has been characterized in *Arabidopsis* (Jung and others 2009). It has been shown to process iPR too, with K_M about half of that for uridine and adenosine. However, the k_{cat} was four orders of magnitude lower. Arabidopsis plants with either increased or decreased expression were constructed, but the levels of cytokinins have not been determined (Möhlmann, personal communication).

It was demonstrated many times that exogenously applied free cytokinin bases are rapidly metabolized into corresponding nucleosides and nucleotides (e.g. Sondheimer and Tzou 1971; Letham and Zhang 1989; Suttle and Banowetz 2000). Reverse phosphorylation of nucleosides to nucleotides could be catalyzed by adenosine kinase (EC 2.7.1.20) as it was manifested for the recombinant enzyme from moss *Physcomitrella patents* (von Schwartzenberg and others 1998). Adenosine kinase isolated from tobacco cells also shows high affinity to cytokinins (Kwade and others

2005). Direct conversion of cytokinin free bases to nucleotides can be contributed to an activity of adenine phosphoribosyltransferases (EC 2.4.2.7; Schnorr and others 1996; Allen and others 2002). Contrary to wild type, *Arabidopsis* male sterile mutants deficient in this enzyme were not able to convert exogenously applied benzyladenine to corresponding ribotide (Moffatt and others 1991). Interestingly, one of the five *Arabidopsis* isoforms identified later shows a higher affinity to benzyladenine than to adenine (Schnorr and others 1996). In general, high concentrations of nucleotides and nucleosides are found in young seedlings and developing organs, probably due to an increased *de novo* synthesis. Therefore the demand for deactivation of free bases by their conversion back to nucleotides *in planta* is still questionable, notwithstanding that mentioned enzymes are usually constitutively expressed without the possibility of pronounced regulation.

However, the recent discovery of cytokinin-specific phosphoribohydrolase (Lonely Guy, LOG; Kurakawa and others 2007) suggests, that also the other reactions may be performed by cytokinin-specific, yet unidentified enzymes.

The Lonely Guy (LOG) was identified in rice plants with altered shoot meristems and reduction in panicle size and abnormal branching patterns as a consequence of loss-of-function mutation (Kurakawa and others 2007). Originally it was annotated as lysine decarboxylase, but the co-occurrence of LOG homologues in operons with CK biosynthetic genes in some bacteria indicated involvement in cytokinin metabolism. Eleven and nine LOG genes have been identified in rice and Arabidopsis genomes, respectively (Kurakawa and others 2007; Kuroha and Sakakibara 2007). The cytokinin phosphoribohydrolase activity of Arabidopsis LOGs has been confirmed *in vitro* and *in vivo* (Kuroha and others 2009). The AtLOG9 misses the N-terminal third of the rice LOG sequence (Kuroha and others 2009) with the highly conserved PGGxGTxxE motif (Jeon and others 2006). Analyzed clone sequence of AtLOG9, together with AtLOG6 showed premature stop codon and thus they are probably pseudogenes. It was shown, that LOG works only with cytokinin nucleotide monophosphates, but not with di- or triphosphates, nucleosides nor with adenosine monophosphate (Kurakawa and others 2007), so the hydrolysis of β- and γ-phosphate by some phosphatase must precede. Regarding the cytokinin preference, the specificity towards isoprenoid AtLOG proteins is obvious (Fig. 17A; Kuroha and others 2009) with DHZRMP being generally the best substrate and tZRMP being slightly better substrate than cZRMP, whereas both were worst substrates, than iPRMP. The reactivity towards aromatic CKs differed more. The relative activities of AtLOG1, 2 and 3 with BARMP were less than 10% of their activity with iPRMP, whereas the activities of the other LOGs were higher, approximately 50% of this with IPRMP (Fig. 17A; Kuroha and others 2009). The plants expressing LOG genes under control of

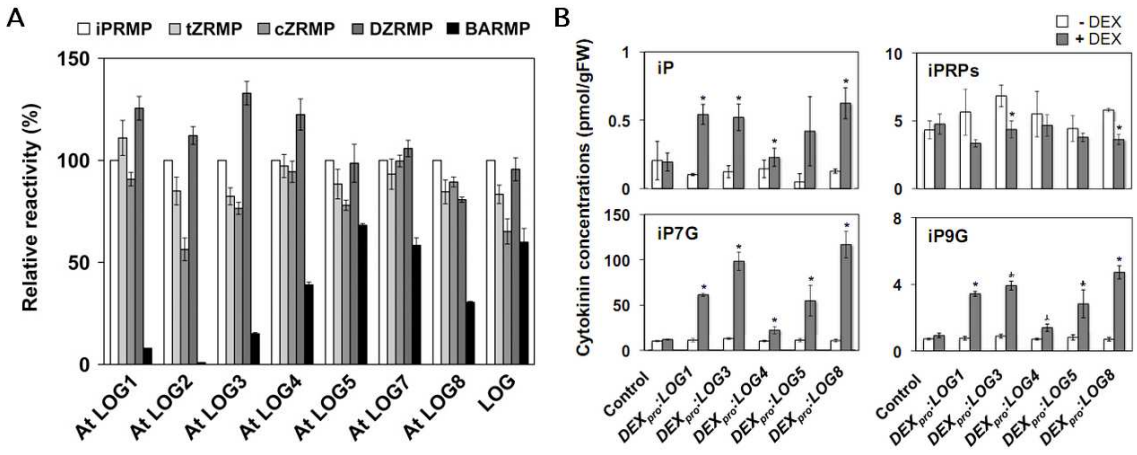

Figure 17: (A) Substrate specificities of recombinant rice LOG and AtLOG proteins. Enzymes were incubated at their optimum pH with 100 μM cytokinin nucleotides. Error bars represent the standard deviation (n = 3). (B) DEX-inducible overexpression of AtLOG genes and changes in cytokinin levels. Transgenic *Arabidopsis* seedlings harboring vector pTA-7001 with indicated insert were grown for 17 days on MGRL agar medium and then transferred to MGRL agar medium with or without 10 μM dexamethasone (DEX). After 5 days, seedling roots were harvested and cytokinin content elucidated. (taken from Kuroha and others 2009)

inducible promoter showed significant increase in iP free base, 9-glucoside and 7-glucoside and nucleotides were decreased, whereas the nucleoside remained unchanged (Fig. 17B).

The biochemical properties of *Arabidopsis* LOGs are similar to the one characterized in rice in details, except of AtLOG2, whose K_M and k_{cat} were 4 and 2 times higher than of rice LOG. Interestingly, AtLOG2 is the least expressed protein of all Arabidopis LOGs (Kuroha and others 2009). Although the k_{cat}/K_M ratio for AtLOG8 was less than 1% of the corresponding value for rice LOG, the AtLOG8 overexpressing *Arabidopsis* plants showed the highest increase in iP, iP7G and iP9G (Fig 17B). It was the most strongly expressed AtLOG in wild-type plants, thus maybe compensating the low processivity. Arabidopis LOG family has differential temporal and spatial pattern of expression with generally higher transcript abundance in shoots than roots. Only AtLOG7 have 5-fold higher expression in root than in shoot (Kuroha and others 2009).

Interestingly, structure of AtLOG3 and AtLOG8 has been determined (Jeon and others 2006), before their CK phosphoribohydrolase activity has been recognized. The overall fold was highly similar and structure based search showed the highest similarity to putative lysine decarboxylases. The CASTp server identified a cleft, which contains the conserved motif PGGxGTxxE (Jeon and others 2006).

Conjugations

As mentioned above, the cytokinin free bases can be de/conjugated to ribosyl(phosphates) by enzymes of adenine metabolism.

The *N7*- and *N9*-glucosides are resistant to hydrolysis and are thought to be inactivation forms. Thus they are discussed shortly below.

Although the *N3*-glucosides have been detected in several plants (Letham and others 1975), their biosynthetic enzymes have not been detected yet. The DHZ and DHZOG, but not Z and ZOG, has been metabolized to DHZ3G in derooted radish seedlings (*Raphanus sativus*; McGaw and others 1985). Contrary to *N7*- and *N9*-glucosides, kinetin-*N3*-glucoside can be deglycosylated together with *O*-glycosides by maize β-glucosidase (Brzobohatý and others 1993).

CKs with hydroxyl group on the side chain are capable to undergo *O*-glycosylations. The genes coding for glucosyltransfarases capable to glycosylate zeatins and topolins have been identified and enzymes has been characterized in many plants, e.g. in *Arabidopsis*, bean, maize and soybean (Hou and others 2004; Martin and others 1999a; 1999b; 2001; Veach and others 2003; Mok and others 2005; Meek and others 2008). The maize enzymes has been shown to utilize only *cis*-zeatin (Martin and others 2001; Veach and others 2003), so they are distinguished as *cis*-zeatin-*O*-β-D-glucosyl-transferase (EC 2.4.1.215). The other group, *trans*-zeatin-*O*-β-D-glucosyltransferase (EC 2.4.1.203), is capable to transfer xylosyl moiety from UDP-Xyl too (Martin and others 1999a; 1999b). Nevertheless, the *Arabidopsis* enzymes did not discriminate between both isomers and are able to efficiently glycosylate both zeatin isomers (Hou and others 2004). Extensive *O*-glycosylations occur in *Phaseolus* and there have been identified many cytokinin *O*-glycosyl-transferases. One of them is narrowly specific to only xylose conjugation (zeatin *O*-xylosyltransferase; EC 2.4.2.40; Turner and others 1987).

The phenotype of maize plants, which generally accumulate predominantly *cis*-zeatin forms (Veach and others 2003; Václavíková and others 2009; Vyroubalová and others 2009), overexpressing *trans*ZOG from *Phaseolus* (Pineda Rodó and others 2008) resembled plants overexpressing the degrading enzyme – CKX by dwarfish shoot and increased root growth (e.g. Werner and others 2001). Interestingly, these plants had also feminized tassel florets (Pineda Rodó and others 2008).

The hydroxylated aromatic cytokinins, topolins, as well as hydroxylated thidiazuron, have been shown to be substrates of *O*-glucosyltransferases (Mok and others 2005). There was preference of *cis*ZOG and *trans*ZOG for *ortho*- and *meta*-topolins, respectively, on the base of structural similarity of respective derivatives with Z-type CKs.

The *O*-glycosyl derivatives are resistant to cytokinin degrading enzyme, CKX (see below) and are proposed to be storage forms as they can be cleaved by β-glucosidase (EC 3.2.1.21; Brzobohatý and others 1993). Both ZOG and β-glucosidase are thought to play important roles in maintaining appropriate levels of active CKs, as the biosynthetic genes work slowly (see above). In agreement, the β-glucosidase has been detected in places of high meristematic activity, e.g. elongation zone of young maize seedlings (Václavíková and others 2009). It is also present around vascular bundles, where it can participate in the cytokinin transport (Kristoffersen and others 2000). Although most β-glucosidases show broad substrate specificity, the one isolated from rape (*Brassica napus*) showed significant activity only towards zeatin *O*-glucoside (Falk and Rask 1995).

Lupinic acid, the conjugate of zeatin and alanine, is produced in lupin seeds (*Lupinus luteus*) by zeatin 9-aminocarboxyethyltransferase (EC 2.5.1.50). The alanyl moiety donor is *O*-acetyl-L-serine and acceptor can be various N^6-derivatives of adenine, but zeatin is the best one (Entsch and others 1983). It was shown, that the enzyme is inhibited by certain urea derivatives, but also by indole-3-acetic acid (auxin) and similar compounds (Parker and others 1986).

