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Biosynthesis of cytokinins

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Abstract Cytokinins are adenine derivatives with an isoprenoid side chain and play an essential role in plant development. Plant isopentenyltransferases that catalyze the first and rate-limiting steps of cytokinin biosynthesis have recently been identified. Unlike bacterial enzymes, which catalyze the transfer of the isopentenyl moiety from dimethylallyldiphosphate (DMAPP) to the N^6 position of adenosine 5′-monophosphate (AMP), plant enzymes catalyze the transfer of the isopentenyl moiety from DMAPP preferentially to ATP and to ADP. The isopentenylated side chain is hydroxylated to form zeatin-type cytokinins. An alternative pathway, in which a hydroxylated side chain is directly added to the N^6 position of the adenine moiety, has also been suggested.

Key words *Arabidopsis* · AtIPTs · Cytokinins · DMAPP : ATP/ADP isopentenyltransferase · Plant hormone

Introduction

This review focuses on the biosynthesis of cytokinins. Naturally occurring cytokinins are adenine derivatives with a side chain at the N^6 position. Depending on the structure of the side chain, cytokinins are classified as isoprenoid or aromatic cytokinins, although aromatic cytokinins are rare. An isoprenoid cytokinin is either an isopentenyladenine (iP)-type cytokinin, which carries an isopentenyl N^6 side chain, or a zeatin-type cytokinin, which carries hydroxylated isopentenyl N^6 side chain. The side chain of a zeatin-type cytokinin occurs in either the *cis* or *trans* configuration, depending on which of the two methyl groups of the side chain is hydroxylated. The activity of *trans*-zeatin is much higher than that of *cis*-zeatin. Cytokinins occur in base, riboside, or ribotide forms, with active cytokinins thought to be in the base form. Yamada et al. (2001) proved this by showing that iP and *trans*-zeatin, but not their ribosides, could bind to the cytokinin receptor CRE1/WOL/AHK4. Many modifications of cytokinins are known; Mok and Mok (2001) have reviewed these in detail. Cytokinin oxidases/dehydrogenases destroy cytokinins by cleaving the side chain (Houba-Herin et al. 1999; Morris et al. 1999).

Levels of cytokinins are spatially and temporally regulated. Cytokinins are abundant in the root tip, the shoot apical meristem, and immature seeds (Letham 1994). It is generally assumed that the root tip is the major site of cytokinin synthesis, but the cambium, the shoot apex, and immature seeds are also thought to synthesize cytokinins (Letham 1994; Emery et al. 2000). Changes in cytokinin levels in association with plant development have been reported (Morris et al. 1993; Benkova et al. 1999; Dewitte et al. 1999; Emery et al. 2000; Yang et al. 2001; Jacqmard et al. 2002). Changes in cytokinin levels are also associated with the cell cycle, the levels being highest in the late S phase and during the M phase (Redig et al. 1996b). Environmental factors also affect cytokinin levels, which are generally positively correlated with levels of mineral nutrients, especially nitrogenous nutrients (Goring and Mardanov 1976; Salama and Wareing 1979; Samuelson and Larsson 1993; Takei et al. 2001b, 2002; Sakakibara and Takei 2002), and decreased by water stress (Yang et al. 2001). The levels of active cytokinins in plants are expected to be regulated by the rates of biosynthesis, inter-conversion, transport, and degradation.

Until recently, the rate-limiting step of cytokinin biosynthesis was assumed to be the addition of the isopentenyl side chain to AMP. Recently, however, cytokinin biosynthetic isopentenyltransferases have been identified (Kakimoto 2001; Takei et al. 2001a), and analysis of these enzymes has revealed that cytokinins are most likely synthesized by

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isopentenylation of ATP and ADP (Kakimoto 2001). An alternative pathway, perhaps involving the addition of a hydroxylated side chain to the adenine moiety, has also been proposed (Astot et al. 2000). Another possible source of cytokinins is tRNA, since particular tRNA species are isopentenylated at an adenosine residue. Because this field is advancing rapidly, it is timely to summarize our current knowledge about cytokinin biosynthesis.

DMAPP : AMP isopentenyltransferase of *Dictyostelium discoideum*

Taya et al. (1978) demonstrated that a partially purified enzyme sample from *Dictyostelium discoideum* catalyzed the transfer of the isopentenyl moiety from dimethylallyl diphosphate (DMAPP) to AMP; this was referred to as DMAPP : AMP isopentenyltransferase activity. ATP, ADP, and cAMP did not function as isopentenyl acceptors. This was the first demonstration of cytokinin biosynthesis in vitro. In *D. discoideum* cells, the reaction product, isopentenyladenosine-5′-monophosphate (iPMP), is converted to iP, and iP is then modified at its N^3 position to form discadenine, a spore germination inhibitor. Interestingly, discadenine shows cytokinin activity in assays of tobacco callus growth (Nomura and Tanaka 1977), but researchers have not examined in detail whether isopentenyladenine itself also has a direct biological role in the development of *D. discoideum*. It would be interesting to examine which processes in the development of *D. discoideum* are affected when the gene for the putative DMAPP : AMP isopentenyltransferase (see Fig. 1) is disrupted. Extracts of *D. discoideum* also possess cytokinin oxidase activity (Armstrong and Firtel 1989). The identification of AMP-isopentenyltransferase activity in *D. discoideum* encouraged investigators to examine similar activity in cytokinin-producing bacteria and in plants.

