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Discoveries and Dilemmas Concerning Cytokinin Metabolism

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Abstract

Natural cytokinin compounds that function as plant hormones are adenine molecules connected to an N^6 side chain. Metabolic pathways produce various structural modifications to the adenine moiety and/or the side chain that affect cytokinin function, activity, stability, and transport in plants. This review focuses on recent research that is elucidating the genes and biochemical pathways involved in cytokinin conjugation, deconjugation, and interconversion. One area of new discovery concerns the process of O-glucoside conjugate formation. Genes have been identified for O-glucosyltransferase enzymes in *Phaseolus* (*ZOG1* and *ZOX1*) and maize (cis*ZOG1*) and have been used to study enzyme activity, structure, and function in plant development. New information about the genetic basis of the adenine salvage pathway and its relationship to cytokinin interconversion and regulation has also become available. However, various dilemmas about cytokinin metabolism remain and need to be resolved using a combination of research approaches.

Key words: Cytokinin; Metabolism; Conjugation; Interconversion pathway; O-glucosyltransferase

Introduction

Cytokinin metabolism pathways produce structural modifications to the natural cytokinins that affect their function, activity, stability, and transport in plants. Recent discoveries about cytokinin biosynthesis, catabolism, and signal transduction have generated excitement and significantly increased the level of knowledge about cytokinins (Kakimoto 2001; Werner and others 2001; Hwang and Sheen 2001; Astot and others 2000). In the area of cytokinin metabolism, genes coding for cytokinin conjugation and deconjugation enzymes are just beginning to be discovered and are providing a somewhat better picture of cytokinin homeostasis. Progress in all aspects of cytokinin biology may be attributable to a fortuitous combination of molecular biology techniques, the *Arabidopsis* genome project, genetic mutants, transgenic plants, sensitive analytical methods for cytokinin quantitation, and international attention to cytokinin research.

For over 30 years, the chemical structures of most cytokinins have been well known, but much less information has been produced about the enzymology of cytokinin regulation, the genetic basis of metabolism, the function of each cytokinin compound *in vivo*, and the occurrence of cytokinins across all members of the plant kingdom. Natural cytokinins are adenine molecules connected to an N^6side chain with either a branched 5-carbon isopentenyl form or an aromatic form (Mok and Mok 2001; Auer 1997; Chen 1997; McGaw and Burch 1995; Jameson 1994; Mok and Martin 1994;

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Koshimizu and Iwamura 1986; Letham and Palni 1983). Their metabolism can be divided into two major categories: structural changes to the adenine moiety or to the side chain. The most common modifications to the adenine molecule are N^9-ribosylation, ribotide formation (5′ mono-, di- or triphosphate), and *N*-glucosylation (N^3, N^7, or N^9 position). Less common is N^9-alanine conjugation. The most common modifications to the isopentenyl side chain include *trans*-hydroxylation of the terminal methyl group (1P—Z), O-β-glucosylation, isomerization (*cis–trans* isomers of (Z), reduction (Z—DHZ), and side chain cleavage by cytokinin oxidase. Less common modifications include O-β-xylosylation, O-acetylation, and methylation.

A recent review (Mok and Mok 2001) and reviews from the past 20 years provide considerable detail on cytokinin structures, metabolic pathways, occurrence in plants, and a historical perspective on cytokinin research (Auer 1997; Chen 1997; McGaw and Burch 1995; Jameson 1994; Mok and Martin 1994; Koshimizu and Iwamura 1986; Letham and Palni 1983). This article focuses on new molecular and biochemical information about cytokinin metabolism, especially regarding O-glucoside conjugate formation and the interconversion pathway. The process of cytokinin metabolism (catabolism) by cytokinin oxidase is examined in a separate review article. Despite some recent discoveries, cytokinin metabolism requires more research to address some of the dilemmas and unanswered questions that remain.