The 2-methylthio derivatives of cytokinins are synthesized by *miaB* gene (for review see Persson and others 1994). It is known to be member of radical S-adenosylmethionine (SAM) enzymes (Gefter 1969; Kaminska and others 2008). This family comprises today more than 2800 members, which contain conserved motif CxxxCxxC nucleating [4Fe-4S] cluster (Pierrel and others 2002; Frey and others 2008; Kaminska and others 2008). MiaB is bifunctional protein involved in both thiolation and methylation of iP in tRNA (Pierrel and others 2004); two molecules of SAM are used, the first is used for thiolation after reductive cleavage of SAM by the [4Fe-4S] cluster and the second serves as source of the methyl group as shown by labeling experiments (Pierrel and others 2004). The enzyme strictly requires isopentenylated adenine in tRNA as substrate. Mutation in the *miaA* gene (tRNA IPT) in *E. coli* results in accumulation of unmodified adenine-37, but not 2-MetS-adenine-37. Adenine in position 37 of $tRNA^{Tyr}$ is a target for activity of both miaA as well as miaB producing 2-MetS-iP (Gefter and Russell 1969). Substrate specificity of miaB, however, seems to be very tight as the $tRNA^{Sec}$ (reading UGA codon) contains iP, but is not methylthiolated (Schön and others 1989).

Majority of cytokinins produced by pathogenic bacteria *Rhodococcus fascians* are methylthio-derivatives (Pertry and others 2009). Besides other cytokinin metabolizing genes, there were identified two ORFs in the pathogenic locus (fas-operon) with significant homology to S-adenosylmethionine-dependent methyltransferases, however, their methylthiolation activity towards free cytokinins has not been studied yet.

Deactivation of cytokinins

The levels of biologically active cytokinins must be precisely maintained. Besides irreversible side chain cleavage the cytokinins can be glucosylated at *N7*- and *N9*-positions. The *N*-glucosides show generally low activity in bioassays (Letham and others 1983, Spíchal and others 2004) and are only weakly metabolized (Parker and Letham 1973; Parker and others 1978). *N*-glucosides showed high indifference to metabolic conversion in radish (*Raphanus sativus*) tissues and thus the *N*-glucosylation is considered as irreversible deactivating process (McGaw and others 1984; 1985). However, recombinant AtCKX proteins showed ability to degrade *N9*-glucosides and two of them even prefer iP9G as a substrate in acidic environment (Galuszka and others 2007).

Irreversible degradation

The adenine derived cytokinins can be irreversibly degraded by oxidative cleavage of the N^6-side chain (Fig. 18). The enzyme was known for long time; it was thought, that it is copper-containing amine oxidase (EC 1.4.3.6 Hare and van Staden 1994). However, the amine oxidases are not able to cleave secondary amines and it was later shown, that the cytokinin degrading enzyme contains FAD as cofactor (Morris and others 1999) leading to classification as cytokinin oxidase (EC 1.4.3.18). Later it was shown that this enzyme works better with other electron acceptors than is oxygen and thus it was named cytokinin dehydrogenase (CKX; cytokinin dehydrogenase; EC 1.5.99.12; Galuszka and others 2001).

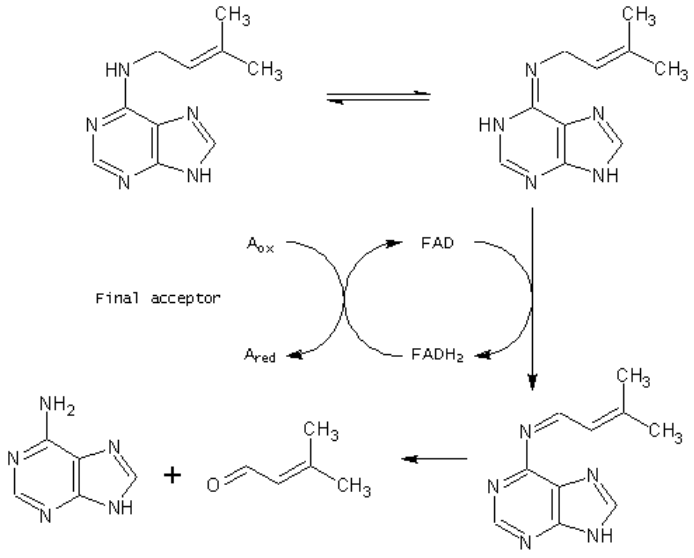

Figure 18: Degradation of cytokinins by CKX leading to adenine and corresponding aldehyde. Adapted from Galuszka and others 2001.

As best electron acceptors has been classified compounds with *p*-quinone structure and 2,6-dichlorophenol indophenol (Galuszka and others 2001). It was shown, that CKX can act in dual mode; in the dehydrogenase mode it highly prefers isoprenoid CKs, whereas in oxidase mode, the turnover rates of isoprenoid and aromatic CKs are similar (Frébortová and others 2004).

The maize CKX has been localized to aleurone layer and accompanion phloem cells in stems. Co-localization with laccases suggests that endogenously produced phenolics could serve as an electron acceptor (Galuszka and others 2005). Recently, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), abundant phenolic compound in maize tissues, it's oxidative cleavage product 4-nitrosoresorcinol-1-monomethylether (coniferron) and potentially also its free radical have been shown to serve as very potent natural electron acceptors of CKX (Frébortová and others 2010).

The CKX proteins have been purified from maize (Morris and others 1999; Houba-Hérin and others 1999), barley and wheat (Galuszka and others 2001). In *Arabidopsis* have been identified 7 CKX genes (Bilyeu and others 2001); and the enzymes were extensively characterized *in vitro* (Galuszka and others 2007). 13 isoforms have been identified in maize (Vyroubalová and others 2009), some of them were heterogously expressed and characterized (Morris and others 1999; Houba-Hérin and others 1999; Massonneau and others 2004; Šmehilová and others 2009).

Mode of Action

The cytokinins act, as other signaling molecules, in very low concentrations (1 to 50 pmol per gram of fresh weight; Galuszka and others 2008). It is accepted that they do not participate physically in processes, which they control, but rather they act through signaling cascade. Cytokinins act both as paracrine local signal in meristematic tissues and as distal signals e.g. for signaling of nutrition availability (Sakakibara 2006; Werner and Schmülling 2009).

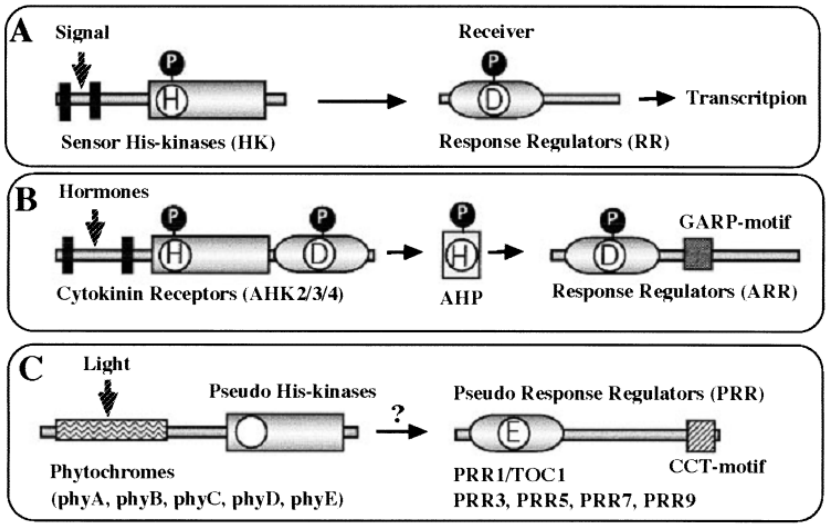

Figure 19: Comparison of prokaryotic (A) and eukaryotic (B) two component system. A presumed view with regard to pseudo two component signal transducers, such as phytochromes and the pseudo response regulator family members of higher plants is shown in C. (taken from Mizuno and Nakamichi 2005)

Two-component signaling – unification of bacterial and plant signaling

The two-component signaling (TCS), also histidyl-to-aspartyl system or His-Asp phosphorelay, gained its name by the pattern of multiple transfer of phosphate group from histidine to aspartate. These cascades are found in bacteria, where genes encoding for two protein components can account up to 1% of whole genome (Mizuno 1997), as well as in plants and fungi, but not in animals. However, the eukaryotic system evolved to be more complicated and contains additional intermediate – histidine phosphotransfer – protein (Fig. 19).

The first component of cytokinin signaling cascade is histidine kinase (HK), which passes the phosphate to the histidine phosphotransfer proteins (HP) and they further pass the signal into nucleus to the last component: response regulators of type B (RR-B). These work as transcription regulators and affect expression of the cytokinin target genes including response regulators of type A (RR-A), which inhibit the phosphotransfer from HPt to RR-B and thus work as negative feedback loop (Fig. 20).

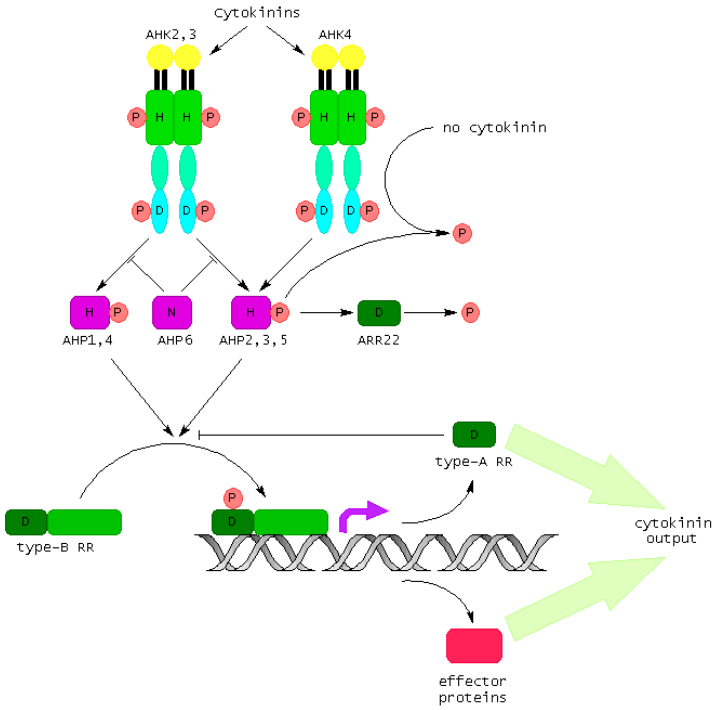

Figure 20: Schematic representation of current state of the art of cytokinin signaling. The cytokinin receptors, the histidine kinases, phosphorylate themselves in presence of cytokinins and transfer further the phosphate to the histidine phosphotransfer proteins (AHPs). However, in absence of cytokinins, AHK4 has phosphatase activity and dephosphorylates all AHPs. The transfer is inhibited by AHP6, which have substituted the conserved Asp for Asn. AHP2,3 and 5 are fastly dephosphorylated by ARR22. The AHPs transfer the phosphate group to type-B response regulators, which work as transcription factors. One of their targets are also type-A response regulators, which inhibit the phosphotransfer from AHPs to type-B RRs. The invariant residues are marked (H - histidine; D - aspartate; N - asparagine). The phosphate group is marked by P in pink circle. The transmembrane domains of the HKs are shown as black dashes, the extracellular CHASE domain in yellow, the transmitter domain is shown in green and receiver and receiver-like domains in light blue with and without marked D, respectively. For more details see the text.