DMAPP : AMP isopentenyltransferases of *Agrobacterium tumefaciens*

The first cytokinin biosynthetic enzyme to be identified came from the gall-forming bacterium *Agrobacterium tumefaciens* (Akiyoshi et al. 1984; Barry et al. 1984). Tissue-cultured *A. tumefaciens*-incited galls are autotrophic for auxin and cytokinin, even after the gall tissue is cured of *A. tumefaciens* (Brown 1958). When *A. tumefaciens* infects a plant, the T-DNA region of the Ti-plasmid is introduced into the plant chromosome. T-DNA carries genes that are expressed in a plant cell and are responsible for deregulated production of auxin and cytokinin and for tumor formation. The *tms* locus is composed of two genes responsible for the production of auxin, and the *tmr* locus consists of a gene

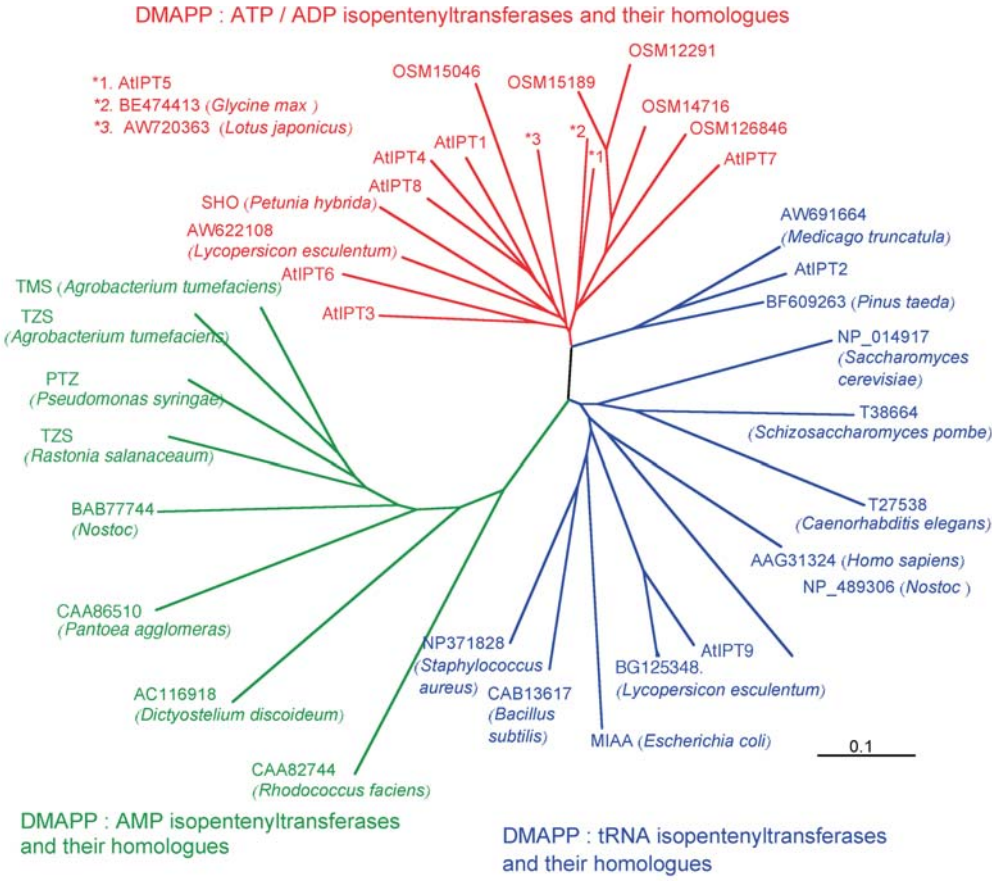

Fig. 1. A phylogenetic tree for conserved regions (regions corresponding to 6–112 amino acid residues of isopentenyltransferases. CLUSTALW program (http://www.ddbj.nig.ac.jp/) was used

responsible for the production of cytokinin. The *tmr* (*ipt*) gene was cloned and expressed in *Escherichia coli*, and extracts of *E. coli* were shown to catalyze production of iPMP from DMAPP and AMP (Akiyoshi et al. 1984; Barry et al. 1984). The purified *tmr* gene product isopentenylated AMP, but not ATP or ADP, and is DMAPP : AMP isopentenyltransferase (Morris et al. 1993).