Discovery of Genes for O-Glucosyltransferase

O-glucoside cytokinin conjugates are common in higher plants and are associated with activity in bioassays, enhanced cytokinin stability, resistance to cytokinin oxidase degradation, and function as a storage form (Mok and Mok 2001; Letham and Palni 1983). O-glucosides of Z and DHZ, and their respective riboside forms, have been identified in higher plants ranging from pine trees to beans, radish, tobacco, and corn, although they have not been reported in lower plants (Auer 1997). In the 1980s, two *Phaseolus* species were used to demonstrate the occurrence of two distinct O-glucosylation enzymes: O-glusosyltransferase and O-xylosyltransferase (Dixon and others 1989; Turner and others 1987). From *Phaseolus lunatus,* an O-glucosyltransferase was isolated that used a *trans*-Z substrate and either UDP-glucose or UDP-xylose as the sugar donor. Although this enzyme could use either sugar donor, the preferred product was zeatin-O-glucoside; it did not recognize *cis*-zeatin, DHZ, or ZR as substrates. From *Phaseolus vulgaris,* an O-xylosyltransferase was identified which preferred *trans*-Z and UDP-xylose. DHZ was also utilized, but *trans*-ZR and *cis*-Z were not acceptable substrates.

Based on this work, zeatin O-glucosyltransferase (*ZOG1)* was the first cytokinin metabolism gene cloned using an expression library from mRNA of immature *P. lunatus* seeds (Martin and others 1999). The second gene, zeatin O-xylosyltransferase (*ZOX1),* was cloned using sequences from *ZOG1* (Martin and others 1999b). *ZOX1* and *ZOG1* are very similar in that they do not contain introns, exhibit 93% identity at the DNA level and 87% identity at the amino acid level, and have similar patterns of expression in *Phaseolus* with high expression in immature seeds and low expression in vegetative tissues.

The isolation of these two highly homologous genes and the production of recombinant protein have allowed studies on substrate specificity and binding sites (Martin and others 2000). By combining sections of the *ZOG1* and *ZOX1* genes, hybrid genes and recombinant hybrid enzymes were produced. The *N*-terminal half of the enzyme probably contains the catalytic site and catalytic activity was affected by the combination of cytokinin and sugar donor; DHZ and the hexose sugar were not compatible, possibly due to steric hindrance. The inability to produce DHZ-O-glucoside with the recombinant protein is interesting because this conjugate has been reported as the major cytokinin in primary leaves of *Phaseolus vulgaris* (Wang and others 1977). Therefore, other undiscovered enzymes or pathways must be involved in production of DHZ-O-glucoside (Martin and others 2000). This shows the importance of combining molecular approaches with protein biochemistry and the characterization of endogenous cytokinins to accurately depict cytokinin metabolism in specific plant species and tissues.

The third O-glucosyltransferase gene was discovered by using the *Phaseolus ZOG1* gene to search a maize expressed sequence tag (EST) database (Martin and others 2001b). The novel maize gene *(cisZOG1)* showed 50% identity at the nucleotide level and 41% identity at the protein level with *ZOG1*. When a recombinant protein was produced, it utilized *cis*-Z and UDP-glucose to produce *cis*-zeatin-O-glucoside. Other cytokinins, including *trans*-Z, *trans*-ZR, *cis*-ZR, and DHZ, did not function as substrates. Of six sugar donors tested, only UDP-glucose was effective. The *cisZOG1* gene had no introns, as reported for *ZOG1* and *ZOX1*. Measurement of *cisZOG1* mRNA showed relatively high levels in corn

roots with lower levels in cob, kernel, and leaf tissue; no significant level of mRNA could be detected in stems. This was the first report of a regulatory enzyme specific to *cis*-Z and supports speculation that the *cis*-Z cytokinins may have a greater importance than previously believed. Surprisingly, there are no published reports of *cis*-Z cytokinins in maize despite extensive studies of this species over the years (Auer 1997). A combination of molecular, biochemical, and analytical techniques would be beneficial in developing a true picture of cytokinin regulation in maize.