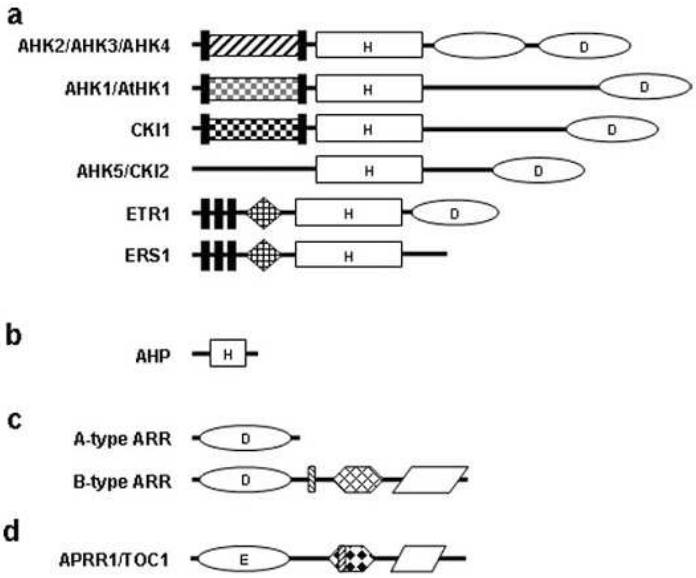

Figure 21: Structural characteristics of canonical histidine kinases (AHKs; a), histidine phosphotransfer proteins (AHP; b), response regulators (ARR; c) and pseudo-response regulators (APRR; d) from *Arabidopsis thaliana*. (a) *Black vertical bars* transmembrane domains; *striped rectancle* extracellular CHASE domain of AHK2, 3 and 4; *checked rectancles* extracellular domains of CKI1 and AtHK1; *open rectancles* transmitter domains; *open ovals* receiver(-like) domains; *crosshatched quadrate* GAF-domain in ETR1 and ERS1; (b) *open quadrate* phosphotransfer domain; (c) *oval* receiver domain; *striped bar* NLS; *rhomboid* GARP DNA binding domain; *open parallelogram* transactivation domain; (d) *oval* receiver-like domain; *striped bar* NLS; *rhomboid* CCT domain; *open parallelogram* transactivation domain. D aspartate; E glutamic acid; H histidine. (taken from Trefen and Harter 2004)

Histidine kinases must determine subtle differences

In the *Arabidopsis* genome was identified 8 canonical histidine kineses, which can be divided into several subclasses (Fig. 21). The first subgroup encompasses ethylene receptors, ethylene resistant 1 (ETR1) and ethylene response sensor 1 (ERS1), which are associated with endoplasmatic reticulum (Guo and Ecker 2004). ERS1 is the only known plant non-hybride HK (Fig. 21); the other subgroup contains AHK1/AtHK1, putative membrane-bound osmosensor (Urao and others 1999), and CKI1 (cytokinin insensitive 1). CKI1 has debatable function; it was proposed to be involved in CK perception, production or having no role in CK signaling. It was shown to be involved in female gametophyte development (Pischke and others 2002) and in CK-independent vascular bundle formation (Hejátko and others 2009). However, plants expressing antisense CKI1 cDNA had altered CK profiles under nutrient stress (Glover and others 2008). AHK5/CKI2 is unique as it lacks any transmembrane domain and is thus predicted to be cytoplasmic hybrid HK. The last subgroup consists of three characterized cytokinin receptors AHK2, AHK3 and AHK4/CRE1/WOL (Inoue and others 2001; Suzuki and others 2001; Ueguchi and others 2001; Higuchi and others 2004; Nishimura and others 2004).

The CK receptors have several transmembrane domains spanning the extracellular domain. It is called cyclases/histidine kinases associated sensory extracellular (CHASE), as it is present in many receptor-like proteins with histidine kinase and nucleotide cyclase domains (Pas and others 2004). The CHASE domain was shown to be sufficient for cytokinin binding and crucial residues for CK binding were identified (Heyl and others 2007). The intracellular part contains a transmitter and receiver domains as well as receiver-like domain positioned between both previously mentioned. The transmitter and receiver domains contain invariant histidine and aspartate, respectively (Fig 21A).

After ligand binding, the receptors form dimers, which phosphorylate each other and the phosphate group is transferred from the transmitter to receiver domain and further to the histidine phosphotransfer protein. In yeast two-hybrid system, interactions between AHK2 with itself, AHK2-AHK3 and AHK3-AHK4 were identified (Dortay and others 2006). Interestingly, AHK4, but not AHK2 and AHK3, was shown to possess phosphatase activity in absence of CKs (Fig. 20; Mähönen and others 2006b).

The AHKs were shown to bind CKs *in vivo*, when expressed in *E. coli* lacking osmosensor resulting in cytokinin-dependent growth (Inoue and others 2001; Suzuki and others 2001; Yamada and others 2001). This system was also used to investigate the specificity of the HKs from *Arabidopsis* and maize (Spíchal and others 2004; Yonekura-Sakakibara and others 2004). In maize, three genes for CK signaling HKs were identified with ZmHK3 being alternatively spliced (Yonekura-Sakakibara and others 2004; Vyroubalová and others 2009). AHK4 accepted only tZ and iP and *meta*-topolin, which is structural homologue of tZ (see ZOG part above) and gave 3-fold lower response. Similarly, its closest ortholog ZmHK1 showed high recognition between particular CKs with affinity: iP >> iPR $\geq$ tZ $\cong$ cZ > tZR > cZR. AHK3 accepted wider range of ligands with tZ and iP being the best, but cZ and DHZ still showed more than 50% affinity. Kinetin and benzyladenine were also accepted. ZmHK3b did not pass the signal further to downstream components of the signaling pathway. It could be involved in fine tunning of CK response. ZmHK2 and ZmHK3a bound all CKs tested with similar affinities, but ZmHK2 showed slightly higher affinity for tZ and tZR and ZmHK3 showed higher affinities for free bases than ribosides in order iP > tZ > cZ.

Histidine phosphotransfer proteins – connecting input and output

The histidine phosphotransfer proteins are responsible for transfer of phosphosignal from the histidine kinase to response regulators in nucleus. They form small gene family in *Arabidopsis* comprising of 6 proteins, from which five (AHP1-5) contain conserved histidine that is the target of phosphorylation and one (AHP6/APHP1),

which lacks this histidine. They were shown to interact directly with other components of cytokinin signaling cascade (e.g. Tanaka and others 2004; Dortay and others 2006) having partially redundant positive effect on cytokinin signal (Hutchison and others 2006) in agreement with overlapping expression pattern (Tanaka and others 2004). However, AHP4, which is close homologue of putative rice pseudo-HPts (Fig. 22), was shown to be partially negative regulator (Hutchison and others 2006). AHP6, which lacks the invariant histidine, was shown to be inhibitor of cytokinin signaling (Mähönen and others 2006a). AHPt1, 2 and 4 were shown to be located in nucleus in response to cytokinin treatment (Hwang and Sheen 2001; Yamada and others 2004).

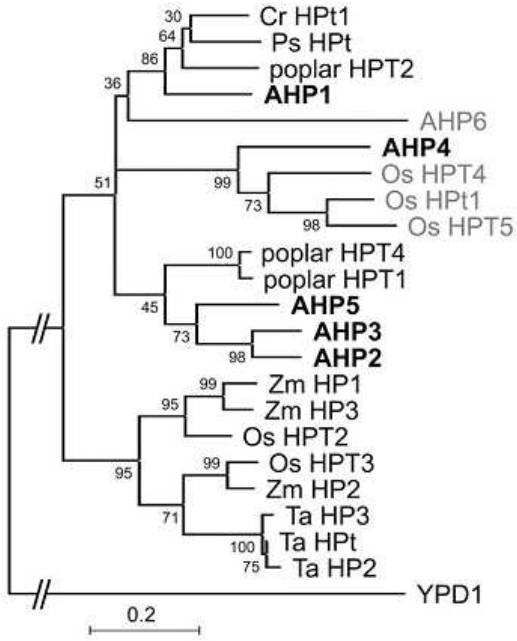

Figure 22: A phylogenetic tree of HPt amino acid sequences from plants. 1000 bootstrap replicates were used to construct the NJ tree, and bootstrap values as percentages are marked on the consensus tree. AHPs are highlighted in bold and predicted pseudo-HPts are in grey. (taken from Hutchison and others 2006)

Response regulators and other transcription factors

The response regulators (RR) are the final components in the cytokinin signaling. They are divided into several classes based on their structure.

The type-A RRs are small proteins consisting of the receiver domain with short C-terminal extension. Although they do not contain nuclear localization signal (NLS), the GFP signal of particular fusion proteins was found in cytosol and nucleus (Sweere and others 2001) or accumulated in nucleus (Kiba and others 2002). Cytokinin treatment results in higher expression (To and others 2004) and in increased half-life (To and others 2007). They are negative regulators of CK signaling due to competition with type-B RRs for phosphosignal delivered by HPts (To and others 2004). Recently, ARR7 and ARR15 were shown to be repressed by auxin (Müller and Sheen 2008).

ARR22 and ARR24 share structural homology with type-A RRs, but sequence alignment revealed closer homology to receiver domains of histidine kinases (Fig. rB; Kiba and others 2004). However, they are not upregulated by CKs as other type-A RRs (Kiba and others 2004). Transcripts of ARR22 were predominantly detected in reproducing organs as flowers and siliques (Kiba and others 2004; Horák and others

2008). It was shown to interact in yeast and tobacco cells with AHP2, AHP3 and AHP5, but not with AHP1, AHP4, AHP6 or the transmitter domain of AHK3 (Horák and others 2008). When ARR22 was incubated with AHP5 labeled with radioactive phosphate, the signal disappeared in 1 min from both proteins, but not in case, when ARR22DN (mutant unable to accept the phosphate group) was used. Meaning that ARR22 has a strong phospho-histidine phosphatase activity, in which the phospho-accepting aspartate residue is critically involved (Kiba and others 2004). As well, essential role of phosphoaccepting aspartate was confirmed in *in vivo* experiment. While, plants overexpressing ARR22DN or ARR22DE (simulating phosphorylated form) did not show any phenotype, plants overexpressing non-mutated ARR22 showed dwarfed growth (Kiba and others 2004).

The type-B RRs have the receiver domain homologous to that in HKs (Kiba and others 2004) and C-terminal extension with NLS, GARP motif and a transactivation domain. The GARP motif (named after presence in proteins Golden2, ARR and Psr1) of ~60 amino acids is related to the Myb repeat motif in Myb transcription factors. The GARP domain binding sites are over-represented in promoter regions of many CK primary response genes, including the type-A RRs (Sakai and others 2000; Rashotte and others 2003). They have been shown to be localized in nucleus and work as redundant positive elements in CK signaling (Hwang and Sheen 2001). Interestingly, ARR14 was shown to interact with AHK2 and ARR10 (type-B; Dortay and others 2006).