Nopaline-producing strains of *A. tumefaciens* possess another gene for DMAPP : AMP isopentenyltransferase, *tzs*, which is present in the Ti-plasmid outside the T-DNA. The *tzs* gene is responsible for the high level of cytokinin production by these *A. tumefaciens* strains (Morris et al. 1993).

Genes that resemble *tmr* and *tzs* are also present in other gall-forming bacteria, including *Pseudomonas syringae* pv. "Savastanoi" (Powell and Morris 1986), *Pseudomonas solanacearum* (*Ralstonia solanacearum*) (Akiyoshi et al. 1989), and *Pantoea agglomerans* (*Erwinia herbicola*) (Lichter et al. 1995), and in the phytopathogenic bacterium *Rhodococcus fascians*, which causes leaf deformation, witches' broom, green patches on laminae, or leafy galls (Crespi et al. 1992; Goethals et al. 2001). These *tmr/tzs*-related genes are also responsible for cytokinin production in these bacteria (Akiyoshi et al. 1987). Interestingly, not only phytopathogenic bacteria, but also the nitrogen-fixing symbiotic cyanobacterium *Nostoc* possesses a gene related to *tmr/tzs* (Fig. 1).

Cytokinin biosynthetic isopentenyltransferases of plants

DMAPP : AMP isopentenyltransferase activity was detected in partially purified enzyme samples from cytokinin-autotrophic cultured cells of tobacco (Chen and Melitz 1979) and from kernels of *Zea mays* (Blackwell and Horgan 1994). In those studies, DMAPP and AMP, either of which was radiolabeled, were incubated with the enzyme samples. After the reaction, the mixtures were treated with a phosphatase and then incorporation of radioactivity into the isopentenyladenosine (iPAdo) fraction was determined as the enzyme activity. However, since then, no significant progress in identifying the corresponding enzyme has been made. Recently, two groups have identified cytokinin biosynthetic isopentenyltransferases by exploiting the *Arabidopsis thaliana* genome database (Kakimoto 2001; Takei et al. 2001a). It had been assumed that the first step in cytokinin biosynthesis was isopentenylation of AMP; however, a standard BLAST search of the database using bacterial cytokinin biosynthetic AMP isopentenyltransferases as a query returned plant sequences with only weak resemblances. Nonetheless, it was still reasonable to think that some kind of isopentenyltransferase was involved in a key step of cytokinin biosynthesis. Therefore, I extracted amino acid residues that were common to both bacterial DMAPP : AMP isopentenyltransferases and DMAPP : tRNA isopentenyltransferases (see below for DMAPP : tRNA isopentenyltransferases) (Kakimoto 2001). The common pattern was as follows:

– G*x*T*xx*GK[ST]*xxxxx*[VLI]*xxxxxxx*[VLI][VLI]*xx*D*xx*Q*x*{57,60}[VLI][VLI]*x*GG[ST]

where *x* denotes any amino acid residue, [ ] denotes any one of the amino acid residues within [ ], and *x*{*m*,*n*} denotes amino acid residues of *m* to *n* in number.