Glucosyltransferase enzymes are abundant in plants and act to transfer sugars to a wide range of acceptor molecules in plants, including plant hormones (Ross and others 2001; Li and others 2001). Glucosylation can activate or inactivate compounds, alter solubility, and change transport. A study of the *Arabidopsis* genome identified a superfamily of over 100 genes that encode UDP-glucosyltransferases and contain a 42-amino-acid consensus sequence. The three O-glucosyltransferase enzymes specific to cytokinin metabolism have some similar features, including size, and some homology in the signature portion of the sequence which may code for the UDP-glucose binding site (M. Mok, personal communication). *cisZOG1* (from a monocot) has higher similarity to *ZOX1* and *ZOG1* (from dicots) than to other genes in the superfamily, making them a distinct subgroup.

Experiments utilizing the *ZOG1* gene have shown that increasing O-glucosyltransferase activity affects cytokinin pools and plant development, but the changes are not always as expected. Transgenic tobacco lines were produced with *Phaseolus ZOG1* under the control of two promoters, tetracycline (Tet) inducible and 35S (Martin and others 2001a; Mok and others 2000). In the presence of Tet, tobacco leaf discs containing the Tet-*ZOG1* construct required an increased level of Z for shoot organogenesis in tissue culture, consistent with increased conversion of Z to the storage form Z-O-glucoside. 35S-*ZOG1* tobacco plants had adventitious roots on stems under greenhouse mist conditions, 2–3 stems, and increased axillary bud development. Measurement of cytokinins showed that Z-O-glucoside increased from very low levels in control plants (<0.9 pmol/gfw) to 25 pmol/gfw in transgenic leaves and 67 pmol/gfw in transgenic roots. It is somewhat surprising that the levels of nine other cytokinins (including Z) did not change in the transgenic plants, producing an overall increase in the cytokinin pool through the accumulation of Z-O-glucoside. The authors suggest that some other steps in cytokinin regulation or biosynthesis may have changed to allow the plant to adjust to the artificial increase in Z conjugation (Martin and others 2001a; Mok and others 2000). Further studies are needed to understand the function and regulation of Z-O-glucoside *in vivo*.

If the O-glucosylation pathway provides an important cytokinin storage mechanism, several dilemmas remain to be investigated. How are cytokinins stored in plant tissues or in species where O-glucoside conjugates do not exist (or are below detection limits)? Since iP cannot be O-glucosylated, what is the storage form for this group of cytokinins? Which enzymes and genes are responsible for deconjugation of O-glucoside conjugates to the ''active'' agylcone forms? With regard to deconjugation, Vevodova and colleagues (2001) argue that the extensive diversity of glucoside conjugates in plants is likely to be paralleled by the presence of various substrate-specific β-glucosidase enzymes. At this time, the only reported β-glucosidase gene for cytokinin is *Zm-p60.1* from maize which can hydrolyze cytokinin O-glucosides and N^3-glucosides (Vevodova and others 2001; Kristoffersen and others 2000; Brzobohaty and others 1993). Transient expression of this β-glucosidase allowed tobacco protoplasts to divide in the presence of cytokinin glucosides (Brzobohaty and others 1993). However, assays indicated a broad range of substrates and the preferred substrate was not a cytokinin conjugate (reviewed in Auer 1997). Recently, *Zm-p60.1* mRNA and protein was shown to be localized to plastids in maize seedling leaf and root cells (Kristoffersen and others 2000). However, the authors do not expect this β-glucosidase to release active cytokinins for chloroplast development because it was found in root amyloplasts, epidermal cells, vascular parenchyma, and sieve elements which do not contain chloroplasts. Clearly, there is insufficient information to determine if specific or nonspecific β-glucosidase enzymes are involved in the conversion to aglycone cytokinins.

Dilemma About the Importance of the Cytokinin Interconversion Pathway

The cytokinin interconversion pathway has been defined as the interchange between cytokinin base, riboside, and ribotide forms (5′ mono-, di-, and triphosphate) (Mok and Mok 2001; Auer 1997; Chen 1997; McGaw and Burch 1995; Jameson 1994; Mok and Martin 1994; Letham and Palni 1983). The cytokinin interconversion pathway has been called part of the most fundamental control

mechanism for enzymic regulation (Chen 1981) and, in contrast, a pathway of primary importance to purine metabolism but only incidental to cytokinin regulation (Mok and Mok 2001). This range of opinions points to a dilemma in understanding cytokinin regulation.