In *Arabidopsis*, six Cytokinin Response Factors (CRFs) were identified as subclass of ethylene response factors, belonging to APETALA2-like superfamily (Rashotte and others 2006). Three of them showed induced expression after CK treatment. Despite, the other three very homologous genes do not directly react on cytokinin signal; their basal expression was significantly higher and thus could resemble cytokinin signaling function of above described respective RR proteins. They are also accumulated in nucleus in HK- and HPt-dependent, but ARR-independent, manner (Rashotte and others 2006).

Recently, another class of transcription factors involved in CK signaling was described (Chevalier and others 2008). They are called GLABROUS1 enhancer-binding protein (GeBP; GPL (GeBP-like)). GeBP/GPL proteins are plant specific transcription factors and 4 out of 21 members share conserved C-terminal domain. They were shown to form homo- and heterodimers through the Leucine-zipper. They are involved in CK response indirectly through type-A RRs, because:

I. The knock-out mutants are less sensitive to CKs and the levels of type-A RRs were elevated.

II. The levels of type-A RRs in the mutants are partially insensitive to exogenous CKs.

III. Plants expressing GPL fused with strong activation domain showed reduced RR levels and increased sensitivity to cytokinins.

In *Arabidopsis* were identified 9 pseudo response regulators (PRRs; Hwang and others 2002). They resemble structure of other RRs, but the phosphoryable aspartate in receiver-like domain is changed for other amino acids (Makino and others 2000). They are involved in circadian rhythm and probably act as nuclear transcription factors downstream of phytochromes, as they contain nuclear localization signal (for review see Grefen and Harter 2004; Mizuno and Nakamichi 2005).

Abbreviations

ABA	abscisic acid
AHK	*Arabidopsis* histidine kinase
AIPT	adenylate isopentenyl transferase (EC 2.5.1.27)
CAM	campesterol
CK	cytokinin
CKX	cytokinin oxidase/dehydrogenase
Con A	concanavalin A (Sepharose chromatography)
cZ	*cis*-zeatin
DCHBS	3,5-dichloro-2-hydroxybenzenesulphonic acid
DHZ	dihydrozeatin (N^6-(4-hydroxy-3-methylbutyl)adenine)
DIMBOA	hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DMAPP	dimethylallyl pyrophosphate
DTT	dithiothreitol
FAD	flavin adenine dinucleotide (oxidised)
$FADH_2$	flavin adenine dinucleotide (reduced)
GFP	green fluorescent protein
HMBDP	(*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate
HP	histidine phosphotransfere protein
iP	N^6-(Δ^2-isopentenyl)adenine
IPT	isopentenyl transferase
LOG	Lonely Guy (cytokinin phosphoribohydrolase)
MEP	methylerythritol phosphate (pathway)
MVA	mevalonate (pathway)
NLS	nuclear localization signal
OPAO	Oat polyamine oxidase
PAGE	polyacrylamide gel electrophoresis
Q_0	2,3-dimethoxy-5-methyl-1,4-benzoquinone
TCA	trichloroacetic acid
WT	wild-type (plant; i.e. non-mutated)
ZI	zeatin *cis-trans* isomerase
ZOG	zeatin *O*-glucosyltransferase or zeatin *O*-glucoside

References

- Abe I, Tanaka H, Abe T and Noguchi H (2007) Enzymatic Formation of Unnatural Cytokinin Analogs by Adenylate Isopentenyltransferase from Mulberry. *Biochem. Biophys. Res. Commun.* **355**, 795-800;
- Akiyoshi DE, Klee H, Amasino RM, Nester EW and Gordon MP (1984) T-DNA of *Agrobacterium tumefaciens* Encodes an Enzyme of Cytokinin Biosynthesis. *PNAS* **81**, 5994-5998;
- Allen M, Qin W, Moreau F and Moffatt B (2002) Adenine Phosphoribosyltransferase Isoforms of *Arabidopsis* and Their Potential Contributions to Adenine and Cytokinin Metabolism. *Physiol. Plant*. **115**, 56-68;
- Artiss JD and Entwistle WM (1981) The Application of a Sensitive Uricase-Peroxidase Couple Reaction to a Centrifugal Fast Analyser for the Determination of Uric Acid. *Clin. Chim. Acta* **116**, 301-309;
- Åstot C, Dolezal K, Nordström A, Wang Q, Kunkel T, Moritz T and Chua N-H (2000) An Alternative Cytokinin Biosynthesis Pathway. *PNAS* **97**, 14778-14783;
- Auer CA (1999) The Arabidopsis mutation *cym* changes cytokinin metabolism, adenosine nucleosidase activity and plant phenotype. *Biol. Plant.* **42**, S3;
- Auer CA (2002) Discoveries and Dilemmas Concerning Cytokinin Metabolism. *J. Plant Growth Regul.* **21**, 24-31;
- Barciszewski J, Mielcarek M, Stobiecki M, Siboska G and Clark BF (2000) Identification of 6-furfuryladenine (kinetin) in human urine. *Biochem. Biophys. Res. Commun.* **279**, 69-73;
- Barciszewski J, Siboska GE, Pedersen BO, Clark BF and Rattan SI (1996) Evidence for the presence of kinetin in DNA and cell extracts. *FEBS Lett.* **393**, 197-200;
- Barry GF, Rogers SG, Fraley RT and Brand L (1984) Identification of a Cloned Cytokinin Biosynthetic Gene. *PNAS* **81**, 4776-4780;
- Bassil NV, Mok DWS and Mok MC (1993) Partial Purification of a *cis-trans*-Isomerase of Zeatin from Immature Seed of *Phaseolus vulgaris* L. *Plant Physiol.* **102**, 867-872;
- Bilyeu KD, Cole JL, Laskey JG, Riekhof WR, Esparza TJ, Kramer MD and Morris RO (2001) Molecular and Biochemical Characterization of a Cytokinin Oxidase from Maize. *Plant Physiol*. **125**, 378-386;
- Binda C, Coda A, Angelini R, Federico R, Ascenzi P and Mattevi A (1999) A 30 Å Long U-Shaped Catalytic Tunnel in the Crystal Structure of Polyamine Oxidase. *Structure* **7**, 265-276;

- Blackwell JR and Horgan R (1991) A Novel Strategy for Production of a Highly Expressed Recombinant Protein in an Active Form. *FEBS Letters* **295**, 10-12;
- Blackwell JR and Horgan R (1993) Cloned *Agrobacterium tumefaciens* ipt1 Gene Product, DMAPP:AMP Isopentenyl Tansferase. *Phytochemistry* **34**, 1477-1481;
- Brzobohatý B, Moore I, Kristoffersen P, Bako L, Campos N, Schell J and Palme K (1993) Release of Active Cytokinin by a Beta-Glucosidase Localized to the Maize Root Meristem. *Science* **262**, 1051-1054;
- Caillet J and Droogmans L (1988) Molecular Cloning of the *Escherichia coli miaA* Gene Involved in the Formation of Δ^2-Isopentenyl Adenosine in tRNA. *J. Bacteriol*. **170**, 4147-4152;
- Chen CM nad Kristopeit SM (1981) Metabolism of Cytokinin: Dephosphorylation of Cytokinin Ribonucleotide by 5'-Nucleotidases from Wheat Germ Cytosol. *Plant Physiol.* **67**, 494-498;
- Chevalier F, Perazza D, Laporte F, Le Hénanff G, Hornitschek P, Bonneville JM, Herzog M and Vachon G (2008) GeBP and GeBP-Like Proteins are Noncanonical Leucine-Zipper Transcription Factors that Regulate Cytokinin Response in *Arabidopsis*. *Plant Physiol*. **146**, 1142-1154;
- Chimnaronk S, Forouhar F, Sakai J, Yao M, Tron CM, Atta M, Fontecave M, Hunt JF and Tanaka I (2009) Snapshots of Dynamics in Synthesizing N^6-Isopentenyladenosine at the tRNA anticodon. *Biochemistry* **48**, 5057-5065;
- Chu HM, Ko TP and Wang AHJ (2010) Crystal structure and substrate specificity of plant adenylate isopentenyltransferase from *Humulus lupulus*: distinctive binding affinity for purine and pyrimidine nucleotides. *Nucleic Acids Res.* **38**, 1738-1748;
- Darwin CR and Darwin F (1880) *The Power of Movement in Plant*, John Murray, London;
- Dortay H, Mehnert N, Bürkle L, Schmülling T and Heyl A (2006) Analysis of Protein Interactions Within the Cytokinin-Signaling Pathway of *Arabidopsis thaliana*. *FEBS J*. **273**, 4631-4644;
- Emanuelsson O, Nielsen H and von Heijne G (1999) ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci.* **8**, 978-984;
- Emery RJN, Leport L, Barton JE, Turner NC and Atkins CA (1998) *Cis*-Isomers of Cytokinins Predominate in Chickpea Seeds Throughout Their Development. *Plant Physiol.* **117**, 1515-1523;
- Emery RJN, Ma Q and Atkins CA (2000) The Forms and Sources of Cytokinins in Developing White Lupine Seeds and Fruits. *Plant Physiol.* **123**, 1593-1604;

- Emery RJN, Hayward AR, Bruce S, Clarke-West C and Bassoriello MMI (2009) The Occurence of *Cis*- and *Trans*-Isomer Cytokinin Systems Among Seeds of Legume Species: Relashionships With Seed Size, Phylogeny and Rhizobial Symbionts. *3rd ACPD International Symposium*, Prague, Czech Rep, July 10-14, 2009.
- Entsch B, Parker CW and Letham DS (1983) An Enzyme from Lupin Seeds Forming Alanine Derivatives of Cytokinins. *Phytochemistry* **22**, 375-381;
- Falk A and Rask L (1995) Expression of a Zeatin-*O*-Glucoside-Degrading β-Glucosidase in *Brassica napus*. *Plant Physiol.* **108**, 1369-1377;
- Forouhar F, Abashidze M, Seetharaman J, Mao L, Xiao R, Maglaqui M, Lee D, Everett JK, Acton TB, Montelione GT, Tong L, Hunt JF (to be published a) Crystal structure of tRNA delta(2)-isopentenylpyrophosphate transferase (SE0981) from *Staphylococcus epidermidis*;
- Forouhar F, Neely H, Abashidze M, Seetharaman J, Shastry R, Janjua H, Cunningham K, Ma L-C, Xiao R, Liu J, Baran MC, Acton TB, Rost B, Montelione GT, Tong L, Hunt JF (to be published b) Crystal structure of tRNA isopentenylpyrophosphate transferase (BH2366) from *Bacillus halodurans*;
- Frébort I, Šebela M, Galuszka P, Werner T, Schmülling T and Peč P (2002) Cytokinin Oxidase/Cytokinin Dehydrogenase Assay: Optimized Procedures and Applications. *Anal. Bioch*. **306**, 1-7;
- Frébortová J, Fraajie MW, Galuszka P, Šebela M, Peč P, Hrbáč J, Novák O, Bilyeu KD, English JT and Frébort I (2004) Catalytic Reaction of Cytokinin Dehydrogenase: Preference for quinones as electron acceptors. *Biochem. J.* **380**, 121-130;
- Frébortová J, Novák O, Frébort I and Jorda R (2010) Degradation of Cytokinins by Maize Cytokinin Dehydrogenase is Mediated by Free Radicals Generated by Enzymatic Oxidation of Natural Benzoxazinones. *Plant J*. **61**, 467-481;
- Frey PA, Hegeman AD and Ruzicka FJ (2008) The Radical SAM Superfamily. *Crit. Rev. Biochem. Mol. Biol.* **43**, 63-88;
- Gajdošová S, Spíchal L, Kamínek M, Hoyerová K, Novák O, Dobrev PI, Galuszka P, Klíma P, Gaudinová A, Žižková E, Hanuš J, Dančák M, Trávníček B, Pešek B, Krupička M, Vaňková R, Strnad M and Motyka V (to be published) Distribution, Biological Activities and Metabolism of *Cis*-Zeatin-Type Cytokinins in Plants;
- Galichet A, Hoyerová K, Kamínek M and Gruissem W (2008) Farnesylation Directs AtIPT3 Subcellular Localization and Modulates Cytokinin Biosynthesis in Arabidopsis. *Plant Physiol.* **146**, 1155-1164;
- Galuszka P, Šebela M, Luhová L, Zajoncová L, Frébort I, Strnad M and Peč P (1998) Cytokinins as Inhibitors of Copper Amine Oxidase. *J. Enz. Inhib.* **13**, 457-463;