Because the common amino acid residues were preserved in these two types of isopentenyltransferases with different substrate specificities, I thought it possible that the pattern would be present even in isopentenyltransferases for unknown substrates. To test this idea, I used the Patmatch program (http://www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl). Unlike the standard BLAST search program, the Patmatch program can calculate homology values for a pattern of specified amino acid residues and ignore intervening, unspecified (nonconserved) amino acid residues. In 1999, the database contained two genes (*AtIPT2* and *AtIPT4*) for products that matched the conserved pattern. When *AtIPT4* was overexpressed in *Arabidopsis* calli, transformed calli underwent typical cytokinin responses – rapid cell division and shoot formation – in the absence of cytokinins. *AtIPT2*, which was later shown to be a gene for DMAPP : tRNA isopentenyltransferase (Golovko et al. 2002), did not have such an effect. Next, biochemical properties were examined. A crude extract of *E. coli* expressing *AtIPT4* exhibited apparent DMAPP : AMP isopentenyltransferase activity: when DMAPP and radiolabeled AMP were incubated with an enzyme sample and the reactant then treated with a phosphatase, incorporation of radioactivity into iPAdo was detected. However, this activity was an artifact: radiolabeled AMP is readily converted to radiolabeled ATP and ADP by the action of *E. coli*-derived factors. Purified AtIPT4 isopentenylated ATP and ADP, but not AMP; therefore, the enzyme should be classified as DMAPP : ATP/ADP isopentenyltransferase. The completed *Arabidopsis* genome database possesses nine genes for putative isopentenyltransferases, which are designated *AtIPT1–9*. Among them, *AtIPT2* (Golovko et al. 2002), and probably *AtIPT9*, code for DMAPP : tRNA isopentenyltransferases. Takei et al. (2001a) also searched the genome database and identified *AtIPT1–8*. *E. coli* expressing *AtIPT1*, *3*, *4*, *5*, *6*, *7*, or *8* secreted iP and *trans*-zeatin into the culture medium, and crude extracts from these *E. coli* exhibited DMAPP : AMP isopentenyltransferase activity. Examining the biochemical properties of purified AtIPT1 showed that although AtIPT1 catalyzed DMAPP : AMP isopentenyltransferase activity, the K_M values were rather high: 185 μM for AMP and 50 μM for DMAPP (Takei et al. 2001a). These values are much higher than the K_M values of the *tmr* product of *Agrobacterium*, which are 85.6 nM for AMP and 8.28 μM for DMAPP (Blackwell and Horgan 1993), and of the *tzs* product of *Agrobacterium*, which are 11.1 μM for AMP and 8.2 μM for DMAPP (Morris et al. 1993). Indeed, AtIPT1, as well as AtIPT4, has been reported to use ATP and ADP much more efficiently than AMP, and the main activity of AtIPT1 is also that of DMAPP : ATP/ADP isopentenyltransferase (Kakimoto 2001). The K_M values of AtIPT4 were 18 μM for ATP and 6.5 μM for DMAPP (Kakimoto

2001). These results, together with the general agreement that the cellular concentration of ATP is much higher than that of AMP – the typical ATP/AMP ratio is about 100 (Hardie et al. 1998) – suggested that the first and rate-limiting steps of cytokinin biosynthesis are mainly the isopentenylation of ATP and ADP. A proposed cytokinin biosynthetic pathway is presented in Fig. 2. The isopentenylated products of ATP and ADP are isopentenyl ATP (iPTP) and isopentenyl ADP (iPDP), respectively. Laloue et al. (1974) detected iPTP in iP-fed plants; however, the presence of the diphosphate and triphosphate forms of cytokinins has since been ignored in studies that examined species and contents of cytokinins in plants. Recently, zeatin diphosphate and zeatin triphosphate have also been detected in normal plants (P. Moritz and G. Sandberg, personal communication), supporting the ATP- and ADP-derived pathway.

A phylogenetic tree revealed three branches: DMAPP : AMP isopentenyltransferases, DMAPP : tRNA isopentenyltransferases, and DMAPP : ATP/ADP isopentenyltransferases (Fig. 1). The DMAPP : ATP/ADP isopentenyltransferase branch is composed of only plant sequences, suggesting that this family diverged after the plant kingdom appeared. Most gene products in the DMAPP : AMP isopentenyltransferase branch belong to phytopathogenic bacteria, but interestingly there are also gene products of *D. discoideum* and a cyanobacterium, *Nostoc*.

Alternative pathway: addition of a hydroxylated side chain

It has generally been thought that the isopentenyl side chain is further hydroxylated to form zeatin-type cytokinins. However, recent studies have suggested that in nopaline-producing *Agrobacterium* strains (Krall et al. 2002), and possibly also in *tms*-transformed and wild-type *Arabidopsis*, zeatin-type cytokinins can also be formed by an alternative pathway. Astot et al. (2000) fed *tms*-transformed *Arabidopsis* with deuterium oxide (^2H_2O) and [$^2H_6$] iPAdo (each of the two methyl groups of the side chain carried three deuterium atoms) simultaneously. The majority of iPMP extracted from the fed plants contained six deuterium atoms, indicating the conversion from fed [$^2H_6$] iPAdo. If iPMP were subsequently hydroxylated, [$^2H_5$]-zeatin riboside 5′-monophosphate (ZMP) would be formed. However, ZMP with 0–4 deuterium atoms predominated, indicating that an iPMP-independent pathway predominated in the *tms*-transformants. Therefore, Astot et al. (2000) proposed the possibility that *trans*-zeatin is formed by the direct addition of a hydroxylated side chain, with 4-hydroxy-3-methyl-2-(*E*)-butenyl diphosphate (HMBDP) as the side-chain donor. Similarly, wild-type *Arabidopsis* was also shown to have an iPMP-independent pathway, although the iPMP-dependent pathway also plays an important role (Astot et