Interconversion is a major feature of cytokinin metabolism in plants and has been observed in various types of experiments (Auer 1997; McGaw and Burch 1995; Jameson 1994; Mok and Martin 1994; Stuchbury and Burch 1986; Letham and Palni 1983; Laloue and Pethe 1982). The ribotide and riboside conjugates of the principal cytokinins (Z, DHZ, iP) have been identified repeatedly as natural endogenous cytokinins in different plant species, and they are believed to be the most abundant natural cytokinins. The interconversion pathway has also been observed in many cytokinin feeding studies using a range of plant species and radiolabeled cytokinins. Because a ribotide form of iP is probably the first product of cytokinin biosynthesis, subsequent conversion to base and riboside forms could be important, especially for controlling transport across cell membranes and interaction with a cytokinin receptor (Kakimoto 2001; Hwang and Sheen 2001; Letham and Palni 1983; Laloue and Pethe 1982). Discovery of an *Arabidopsis* gene family (*AtPUP*) for small membrane proteins, which are high-affinity transporters for purines and cytokinins (Z and kinetin), indicates that interconversion may be important in controlling cytokinin transport (Gillissen and others 2000). It has been suggested that riboside deconjugation may be the final step in producing a biologically active cytokinin and that the rate of riboside:base conversion may control ''active'' cytokinin levels in plant cells (Schulz and others 2001; Kaminek 1992).

The purine nucleotides in plants perform a variety of functions, including energy transfer, subunits of nucleic acids, and precursors of nucleotide cofactors, and can be produced through well-characterized salvage pathways (Moffatt and Ashihara 200l). Of particular interest is the adenine salvage pathway which parallels the interconversion pathway for cytokinin metabolism. The five enzymes associated with adenine salvage (interconversion) include adenine phosphoribosyl transferase (Ade→AMP), 5'nucleotidase (AMP→Ado), adenosine kinase (Ado→AMP), adenosine nucleosidase (Ado→Ade), and purine nucleoside phosphorylase (Ade→Ado) (Moffatt and Ashihara 2001; von Schwartzenberg and others 1998). For each of these metabolic steps, proteins have been extracted from plants which can utilize cytokinin substrates (reviewed in Chen 1997; Mok and Martin 1994). A consistent feature of these experiments was that the enzymes exhibited higher substrate specificity for the adenine-type substrate than did the cytokinins tested (Mok and Martin, 1994). This is cited as evidence that the interconversion pathway predominantly functions to manage adenine (purine) salvage and is incidental to cytokinin regulation (Mok and Mok 2001).

Recently, small gene families have been identified for two enzymes in the adenine salvage pathway: adenosine phosphoribosyl transferase (APT) and adenosine kinase (ADK) (Allen and others 2001; von Schwartzenberg and others l998). The discovery of the APT genes began with the selection of *Arabidopsis* mutants (*aptI*) resistant to 2,6–diaminopurine and the observation that cytokinin metabolism was altered in feeding studies with a decrease in ribotide conjugates (Moffat and others 1991; Moffatt and Somerville 1988). Reduction in APT in the mutants produced male sterility and a reduced capacity to produce callus cells in culture (Gaillard and others 1998: Lee and Moffatt 1994; Moffatt and Somerville 1998). APT is constitutively expressed and the *Arabidopsis* genome contains five sequences encoding APT or APT-like enzymes (Allen and others 2001). Recombinant proteins of *APT1* and *APT2* were produced and showed preference for adenine as a substrate compared with the cytokinin benzyladenine (Schnorr and others 1996). More recently, three of these genes (*APT1, APT2, APT3*) have been cloned, expressed in *E. coli,* and their activity studied with adenine and cytokinin substrates (Z, iP, and benzyladenine) (Allen and others 2001). *APT1* and *APT3* were localized in the cytosol of *Arabidopsis* leaf cells, but there was no evidence that *APT4* and *APT5* were expressed. Based on V_{max} and K_m values, all three APT enzymes used adenine efficiently, and the authors suggest that they would be about equal in the metabolism of Z and iP, although the relevance of this study to *in vivo* conditions is unknown (Allen and others 2001).