- Galuszka P, Frébort I, Šebela M, Sauer P, Jacobsen S and Peč P (2001) Cytokinin Oxidase or Dehydrogenase? Mechanism of Cytokinin Degradation in Cereals. *Eur. J. Biochem.* **268**, 450-461;
- Galuszka P, Frébortová J, Luhová L, Bilyeu KD, English JT and Frébort I (2005) Tissue Localization of Cytokinin Dehydrogenase in Maize: Possible Involvement of Quinone Species Generated from Plant Phenolics by Other Enzymatic Systems in the Catalytic Reaction. *Plant Cell Physiol*. **46**, 716-728;
- Galuszka P, Popelková H, Werner T, Frébortová J, Pospíšilová H, Mik V, Köllmer I, Schmülling T and Frébort I (2007) Biochemical Characterization of Cytokinin Oxidases/Dehydrogenases from *Arabidopsis thaliana* Expressed in *Nicotiana tabacum* L. *J. Plant Growth Regul.* **26**, 255-267;
- Galuszka P, Spíchal L, Kopečný D, Tarkowski P, Frébortová J, Šebela M and Frébort I (2008) Metabolism of plant hormones cytokinins and their function in signaling, cell differentiation and plant development. In *Studies in Natural Products Chemistry, Bioactive Natural Products (Part N)* (Atta-ur-Rahman ed.) **34**, 203-264; Elsevier, The Netherlands;
- Gaudinová A, Dobrev PI, Šolcová B, Novák O, Strnad M, Friedecký D and Motyka V (2005) The Involvement of Cytokinin Oxidase/Dehydrogenase and Zeatin Reductase in Regulation of Cytokinin Levels in Pea (*Pisum sativum* L.) Leaves. *J. Plant Growth Regul.* **24**, 188-200;
- Ge L, Yong JW, Goh NK, Chia LS, Tan SN and Ong ES (2005) Identification of kinetin and kinetin riboside in coconut (*Cocos nucifera* L.) water using a combined approach of liquid chromatography-tandem mass spectrometry, high performance liquid chromatography and capillary electrophoresis. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **829**, 26-34;
- Gefter ML (1969) The in vitro Synthesis of 2'-omethylguanosine and 2-Methylthio ^{6}N (γ,γ, Dimethylallyl) Adenosine in Transfer RNA of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **36**, 435-441;
- Gefter ML and Russell RL (1969) Role of Modifications in Tyrosine Transfer RNA: A Modified Base Affecting Ribosome Binding. *J. Mol. Biol*. **39**, 145-154;
- Glover BJ, Torney K, Wilkins CG and Hanke DE (2008) *CYTOKININ INDEPENDENT-1* Regulates Levels of Different Forms of Cytokinin in Arabidopsis and Mediates Response to Nutrient Stress. *J. Plant Physiol*. **165**, 251-261;
- Golovko A, Hjälm G, Sitbon F and Nicander B (2000) Cloning of a Human tRNA Isopentenyl Transferase. *Gene* **258**, 85-93;

- Golovko A, Sitbon F, Tillberg E and Nicander B (2002) Identification of a tRNA Isopentenyltransferase Gene from *Arabidopsis thaliana*. *Plant Mol. Biol.* **49**, 161-169;
- Grefen C and Harter K (2004) Plant Two-Component Systems: Principles, Functions, Complexity and Cross Talk. *Planta* **219**, 733-742;
- Guo H and Ecker JR (2004) The Ethylene Signaling Pathway: New Insights. *Curr. Opin. Plant Biol.* **7**, 40-49;
- Haagen-Smith AJ, Dadliker WB and Witner y Murneek AE (1946) Isolation of 3-indolacetic acid from immature corn kernels. *Am. J. Botany* **33**, 118-127;
- Hare PD and van Staden J (1994) Cytokinin Oxidase: Biochemical Features and Physiological Significance. *Physiol. Plant*. **91**, 128-136;
- Hejátko J, Ryu H, Kim GT, Dobesová R, Choi S, Choi SM, Soucek P, Horák J, Pekárová B, Palme K, Brzobohaty B and Hwang I (2009) The Histidine Kinases CYTOKININ-INDEPENDENT1 and ARABIDOPSIS HISTIDINE KINASE2 and 3 Regulate Vascular Tissue Development in *Arabidopsis* Shoots. *Plant Cell* **21**, 2008-2021;
- Heyl A, Wulfetange K, Pils B, Nielsen N, Romanov GA and Schmülling T (2007) Evolutionary Proteomics Identifies Amino Acids Essential for Ligand-Binding of the Cytokinin Receptor CHASE Domain. *BMC Evol. Biol*. **7**:62;
- Higuchi M, Pischke MS, Mähönen AP, Miyawaki K, Hashimoto Y, Seki M, Kobayashi M, Shinozaki K, Kato T, Tabata S, Helariutta Y, Sussman MR and Kakimoto T (2004) *In planta* Functions of the *Arabidopsis* Cytokinin Receptor Family. *PNAS* **101**, 8821-8826;
- Hirai N, Yoshida R, Todoroki Y and Ohigashi H (2000) Biosynthesis of Abscisic Acid by the Non-Mevalonate Pathway in Plants, and by the Mevalonate Pathway in Fungi. *Biosci. Biotechnol. Biochem*. **64**, 1448-1458;
- Hirose N, Takei K, Kuroha T, Kamada-Nobusada T, Hayashi H and Sakakibara H (2008) Regulation of Cytokinin Biosynthesis, Compartmentalization and Translocation. *J. Exp. Bot.* **59**, 75-83;
- Hluska T (2008) Expression and Characterization of Plant Adenylate Isopentenyl Transferase, *Bachelor Thesis, Faculty of Science, Palacký University*;
- Horák J, Grefen C, Berendzen KW, Hahn A, Stierhof YD, Stadelhofer B, Stahl M, Koncz C and Harter K (2008) The *Arabidopsis thaliana* Response Regulator ARR22 is a Putative AHP Phospho-Histidine Phosphatase Expressed in the Chalaza of Developing Seeds. *BMC Plant Biol*. **8**:77;
- Horgan R, Hewett EW, Horgan JM, Purse J and Wareing PF (1975) A New Cytokinin from *Populus x robusta. Phytochemistry* **14**, 1005-1008;

- Horgan R, Hewett EW, Purse JG, and Wareing PF (1973) A New Cytokinin from *Populus robusta*. *Terahedron Lett*. **14**, 2827-2828;
- Hou B, Lim EK, Higgins GS and Bowles DJ (2004) N-Glucosylation of Cytokinins by Glycosyltransferases of *Arabidopsis thaliana*. *J. Biol. Chem.* **279**, 47822-47832;
- Houba-Hérin N, Pethe C, d'Alayer J and Laloue M (1999) Cytokinin Oxidase from Zea mays: Purification, cDNA Cloning and Expression in Moss Protoplasts. *Plant J.* **17**, 615-626;
- Hutchison CE, Li J, Argueso C, Gonzalez M, Lee E, Lewis MW, Maxwell BB, Perdue TD, Schaller GE, Alonso JM, Ecker JR and Kieber JJ (2006) The *Arabidopsis* Histidine Phosphotransfer Proteins are Redundant Positive Regulators of Cytokinin Signaling. *Plant Cell* **18**, 3073-3087;
- Hwang I, Chen HC and Sheen J (2002) Two-Component Signal Transduction Pathways in *Arabidopsis*. *Plant Physiol*. **129**, 500-515;
- Hwang I and Sheen J (2001) Two-Component Circuitry in *Arabidopsis* Cytokinin Signal Transduction. *Nature* **413**, 383-389;
- Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K and Kakimoto T (2001) Identification of CRE1 as a Cytokinin Receptor from *Arabidopsis*. *Nature* **409**, 1060-1063;
- Jeon WB, Allard ST, Bingman CA, Bitto E, Han BW, Wesenberg GE and Phillips GN Jr. (2006) X-Ray Crystal Structures of the Conserved Hypothetical Proteins from *Arabidopsis thaliana* Gene Loci At5g11950 and At2g37210. *Proteins* **65**, 1051-1054;
- Jung B, Flörchinger M, Kunz HH, Traub M, Wartenberg R, Jeblick W, Neuhaus HE and Möhlmann T (2009) Uridine-Ribohydrolase is a Key Regulator in the Uridine Degradation Pathway of *Arabidopsis*. *Plant Cell* **21**, 876-891;
- Kakimoto T (2001) Identification of Plant Cytokinin Biosynthetic Enzymes as Dimethylallyl Diphosphate:ATP/ADP Isopentenyltransferases. *Plant Cell Physiol.* **42**, 677-685;
- Kakimoto T (2003a) Biosynthesis of Cytokinins. *J. Plant Res.* **116**, 233-239;
- Kakimoto T (2003b) Perception and Signal Transduction of Cytokinins. *Annu. Rev. Plant Biol.* **54**, 605-627;
- Kamada-Nobusada T and Sakakibara H (2009) Molecular Basis for Cytokinin Biosynthesis. *Phytochemistry* **70**, 444-449;
- Kaminska KH, Baraniak U, Boniecki M, Nowaczyk K, Czerwoniec A and Bujnicki JM (2008) Structural Bioinformatics Analysis of Enzymes Involved in the Biosynthesis Pathway of the Hypermodified Nucleoside ms^2io^6A37 in tRNA. *Proteins* **70**, 1-18;