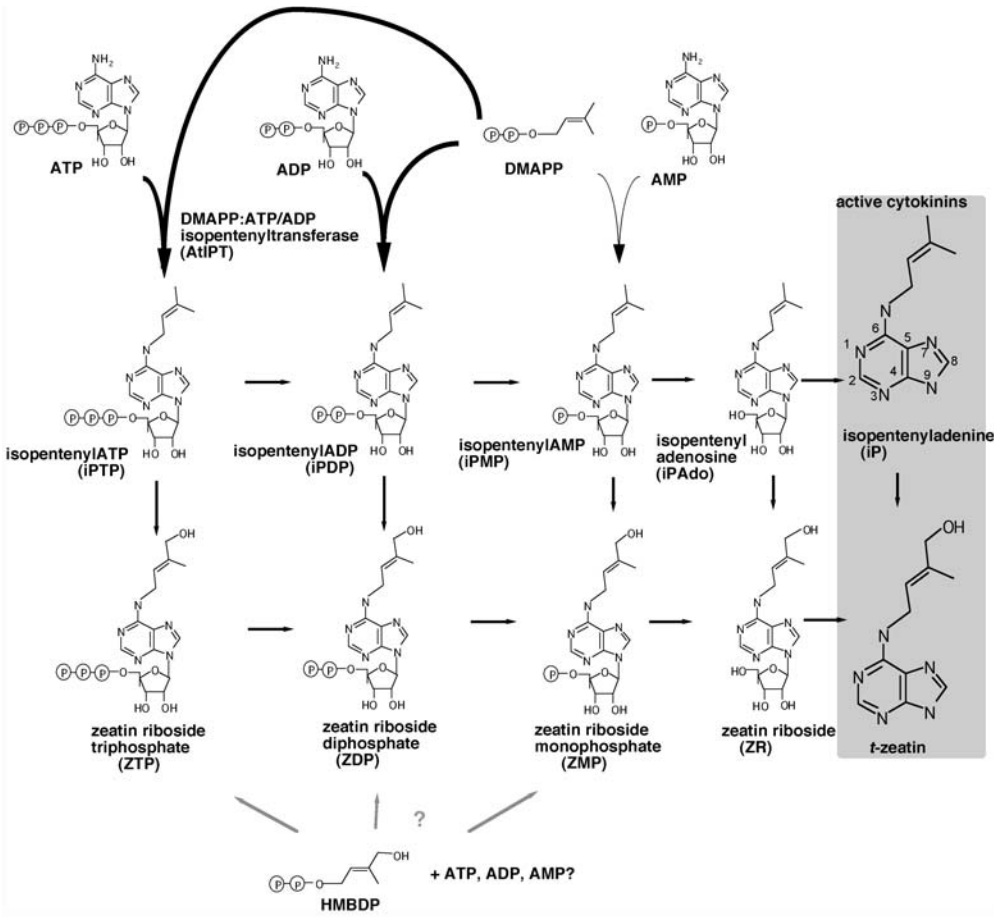

Fig. 2. A model for cytokinin biosynthesis in plants. Cytokinins that directly bind to cytokinin receptors are *shaded*

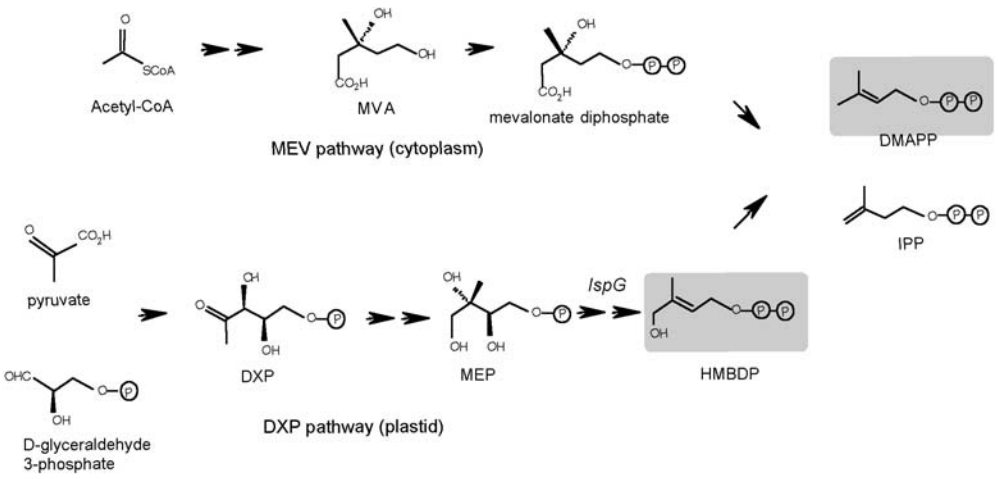

Fig. 3. An outline of the mevalonate (*MEV*) pathway and the deoxyxylulose (*DXP*) pathway for isoprenoid biosynthesis. Possible side-chain donors for cytokinins are *shaded*. *IPP*, Δ^3 isopentenyldiphosphate. *MEP*, 2-*C*-methyl-D-erythritol 4-phosphate

al. 2000). HMBDP is an intermediate of the deoxyxylulose (DXP) pathway of DMAPP and Δ^3-isopentenyldiphosphate (IPP) synthesis, and is formed by the action of the *IspG* gene product in the plastid (Fig. 3) (Hecht et al. 2001). This pathway is present in bacteria and chloroplasts, and a full set of genes for the enzymes required for the pathway is present in *Arabidopsis* (Rohdich et al. 2001). DMAPP is also synthesized by the mevalonate (MEV) pathway, which does not involve HMBDP. Conversion from iPMP to *trans*-zeatin, which is involved in the iPMP-dependent pathway, was completely inhibited by metyrapone, an inhibitor of cytochrome P450. Enzyme activity that hydroxylates the side chains of iP and iPAdo, a process which requires NADPH, was detected in a microsomal fraction of cauliflower (Chen and Leisner 1984).

As the names *tzs* (*trans*-zeatin secretion) of *Agrobacterium tumefaciens* and *ptz* (*Pseudomonas trans*-zeatin producing) of *P. syringae* indicate, bacteria expressing these genes secrete *trans*-zeatin (Beaty et al. 1986; Powell and Morris 1986; Akiyoshi et al. 1987). This can be explained either by the possible presence of an isopentenyladenine hydroxylase, or by the presence of an iPMP-independent pathway. Recently, purified tzs protein was shown to catalyze the transfer of a hydroxylated side chain from HMBDP to AMP, producing ZMP (Krall et al. 2002), but it is not known whether plant isopentenyltransferases also use HMBDP. Overexpression of the *tmr* gene in tobacco (Redig et al. 1996a; Faiss et al. 1997; McKenzie et al. 1998) and in *Arabidopsis* (Astot et al. 2000) resulted in large increases in zeatin-type cytokinins, but only subtle or modest increases in iP-type cytokinins. By contrast, iP-type cytokinins were predominantly increased when an endogenous plant cytokinin biosynthetic isopentenyltransferase gene *SHO* of *Petunia hybrida* (Zubko et al. 2002) or *AtIPT8* of *Arabidopsis* (Sun et al. 2003) was overexpressed. These results suggest that plant cytokinin biosynthetic isopentenyltransferases, at least in the case of petunia SHO and *Arabidopsis* AtIPT8, prefer DMAPP as the side-chain donor. Obviously, it should be tested whether plant cytokinin biosynthetic isopentenyltransferases are able to use HMBDP as a side-chain donor.

tRNA as a possible source of cytokinins

In 1966, iPAdo and 6-(3-methylbut-2-enylamino)purine, both of which were known to have cytokinin activity, were found as constituents of two serine tRNAs. Cytokinin moieties occur as a modified adenosine residue immediately 3′ to the anticodon of tRNAs that recognize the codon UNN (Skoog and Armstrong 1970). Cytokinin moieties in tRNA have been found in virtually all organisms tested, and plant tRNAs also contain iPAdo, *cis*-zeatin riboside (*cis*-ZR), *trans*-ZR, 2-methylthio-iPA, and 2-methylthio-ZR (Horgan 1984). It is generally thought that these tRNAs are first isopentenylated and that the isopentenyl side chain may be then further modified (Cherayil and Lipsett 1977). It is possible that degradation products of cytokinin-containing tRNAs are sources of cytokinins in plants. However, tRNAs are estimated to account for at most 40% of the cytokinin biosynthesis when calculated from the tRNA turnover rate, and it is generally assumed that tRNAs play only a minor role, if any, for cytokinin precursors (Barnes et al. 1980).

Perspectives

AtIPTs, or at least AtIPT1 and AtIPT4, preferentially isopentenylate ATP and ADP. For the complete understanding of the biosynthetic route of cytokinins, substrate specificities of all isopentenyltransferases of *Arabidopsis* should be examined. Also, it is an open question whether direct addition of a hydroxylated side chain to form *trans*-zeatin occurs in plants. Another important question is whether AtIPT1, 3, 4, 5, 6, 7, and 8 are indeed responsible for the production of major part of cytokinins in plants, because we can not exclude a t-RNA derived pathway or an unidentified pathway for cytokinin biosynthesis. The only means to answer this question will be analyses of knockout plants for AtIPTs. Finally, in order to understand how cytokinins regulate plant development, we have to

clarify how biosynthesis, inter-conversion, transport, and degradation are regulated by internal and environmental signals and are coordinated.