- Kasahara H, Takei K, Ueda N, Hishiyama S, Yamaya T, Kamiya Y, Yamaguchi S and Sakakibara H (2004) Distinct Isoprenoid Origins of *cis*- and *trans*-Zeatin Biosyntheses in *Arabidopsis*. *J. Biol. Chem.* **279**, 14049-14054;
- Kiba T, Aoki K, Saakibara H and Mizuno T (2004) *Arabidopsis* Response Regulator, ARR22, Ectopic Expression of which Results in Phenotypes Similar to the *wol* Cytokinin-Receptor Mutant. *Plant Cell Physiol.*, **45**, 1063-1077;
- Kiba T, Yamada H and Mizuno T (2002) Characterization of the ARR15 and ARR16 Response Regulators with Special Reference to the Cytokinin Signaling Pathway Mediated by the AHK4 Histidine Kinase in Roots of *Arabidopsis thaliana*. *Plant Cell Physiol*. **43**, 1059-1066
- Klämbt D (1992) The Biogenesis of Cytokinins in Higher Plants: Our Present Knowledge. In *Physiology and Biochemistry of Cytokinins in Plants* (Kamínek M, Mok DWS and Zažímalová E eds.), 25; SPB Academic Publishing, The Hague, The Netherlands;
- Klimasauskas S, Kumar S, Roberts RJ and Cheng X (1994) HhaI Methyltransferase Flips its Target Base out of the DNA Helix. *Cell* **76**, 357-369;
- Kobayashi H, Morisaki N, Tago Y, Hashimoto Y, Iwasaki S, Kawachi E, Nagata R and Shudo K (1995) Identification of a Major Cytokinin in Coconut Milk. *Experientia* **51**, 1081-1084;
- Kögl F and Kostermans DGFR (1934) Hetero-Auxin als Stoffwechselprodukt Niederer Pflanzicher Organismen. Isolierung aus Hefe. *Zeit Physiol. Chem.* **228**, 113-121;
- Komath SS, Kavitha M, Swamy MJ (2006) Beyond Carbohydrate Binding: New Directions in Plant Lectin Research. *Org. Biomol. Chem.* **4**, 973-988;
- Krall L, Raschke M, Zenk MH and Baron C (2002) The Tzs Protein from *Agrobacterium tumefaciens* C58 produces Zeatin riboside 5'-phosphate from 4-hydroxy-3-methyl-2-(*E*)-butenyl diphosphate and AMP. *FEBS Letters* **527**, 315-318;
- Kristoffersen P, Brzobohaty B, Höhfeld I, Bako L, Melkonian M and Palme K (2000) Developmental Regulation of the Maize *Zm-p60.1* Gene Encoding a β-Glucosidase Located to Plastids. *Planta* **210**, 407-415;
- Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H and Kyozuka J (2007) Direct Control of Shoot Meristem Activity by a Cytokinin-Activating Enzyme. *Nature* **445**, 652-655;
- Kuroha T, Kato H, Asami T, Yoshida S, Kamada H and Satoh S (2002) A *trans*-Zeatin Riboside in Root Xylem Sap Negatively Regulates Adventitious Root Formation on Cucumber Hypocotyls. *J. Exp. Bot*. **53**, 2193-2200;

- Kuroha T and Sakakibara H (2007) Functional Characterization of Cytokinin Activating Enzymes in *Arabidopsis*. *19th Annual Meeting IPGSA*, Puerto Vallarta México, July 21-25, 2007;
- Kuroha T, Tokunaga H, Kojima M, Ueda N, Ishida T, Nagawa S, Fukuda H, Sugimoto K and Sakakibara H (2009) Functional Analyses of LONELY GUY Cytokinin-Activating Enzymes Reveal the Importance of the Direct Activation Pathway in *Arabidopsis. Plant Cell* **21**, 3152-3169;
- Kwade Z, Swiatek A, Azmi A, Goossens A, Inzé D, Van Onckelen H and Roef L (2005) Identification of Four Adenosine Kinase Isoforms in Tobacco By-2 Cells and Their Putative Role in the Cell Cycle-Regulated Cytokinin Metabolism. *J. Biol. Chem.* **280**, 17512-17519;
- Laule O, Fürholz A, Chang H-S, Zhu T, Wang X, Heifetz PB, Gruissem W and Lange BM (2003) Crosstalk Between Cytosolic and Plastidial Pathways of Isoprenoid Biosynthesis in *Arabidopsis thaliana*. *PNAS* **100**, 6866-6871
- Laureys F, Dewitte W, Witters E, Van Montagu M, Inzé D and Van Onckelen H (1998) Zeatin is Indispensable for the G2-M Transition in Tobacco BY-2 Cells. *FEBS Lett*. **426**, 29-32;
- Laureys F, Smets R, Lenjou M, Van Bockstaele D, Inzé D and Van Onckelen H (1999) A Low Content in Zeatin Type Cytokinins Is Not Restrictive for the Occurrence of G1/S Transition in Tobacco BY-2 cells. *FEBS Lett*. **460**, 123-128;
- Lei HY and Chang CP (2009) Lectin of Concanavalin A as an Anti-Hepatoma Therapeutic Agent. *J. Biomed. Sci.* **16**:10;
- Letham DS (1963) Zeatin, a Factor Inducing Cell Division Isolated from *Zea mays*. *Life Sci.* **8**, 569-573;
- Letham DS, Palni LMS, Tao GQ, Gollnow BI and Bates CM (1983) Regulators of Cell Division in Plant Tissues XXIX. The Activities of Cytokinin Glucosides and Alanine Conjugates in Cytokinin Bioassays. *J. Plant Growth Regul*. **2**, 103-115;
- Letham DS, Wilson MM, Parker CW, Jenkins ID, Macleod JK and Summons RE (1975) Regulators of Cell Division in Plant Tissue. XXIII. The Identity of an Unusual Metabolite of 6-Benzylaminopurine. *Biochim. Biophys. Acta* **399**, 61-70;
- Letham DS and Zhang R (1989) Cytokinin Translocation and Metabolism in Lupin Species II. New Nucleotide Metabolites of Cytokinins. *Plant Sci*. **64**, 161-165;
- Liavonchanka A, Hornung E, Feussner I and Rudolph MG (2006) Structure and mechanism of the *Propionibacterium acnes* polyunsaturated fatty acid isomerase. *PNAS* **103**, 2576-2581;
- Lichtenstein C, Klee H, Montoya A, Garfinkel D, Fuller S, Flores C, Nester E and Gordon M (1984) Nucleotide Sequence and Transcript Mapping of the *tmr* Gene

of the pTiA6NC Octopine T_i-plasmid: a Bacterial Gene Involved in Plant Tumorigenesis. *J. Mol. Appl. Genet*. **2**, 354-362;

- Lichtenthaler HK (1999) The 1-Deoxy-D-xylulose-5-phosphate Pathway of Isoprenoid Biosynthesis in Plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 47-65;
- Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, Törmäkangas K, Ikeda Y, Oka A, Kakimoto T and Helariutta Y (2006a) Cytokinin Signaling and its Inhibitor AHP6 Regulate Cell Fate During Vascular Development. *Science* **311**, 94-98;
- Mähönen AP, Higuchi M, Törmäkangas K, Miyawaki K, Pische MS, Sussman MR, Helariutta Y and Kakimoto T (2006b) Cytokinins Regulate a Bidirectional Phosphorelay Network in *Arabidopsis*. *Curr. Biol.* **16**, 1116-1122;
- Makino S, Kiba T, Imamura A, Hanaki N, Nakamura A, Suzuki T, Taniguchi M, Ueguchi C, Sugiyama T and Mizuno T (2000) Genes Encoding Pseudo-Response Regulators: Insight into His-to-Asp Phosphorelay and Circadian Rhytm in *Arabidopsis thaliana*. *Plant Cell Physiol*. **41**, 791-803;
- Martin RC, Mok MC, Habben JE and Mok DWS (2001) A Maize Cytokinin Gene Encoding an *O*-Glucosyltransferase Specific to *cis*-Zeatin. *PNAS* **98**, 5922-5926;
- Martin RC, Mok MC and Mok DWS (1999a) Isolation of a Cytokinin Gene, ZOG1, Encoding Zeatin *O*-Glucosyltransferase from *Phaseolus lunatus*. *PNAS* **96**, 284-289;
- Martin RC, Mok MC and Mok DWS (1999b) A Gene Encoding the Cytokinin Enzyme Zeatin *O*-Xylosyltransferase of *Phaseolus vulgaris*. *Plant Physiol*. **120**, 553-558;
- Martin RC, Mok MC, Shaw G and Mok DWS (1989) An Enzyme Mediating the Conversion of Zeatin to Dihydrozeatin in *Phaseolus* Embryos. *Plant Physiol*. **90**, 1630-1635;
- Massonneau A, Houba-Hérin N, Pethe C, Madzak C, Falque M, Mercy M, Kopecny D, Majira A, Rogowsky P and Laloue M (2004) Maize Cytokinin Oxidase Genes: Differential Expression and Cloning of Two New cDNAs. *J. Exp. Bot*. **55**, 2549-2557;
- Mathevon C, Pierrel F, Oddou JL, Garcia-Serres R, Blondin G, Latour JM, Ménage S, Gambarelli S, Fontecave M and Atta M (2007) tRNA-Modifying MiaE Protein from *Salmonella typhimurium* is a nonheme diiron monooxygenase. *PNAS* **104**, 13295-13300;
- Mauk CS and Langille AR (1978) Physiology of Tuberization in *Solanum tuberosum* L: *Cis*-Zeatin Riboside in the Potato Plant: Its Identification and Changes in Endogenous Levels as Influenced by Temperature and Photoperiod. *Plant Physiol.* **62**, 438-442;

- McGaw BA, Heald JK and Horgan R (1984) Dihydrozeatin Metabolism in Radish Seedlings. *Phytochemistry* **23**, 1373-1377;
- McGaw BA, Horgan R and Heald JK (1985) Cytokinin Metabolism and the Modulation of Cytokinin Activity in Radish. *Phytochemistry* **24**, 9-13;
- Meek L, Martin RC, Shan X, Karplus PA, Mok DWS and Mok MC (2008) Isolation of Legume Glycosyltransferases and Active Site Mapping of the *Phaseolus lunatus* Zeatin O-Glucosyltransferase ZOG1. *J. Plnat Growth Regul*. **27**, 192-201;
- Miller CO, Skoog F, von Saltza MH and Strong FM (1955) Kinetin, a Cell Division Factor from Deoxyribonucleic Acid. *J. Am. Chem. Soc.* **77**, 1392-1392;
- Miyawaki K, Matsumoto-Kitano M and Kakimoto T (2004) Expression of Cytokinin Biosynthetic Isopentenyltransferase Genes in *Arabidopsis*: Tissue Specificity and Regulation by Auxin, Cytokinin and Nitrate. *Plant J.* **37**, 128-138;
- Miyawaki K, Tarkowski P, Matsumoto-Kitano M, Kato T, Sato S, Tarkowska D, Tabata S, Sandberg G and Kakimoto T (2006) Roles of *Arabidopsis* ATP/ADP Isopentenyltransferases and tRNA Isopentenyltransferases in Cytokinin Biosynthesis. *PNAS* **103**, 16598-16603;
- Mizuno T (1997) Compilation of all Genes Encoding Two-Component Phosphotransfer Signal Transducers in the Genome of *Escherichia coli*. *DNA Res.* **4**, 161-168;
- Mizuno T and Nakamichi N (2005) *Pseudo*-Response Regulators (PRRs) or *True* Oscillator Components (TOCs). *Plant Cell Physiol.* **46**, 677-685;
- Moffatt B, Pethe C and Laloue M (1991) Metabolism of Benzyladenine is Impaired in a Mutant of *Arabidopsis thaliana* Lacking Adenine Phosphoribosyltransferase Activity. *Plant Physiol*. **95**, 900-908;
- Mok MC, Mok DW and Armstrong DJ (1978) Differential Cytokinin Strukture-Activity Relationships in *Phaseolus. Plant Physiol*. **61**, 72-75;
- Mok DWS, Mok MC, Shaw G, Dixon SC and Martin RC (1990) Genetic Differences in the Enzymatic Regulation of Zeatin Metabolism in *Phaseolus* Embryos. In *Plant Growth Substances 1988* (Pharis RP and Rood SB eds.), 267-274; Springer-Verlag, Berlin, Germany;
- Mok MC, Martin RC, Dobrev PI, Vaňková R, Ho PS, Yonekura-Sakakibara K, Sakakibara H and Mok DW (2005) Topolins and Hydroxylated Thidiazuron Derivatives are Substrates of Cytokinin *O*-Glucosyltransferase with Position Specificity Related to Receptor Recognition. *Plant Physiol*. **137**, 1057-1066;
- Morris RO, Bilyeu KD, Laskey JG and Cheikh NN (1999) Isolation of a Gene Encoding a Glycosylated Cytokinin Oxidase from Maize. *Biochem. Biophys. Res. Commun.* **255**, 328-333;