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References

Akiyoshi DE, Klee H, Amasino RM, Nester EW, Gordon MP (1984) T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. Proc Natl Acad Sci USA 81:5994–5998

Akiyoshi DE, Regier DA, Gordon MP (1987) Cytokinin production by *Agrobacterium* and *Pseudomonas* spp. J Bacteriol 169:4242–4248

Akiyoshi DE, Regier DA, Gordon MP (1989) Nucleotide sequence of the tzs gene from *Pseudomonas solanacearum* strain K60. Nucleic Acids Res 17:88–86

Armstrong DJ, Firtel RA (1989) Cytokinin oxidase activity in the cellular slime mold, *Dictyostelium discoideum*. Dev Biol 136:491–499

Astot C, Dolezal K, Nordstrom A, Wang Q, Kunkel T, Moritz T, Chua NH, Sandberg G (2000) An alternative cytokinin biosynthesis pathway. Proc Natl Acad Sci USA 97:14778–14783

Barnes MF, Tien CL, Gray JS (1980) Biosynthesis of cytokinins by potato cell cultures. Phytochemistry 19:409–412

Barry GF, Rogers SG, Fraley RT, Brand L (1984) Identification of a cloned cytokinin biosynthetic gene. Proc Natl Acad Sci USA 81:4776–4780

Beaty JS, Powell GK, Lica DA, Regier DA, MacDonald EMS, Hommes NG, Morris RO (1986) Tzs, a nopaline Ti plasmid gene from *Agrobacterium tumefaciens* associated with *trans*-zeatin biosynthesis. Mol Gen Genet 203:274–280

Benkova E, Witters E, Van Dongen W, Kolar J, Motyka V, Brzobohaty B, Van Onckelen HA, Machackova I (1999) Cytokinins in tobacco and wheat chloroplasts: occurrence and changes due to light/dark treatment. Plant Physiol 121:245–252

Blackwell JR, Horgan R (1993) Cloned *Agrobacterium tumefaciens ipt1* gene product, DMAPP : AMP isopentenyltransferase. Phytochemistry 34:1477–1481

Blackwell JR, Horgan R (1994) Cytokinin biosynthesis by extracts of *Zea mays*. Phytochemistry 35:339–342

Brown AC (1958) A physiological basis for the autonomous growth of the crown gall tumor cell. Proc Natl Acad Sci USA 44:344–349

Chen C, Leisner S (1984) Modification of cytokinins by cauliflower microzomal enzymes. Plant Physiol 75:442-226

Chen CM, Melitz DK (1979) Cytokinin biosynthesis in a cell-free system from cytokinin-autotrophic tobacco tissue cultures. FEBS Lett 107:15–20

Cherayil JD, Lipsett MN (1977) Zeatin ribonucleosides in the transfer ribonucleic acid of *Rhizobium leguminosarum*, *Agrobacterium tumefaciens*, *Corynebacterium fascians*, and *Erwinia amylovora*. J Bacteriol 131:741–744

Crespi M, Messens E, Caplan AB, Van Montagu M, Desomer J (1992) Fasciation induction by the phytopathogen *Rhodococcus fascians* depends upon a linear plasmid encoding a cytokinin synthase gene. EMBO J 11:795–804

Dewitte W, Chiappetta A, Azmi A, Witters E, Strnad M, Rembur J, Noin M, Chriqui D, Van Onckelen H (1999) Dynamics of cytokinins in apical shoot meristems of a day-neutral tobacco during floral transition and flower formation. Plant Physiol 119:111–122

Emery RJ, Ma Q, Atkins CA (2000) The forms and sources of cytokinins in developing white lupine seeds and fruits. Plant Physiol 123:1593–1604

Faiss M, Zalubilova J, Strnad M, Schmülling T (1997) Conditional transgenic expression of the *ipt* gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. Plant J 12:401–415

Goethals K, Vereecke D, Jaziri M, Van Montagu M, Holsters M (2001) Leafy gall formation by *Rhodococcus fascians*. Annu Rev Phytopathol 39:27–52

Golovko A, Sitbon F, Tillberg E, Nicander B (2002) Identification of a tRNA isopentenyltransferase gene from *Arabidopsis thaliana*. Plant Mol Biol 49:161–169

Goring H, Mardanov AA (1976) Influence of nitrogen deficiency on K/Ca ratio and cytokinin content of pumpkin seedlings. Biochem Physiol Pflanz 170:261–264

Hardie DG, Carling D, Carlson M (1998) The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem 67:821–855

Hecht S, Eisenreich W, Adam P, Amslinger S, Kis K, Bacher A, Arigoni D, Rohdich F (2001) Studies on the nonmevalonate pathway to terpenes: the role of the GcpE (IspG) protein. Proc Natl Acad Sci USA 98:14837–14842

Horgan R (1984) Cytokinins. In: Wilkins MB (ed) Advanced plant physiology. Longmans, London, pp 89–101

Houba-Herin N, Pethe C, d'Alayer J, Laloue M (1999) Cytokinin oxidase from *Zea mays*: purification, cDNA cloning and expression in moss protoplasts. Plant J 17:615–626