- Morris RO, Blevins DG, Dietrich JT, Durley RC, Gelvin SB, Gray J, Hommes NG, Kaminek M, Mathews LJ, Meilan R, Reinbott TM and Sayavedra-Sato L (1993) Cytokinins in Plant Pathogenic Bacteria and Developing Cereal grains. *Aust. J. Plant Physiol.* **20**, 621-637;
- Müller B and Sheen J (2008) Cytokinin and Auxin Interaction in Root Stem-Cell Specification During Early Embryogenesis. *Nature* **453**, 1094-1097;
- Mýtinová Z, Haisel D and Wilhelmová N (2006) Phytosynthesis and Protective Mechanisms During Ageing in Transgenic Tobacco Leaves with Over-Expressed Cytokinin Oxidase/Dehydrogenase and thus Lowered Cytokinin Content. *Photosynth*. **44**, 599-605;
- Nandi SK, Letham DS, Palni LMS, Wong OC and Summons RE (1989) 6-Benzylaminopurine and its Glycosides as Naturally Occuring Cytokinins. *Plant Sci*. **61**, 189-196;
- Nandi SK and Palni LMS (1997) Metabolism of Zeatin Riboside in a Hormone Autonomous Genetic Tumour Line of Tobacco. *Plant Growth Regul*. **23**, 159-166;
- Nishimura C, Ohashi Y, Sato S, Kato T, Tabata S and Ueguchi C (2004) Histidine Kinase Homologs that Act as Cytokinin Receptors Possess Overlapping Functions in the Regulation of Shoot and Root Growth in *Arabidopsis*. *Plant Cell* **16**, 1365-1377;
- Nomura T, Tanaka Y, Abe H and Uchiyama M (1977) Cytokinin Activity of Discadenine: A Spore Germination Inhibitor of *Dictyostelium discoideum*. *Phytochemistry* **16**, 1819-1820;
- Nordström A, Tarkowski P, Tarkowská D, Norbaek R, Åstot C, Doležal K and Sandberg G (2004) Auxin Regulation of Cytokinin Biosynthesis in *Arabidopsis thaliana*: A Factor of Potential Importance for Auxin-Cytokinin-Regulated Development. *PNAS* **101**, 8039-8044;
- Novák O, Tarkowski P, Tarkowská D, Doležal K, Lenobel R and Strnad M (2003) Quantitative analysis of cytokinins in plants by liquid chromatography-single quadrupole mass spectrometry. *Anal. Chim. Acta* **480**, 207-218;
- Novák O, Hauserová E, Amakorová P, Dolezal K and Strnad M (2008) Cytokinin Profiling in Plant Tissues Using Ultra-Performance Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Phytochemistry* **69**, 2214-2224;
- Parker CW, Entsch B and Letham DS (1986) Inhibitors of two Enzymes which Metabolize Cytokinins. *Phytochemistry* **25**, 303-310;
- Parker CW and Letham DS (1973) Regulators of Cell Division in Plant Tissues XVI. *Planta* **114**, 199-218;

- Parker CW, Letham DS, Gollnow BI, Summons RE, Duke CC and MacLeod JK (1978) Regulators of Cell Division in Plant Tissues. *Planta* **142**, 239-251;
- Pas J, von Grotthuss M, Wyrwicz LS, Rychlewski L and Barciszewski J (2004) Structure Prediction, Evolution and Ligand Interaction of CHASE Domain. *FEBS Lett*. **576**, 287-290;
- Persson BC and Björk GR (1993) Isolation of the Gene (*miaE*) Encoding the Hydroxylase Involved in the Synthesis of 2-Methylthio-*cis*-Ribozeatin in tRNA of *Salmonella typhimurium* and characterization of Mutants. *J. Bacteriol.* **175**, 7776-7785;
- Persson BC, Esberg B, Ólafsen Ó and Björk GR (1994) Synthesis and Function of Isopentenyl Adenosine Derivatives in tRNA. *Biochimie* **76**, 1152-1160;
- Persson BC, Ólafsson Ó, Lundgren HK, Hederstedt L and Björk GR (1998) The ms^2io^6A37 Modification of tRNA in *Salmonella typhimurium* Regulates Growth on Citric Acid Cycle Intermediates. *J. Bacteriol*. **180**, 3144-3151;
- Piaggesi A, Picciarelly P, Ceccarelli N and Lorenzi R (1997) Cytokinin Biosynthesis in Endosperm of *Sechium edule* Sw. *Plant Sci.* **129**, 131-140;
- Pierrel F, Björk GR, Fontecave M and Atta M (2002) Enzymatic Modification of tRNAs: MiaB is an Iron-Sulfur Protein. *J. Biol. Chem*. **277**, 13367-13370;
- Pierrel F, Douki T, Fontecave M and Atta M (2004) MiaB Protein is a Bifunctional Radical-S-Adenosylmethionine Enzyme Involved in Thiolation and Methylation of tRNA. *J. Biol. Chem*. **279**, 47555-47563;
- Pineda Rodó A, Brugière N, Vaňková R, Malbeck J, Olson JM, Haines SC, Martin RC, Habben JE, Mok DW and Mok MC (2008) Over-Expression of a Zeatin *O*-Glucosylation Gene in Maize Leads to Growth Retardation and Tasselseed Formation. *J. Exp.Bot*. **59**, 2673-2686;
- Pischke MS, Jones LG, Otsuga D, Fernandez DE, Drews GN and Sussman MR (2002) An Arabidopsis Histidine Kinase is Essential for Megagametogenesis. *PNAS* **99**, 15800-15805;
- Proteau PJ (2004) 1-Deoxy-D-xylulose-5-phosphate Reductoisomerase: An Overview. *Bioorg. Chem.* **32**, 483-493;
- Quesnelle PE and Emery RJN (2007) *Cis*-Cytokinins That Predominate in Pisum sativum During Early Embryogenesis Will Accelerate Embryo Growth *in vitro*. *Can. J. Bot.* **85**, 91-103;
- Raman N and Elumalai S (1996) Presence of Cytokinin in the Root Nodules of *Casuarina equisetifolia*. *Indian J. Exp. Biol.* **34**, 577-579;
- Rashotte AM, Carson SD, To JP and Kieber JJ (2003) Expression Profiling of Cytokinin Action in *Arabidopsis*. *Plant Physiol.* **132**, 1998-2011;

- Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE and Kieber JJ (2006) A Subset of *Arabidopsis* AP2 Transcription Factors Mediates Cytokinin Responses in Concert with a Two-Component Pathway. *PNAS* **103**, 11081-11085;
- Rasmussen HN, Veierskov B, Hansen-Moeller J and Noerbaek (2009) Cytokinin Composition in Bud Determination of a Conifer Tree. *3rd ACPD International Symposium*, Prague, Czech Rep, July 10-14, 2009.
- Rohmer M, Knani M, Simonin P, Sutter B and Sahm H (1993) Isoprenoid Biosynthesis in Bacteria: a Novel Pathway for Early Steps Leading to Isopentenyl Diphosphate. *Biochem. J.* **295**, 517-524;
- von Sachs J (1887) *Vorlesungen über Pflanzenphysiologie*, Oxford Clarendon Press;
- Sakai H, Aoyama T and Oka A (2000) *Arabidopsis* ARR1 and ARR2 Response Regulators Operate as Transcriptional Activators. *Plant J.* **24**, 703-711;
- Sakakibara H (2006) Cytokinins: Activity, Biosynthesis and Translocation. *Annu. Rev. Plant Biol.* **57**, 431-449;
- Sakakibara H, Kasahara H, Ueda N, Kojima M, Takei K, Hishiyama S, Asami T, Okada K, Kamiya Y, Yamaya T and Yamaguchi S (2005) *Agrobacterium tumefaciens* Increases Cytokinin Production in Plastids by Modifying the Biosynthetic Pathway in the Host Plant. *PNAS* **102**, 9972-9977;
- Sakano Y, Okada Y, Matsunaga A, Suwama T, Kaneko T, Ito K, Noguchi H and Abe I (2004) Molecular Cloning, Expression and Characterization of Adenylate Isopentenyltransferase from Hop (*Humulus lupulus* L.). *Phytochemistry* **65**, 2439-2446;
- Schmitz RY and Skoog F (1972) Cytokinins: Synthesis and Biological Activity of Geometric and Position Isomers of Zeatin. *Plant Physiol*. **50**, 702-705;
- Schnorr KM, Gaillard C, Biget E, Nygaard P and Laloue M (1996) A Second Form of Adenine Phosphoribosyltransferase in *Arabidopsis thaliana* with Relative Specificity Towards Cytokinins. *Plant J*. **9**, 891-898;
- Schön A, Böck A, Ott G, Sprinzl M and Söll D (1989) The Selenocysteine-Inserting Opal Suppressor Serine tRNA from *E. coli* is Highly Unsual in Structure and Modification. *Nucleic Acids Res*. **17**, 7159-7165;
- von Schwartzenberg K, Kruse S, Reski R, Moffatt B and Laloue M (1998) Cloning and Characterization of an Adenosine Kinase from *Physcomitrella* involved in Cytokinin Metabolism. *Plant J*. **13**, 249-257;
- Séguéla M, Briat JF, Vert G and Curie C (2008) Cytokinins Negatively Regulate the Root Iron Uptake Machinery in *Arabidopsis* Through a Growth-Dependent Pathway. *Plant J.* **55**, 289-300;