Jacqmard A, Detry N, Dewitte W, Van Onckelen H, Bernier G (2002) In situ localisation of cytokinins in the shoot apical meristem of *Sinapis alba* at floral transition. Planta 214:970–973

Kakimoto T (2001) Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyltransferases. Plant Cell Physiol 42:677–685

Krall L, Raschke M, Zenk MH, 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 Lett 527:315–318

Laloue M, Terrine C, Gawer M (1974) Cytokinins: formation of the nucleoside-5′-tri phosphate in *Tobacco* and *Acer* cells. FEBS Lett 46:45–50

Letham DS (1994) Cytokinins as phytohormones: sites of biosynthesis, translocation, and function of translocated cytokinin. CRC, Boca Raton

Lichter A, Barash I, Valinsky L, Manulis S (1995) The genes involved in cytokinin biosynthesis in *Erwinia herbicola* pv. *gypsophilae*: characterization and role in gall formation. J Bacteriol 177:4457–4465

McKenzie MJ, Mett VV, Stewart Reynolds PH, Jameson PE (1998) Controlled cytokinin production in transgenic tobacco using a copper-inducible promoter. Plant Physiol 116:969–977

Mok DW, Mok MC (2001) Cytokinin metabolism and action. Annu Rev Plant Physiol Plant Mol Biol 52:89–118

Morris RO, Blevins DG, Dietrich JT, Durley RC, Gelvin SB, Gray J, Hommes NG, Kaminek M, Mathesius U, Meilan R, Reinbott TM, Sayavedra-Soto L (1993) Cytokinins in plant pathogenic bacteria and developing cereal grains. Aust J Plant Physiol 20:621–637

Morris RO, Bilyeu KD, Laskey JG, Cheikh NN (1999) Isolation of a gene encoding a glycosylated cytokinin oxidase from maize. Biochem Biophys Res Commun 255:328–333

Nomura T, Tanaka Y (1977) Cytokinin activity of discadenine: a spore germination inhibitor of *Dictyostelium discoideum*. Phytochemistry 16:1819–1820

Powell GK, Morris RO (1986) Nucleotide sequence and expression of a *Pseudomonas savastanoi* cytokinin biosynthetic gene: homology with *Agrobacterium tumefaciens tmr* and *tzs* loci. Nucleic Acids Res 14:2555–2565

Redig P, Schmülling T, Van Onckelen H (1996a) Analysis of cytokinin metabolism in *ipt* transgenic tobacco by liquid chromatography-tandem mass spectrometry. Plant Physiol 112:141–148

Redig P, Shaul O, Inze D, Van Montagu M, Van Onckelen H (1996b) Levels of endogenous cytokinins, indole-3-acetic acid and abscisic acid during the cell cycle of synchronized tobacco BY-2 cells. FEBS Lett 391:175–180

Rohdich F, Kis K, Bacher A, Eisenreich W (2001) The non-mevalonate pathway of isoprenoids: genes, enzymes and intermediates. Curr Opin Chem Biol 5:535–540

Sakakibara H, Takei K (2002) Identification of cytokinin biosynthesis genes in *Arabidopsis*: a breakthrough for understanding the metabolic pathway and the regulation in higher plants. J Plant Growth Regul 21:17–23

Salama AM, Wareing PF (1979) Effects of mineral nutrition on endogenous cytokinins in plants of sunflower. J Exp Bot 30:971–981

Samuelson ME, Larsson C-M (1993) Nitrate regulation of zeatin riboside levels in barley roots: effects of inhibitors of N assimilation and comparison with ammonium. Plant Sci 93:77–84

Skoog F, Armstrong DJ (1970) Cytokinins. Annu Rev Plant Physiol 21:359–384

Sun J, Niu QW, Tarkowski P, Zheng B, Tarkowska D, Sandberg G, Chua NH, Zuo J (2003) The *Arabidopsis AtIPT8/PGA22* gene encodes an isopentenyl transferase that is involved in de novo cytokinin biosynthesis. Plant Physiol 131:167–176

Takei K, Sakakibara H, 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, 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 regulator. Plant Cell Physiol 42:85–93

Takei K, Takahashi T, Sugiyama T, Yamaya T, Sakakibara H (2002) Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. J Exp Bot 53:971–977

Taya Y, Tanaka Y, Nishimura S (1978) 5′-AMP is a direct precursor of cytokinin in *Dictyostelium discoideum*. Nature 271:545–547

Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, Miwa K, Yamashino T, 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

Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001) Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol 127:315–323

Zubko E, Adams CJ, Machaekova I, Malbeck J, Scollan C, Meyer P (2002) Activation tagging identifies a gene from *Petunia hybrida* responsible for the production of active cytokinins in plants. Plant J 29:797–808