- Seif E and Hallberg BM (2009) RNA-Protein Mutually Induced Fit: Structure of *Escherichia coli* Isopentenyl-tRNA Transferase in Complex with tRNA(Phe). *J. Biol. Chem.* **284**, 6600-6604;
- Shine WE and Loomis WD (1974) Isomerization of Geraniol and Geranyl Phosphate by Enzymes from Carrot and Peppermint. *Phytochemistry* **13**, 2095-2101;
- Skoog F and Armstrong DJ (1970) Cytokinins. *Annu. Rev. Plant Physiol.* **21**, 359-384;
- Slupphaug G, Mol CD, Kavli B, Arvai AS, Krokan HE and Trainer JA (1996) A Nucleotide-Flipping Mechanism From the Structure of Human Uracil-DNA Gylcosylase Bound to DNA. *Nature* **384**, 87-92;
- Smith TA (1983) Polyamine oxidase (oat seedlings) In *Methods in Enzymology* (Tabor H and Tabor CW eds.) **94**, 311-314; Elsevier, The Netherlands;
- Sondheimer E and Tzou DS (1971) The Metabolism of Hormones During Seed Germination and Dormancy: II The Metabolism of 8-^{14}C-Zeatin in Bean Axes. *Plant Physiol*. **47**, 516-520;
- Spíchal L, Rakova NY, Riefler M, Mizuno T, Romanov GA, Strnad M and Schmülling T (2004) Two Cytokinin Receptors of *Arabidopsis thaliana*, CRE1/AHK4 and AHK3, differ in their Ligand Specificity in a Bacterial Assay. *Plant Cell Physiol*. **45**, 1299-1305;
- Stránská J, Šebela M, Tarkowski P, Řehulka P, Chmelík J, Popa I and Peč P (2007) Inhibition of plant amine oxidases by novel series of diamine derivatives. *Biochimie* **89**, 135-144;
- Sugawara H, Ueda N, Kojima M, Makita N, Yamaya T and Sakakibara H (2008) Structural insight into the reaction mechanism and evolution of cytokinin biosynthesis. *PNAS* **105**, 2734-2739;
- Suttle JC and Banowetz GM (2000) Changes in *cis*-Zeatin and *cis*-Zeatin Riboside Levels and Biological Activity During Potato Tuber Dormancy. *Physiol. Plant.* **109**, 68-74;
- Suzuki T, Miwa K, Ishikawa K, Yamada H, Aiba H and Mizuno T (2001) The *Arabidopsis* Sensor His-Kinase, AHK4, Can Respond to Cytokinins. *Plant Cell Physiol.* **42**, 107-113;
- Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Bäurle I, Kudla J, Nagy F, Schafer E and Harter K (2001) Interaction of the Response Regulator ARR4 with Phytochrome B in Modulating Red Light Signaling. *Science* **294**, 1108-1111;
- Šebela M, Štosová T, Havliš J, Wielsch N, Thomas H, Zdráhal Z and Shevchenko A (2006) Thermostable Trypsin Conjugates for High-Throughput Proteomics: Synthesis and Performance Evaluation. *Proteomics* **6**, 2959-2963;

- Šmehilová M, Galuszka P, Bilyeu KD, Jaworek P, Kowalska M, Šebela M, Sedlářová M, English JT and Frébort I (2009) Subcellular Localization and Biochemical Comparison of Cytosolic and Secreted Cytokinin Dehydrogenase Enzymes from Maize. *J. Exp. Bot*. **60**, 2701-2712;
- Štosová T, Šebela M, Řehulka P, Šedo O, Havliš J and Zdráhal Z (2008) Evaluation of the Possible Proteomic Application of Trypsin from *Streptomyces griseus*. *Anal. Biochem.* **376**, 94-102;
- Takagi M, Yokota T, Murofushi N, Ota Y and Takahashi N (1985) Fluctuation of Endogenous Cytokinin Contents in Rice During its Life Cycle-Quantification of Cytokinins by Selected Ion Monitoring Using Deuterium-Labelled Internal Standards. *Agric. Biol. Chem*. **49**, 3271-3277;
- Takei K, Sakakibara H and Sugiyama T (2001a) Identification of Genes Encoding Adenylate Isopentenyltransferase, a Cytokinin Biosynthesis Enzyme, in *Arabidopsis thaliana*. *J. Biol. Chem.* **276**, 26405-26410;
- Takei K, Sakakibara H, Taniguchi M and Sugiyama T (2001b) Nitrogen-Dependent Accumulation of Cytokinins in Root and the Translocation to Leaf: Implication of Cytokinin Species that Induces Gene Expression of Maize Response Regulatore. *Plant Cell Physiol*. **42**, 85-93;
- Takei K, Yamaya T and Sakakibara H (2004a) *Arabidopsis* CYP735A1 and CYP735A2 Encode Cytokinin Hydroxylases That Catalyze the Biosynthesis of *trans*-Zeatin. *J. Biol. Chem.* **279**, 41866-41872;
- Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T and Sakakibara H (2004b) AtIPT3 is a Key Determinant of Nitrate-Dependent Cytokinin Biosynthesis in *Arabidopsis*. *Plant Cell Physiol.* **45**, 1053-1062;
- Taller BJ, Murai N and Skoog F (1987) Endogenous Cytokinins in the Ribosomal RNA of Higher Plants. *Plant Physiol.* **83**, 755-760;
- Tanaka Y, Suzuki T, Yamashino T and Mizuno T (2004) Comparative Studies of the AHP Histidine-Containing Phosphotransmitters Implicated in His-to-Asp Phosphorelay in *Arabidopsis thaliana*. *Biosci. Biotechnol. Biochem*. **68**, 462-465;
- Tanaka M, Takei K, Kojima M, Sakakibara H and Mori H (2006) Auxin Controls Local Cytokinin Biosynthesis in the Nodal Stem in Apical Dominance. *Plant J.* **45**, 1028-1036;
- Taya Y, Tanaka Y, Nishimura S (1978) 5'-AMP is a Direct Precursor of Cytokinin in *Dictyostelium discoideum*. *Nature* **271**, 545-547;
- Taylor JS, Koshioka M, Pharis RP and Sweet GB (1984) Changes in Cytokinins and Gibberellin-Like Substances in *Pinus radiata* Buds during Lateral Shoot Initiation

and the Characterization of Ribosyl Zeatin and a Novel Ribosyl Zeatin Glycoside. *Plant Physiol*. **74**, 626-631;

- To JP, Deruère J, Maxwell BB, Morris VF, Hutchison CE, Ferreira FJ, Schaller GE and Kieber JJ (2007) Cytokinin Regulates Type-A *Arabidopsis* Response Regulator Activity and Protein Stability via Two-Component Phosphorelay. *Plant Cell* **19**, 3901-3914;
- To JP, Haberer G, Ferreira FJ, Deruère J, Mason MG, Schaller GE, Alonso JM, Ecker JR and Kieber JJ (2004) Type-A *Arabidopsis* Response Regulators are Partially Redundant Negative Regulators of Cytokinin Signaling. *Plant Cell* **16**, 658-671;
- Turner JE, Mok DWS, Mok MC and Shaw G (1987) Isolation and Partial Purification of an Enzyme Catalyzing the Formation of *O*-xylosylzeatin in *Phaseolus vulgaris* embryos. *PNAS* **84**, 3714-3717;
- Ueguchi C, Sato S, Kato T and Tabata S (2001) The AHK4 Gene Involved in the Cytokinin-Signaling Pathway as a Direct Receptor Molecule in *Arabidopsis thaliana*. *Plant Cell Physiol*. **42**, 751-755;
- Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T and Shinozaki K (1999) A Transmembrane Hybrid-Type Histidine Kinase in *Arabidopsis* Functions as an Osmosensor. *Plant Cell* **11**, 1743-1754;
- Václavíková K, Hluska T, Floková K, Slováková K, Galuszka P and Tarkowski P (2009) The Cytokinin Metabolism in Maize. 3rd *ACPD International Symposium,* Prague, Czech Rep, July 10-14, 2009.
- Veah YK, Martin RC, Mok DWS, Malbeck J, Vaňková R, Mok MC (2003) *O*-Glucosylation of *Cis*-Zeatin in Maize. Characterization of Genes, Enzymes and Endogenous Cytokinins. *Plant Physiol.* **131**, 1374-1380;
- Vreman HJ, Thomas R and Corse J (1978) Cytokinins in tRNA Obtained from *Spinacia olearacea* L. Leaves and Isolated Chloroplasts. *Plant Physiol.* **61**, 296-306;
- Vyroubalová S, Václavíková K, Turecková V, Novák O, Smehilová M, Hluska T, Ohnoutková L, Frébort I and Galuszka P (2009) Characterization of New Maize Genes Putatively Involved in Cytokinin Metabolism and Their Expression During Osmotic Stress in Relation to Cytokinin Levels. *Plant Physiol*. **151**, 433-447;
- Watanabe N, Yokota T and Takahashi N (1982) Transfer RNA, a Possible Supplier of Free Cytokinins, Ribosyl-*Cis*-Zeatin and Ribosyl-2-Methylthiozeatin: Quantitative Comparison Between Free and Transfer Cytokinins in Various Tissues of the Hop Plant. *Plant Cell Physiol.* **23**, 479-488;
- Went FW (1928) Wuchsstoff und Wachstum. *Rec. Trav. Bot. Neerl.* **25**, 1-116;

- Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H and Schmülling T (2003) Cytokinin-Deficient Transgenic *Arabidopsis* Plants Show Multiple Developmental Alterations Indicating Opposite Functions of Cytokinins in the Regulation of Shoot and Root Meristem Activity. *Plant Cell* **15**, 2532-2550;
- Werner T, Motyka V, Strnad M and Schmülling T (2001) Regulation of Plant Growth by Cytokinin. *PNAS* **98**, 10487-10492;
- Werner T and Schmülling T (2009) Cytokinin Action in Plant Development. *Curr. Opin. Plant Biol.* **12**, 527-538;
- Xie W, Liu X and Huang RH (2003) Chemical Trapping and Crystal Structure of a Catalytic tRNA Guanine Transglycosylase Covalent Intermediate. *Nature Struct. Biol.* **10**, 781-788;
- Xie W, Zhou Ch and Huang RH (2007) Structure of tRNA Dimethylallyltransferase: RNA Modification through a Channel. *J. Mol. Biol.* **367**, 872-881;
- Yamada H, Koizumi N, Nakamichi N, Kiba T, Yamashino T and Mizuno T (2004) Rapid Response of *Arabidopsis* T87 Cultured Cells to Cytokinin Through His-to-Asp Phosphorelay Signal Transduction. *Biosci. Biotechnol. Biochem*. **68**, 1966-1976;
- Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, Miwa K, Yamashino T and Mizuno T (2001) The *Arabidopsis* AHK4 Histidine Kinase is a Cytokinin-Binding Receptor that Transduces Cytokinin Signals Across the Membrane. *Plant Cell Physiol.* **42**, 1017-1023;
- Ye C, Wu S, Kong F, Zhou C, Yang Q, Sun Y and Wang B (2006) Identification and Characterization of an Isopentenyltransferase (*IPT*) Gene in Soybean (*Glycine max* L.). *Plant Science* **170**, 542-550;
- Yevdakova NA, von Schwartzenberg K (2007) Characterisation of a Prokaryote-Type tRNA Isopentenyltransferase Gene from the Moss *Physcomitrella patens*. *Planta* **226**, 683-695;
- Yonekura-Sakakibara K, Kojima M, Yamaya T and Sakakibara H (2004) Molecular Characterization of Cytokinin-Responsive Histidine Kinases in Maize. Differential Ligand Preferences and Response to Cis-Zeatin. *Plant Physiol*. **134**, 1654-1661;
- Zhang H, Horgan KJ, Reynolds PSH, Norris GE and Jameson PE (2001) Novel Cytokinins: The Predominant Forms in Mature Buds of *Pinus radiata*. *Physiol. Plant.* **112**, 127-134;
- Zhou C and Huang RH (2008) Crystallographic Snapshots of Eukaryotic Dimethyl-allyltransferase Acting on tRNA: Insight into tRNA Recognition and Reaction Mechanism. *PNAS* **105**, 16142-16147;