Adenosine kinase (ADK) is responsible for the conversion of adenosine to AMP (Moffatt and Ashihara 2001). The first plant gene for ADK was cloned from a cDNA library for the moss *Physcomitrella patens* and function was demonstrated by complementation in an *E. coli* purine auxotrophic line (von Schwartzenberg and others 1998). Adenosine was converted to AMP using ATP as the source of the phosphate group. Using either recombinant protein or moss enzyme, a cytokinin riboside (isopentenyladenosine) could be converted to the monophosphate form, leading to the suggestion that ADK could play a role in cytokinin interconversion. Subsequently, two isoforms of ADK were found in *Arabidopsis* (Moffatt and others

2000). ADK1 and ADK2 were similar with regard to DNA and amino acid sequences and had constitutive expression as expected for a housekeeping enzyme. Both ADK isoforms had a preference for adenosine in comparison with isopentenyladenosine. Although the primary role of this enzyme is adenylate and methyl recycling, a role in cytokinin interconversion *in vivo* cannot be excluded (Moffatt and others 2000).

The interconversion step of deribosylation—potentially the most important mechanism for regulating the deconjugation of a cytokinin riboside to an ''active'' base—has recently been studied in three model plant systems, *Physcomitrella, Arabidopsis*, and *Mercurialis.* With regard to the adenine salvage pathway, no plant genes have been identified for adenosine nucleosidase (Ado→Ade). Schulz and colleagues (2001) utilized the *ove* mutants of the moss *Physcomitrella patens* to study cytokinin regulation and deribosylation. Recessive *ove* mutant lines have been isolated because of their phenotype of increased bud formation. Studies have shown that the *ove* phenotype is associated with mutations in three different complementation groups, increased extracellular cytokinin levels, and increased endogenous iP-type cytokinin, although the genetic basis for these changes is unknown. Schultz and coworkers (2001) used five *ove* mutant lines in the chloronema stage in feeding studies with ^{3}H-isopentenyladenosine. Although feeding studies always introduce some complications for data analysis, the results showed an increased rate of riboside:base conversion in all *ove* lines compared with wild type. The authors noted that, unlike the *ove* mutants, *ipt* transgenic moss lines with increased cytokinin biosynthesis did not have an increased rate of riboside:base conversion. Therefore, the change in the riboside:base conversion rate in *ove* lines is not due to increased cytokinin biosynthesis. Schultz and co-workers (2001) supported the hypothesis that the *ove* gene products code for negative regulators of cytokinin biosynthesis and may also code for negative regulators of the enzyme(s) responsible for deribosylation. Surprisingly, ribohydrolase (adenosine nucleosidase) enzyme activity could not be detected in extracts of *Physcomitrella* tissue, although activity has been found in protein extracts from other plant species (Auer 1999; Yang and others l998; reviewed in Mok and Martin 1994).

The interconversion pathway for cytokinin regulation has also been studied in an *arabidopsis* mutant (*cym*) which has resistance to cytokinin ribosides, a cytokinin-overproduction phenotype, and a reduction in both cytokinin ribohydrolase activity and adenosine nucleosidase activity (Auer 1999). Compared with wild type plants, protein extracts from the *cym* mutant had only 50% of ribohydrolase enzyme activity using the substrate isopentenyladenosine and 68% with the substrate adenosine. Competition assays showed that the ribohydrolase could hydrolyze riboside conjugates of Z, iP, or DHZ but not the nucleosides guanosine, uridine,or cytidine. Furthermore, *cym* plants had increased cytokinin riboside and ribotide accumulation in shoot tissue, although cytokinin levels in roots were similar to wild type (C. Auer, A. Nordstrom, G. Sandberg, unpublished data). Therefore, it appears that the *cym* line contains a single recessive mutation on chromosome 2 that affects ribohydrolase activity and cytokinin accumulation. In a study of the flowering process in *Mercurialis annua,* an enzyme with ribohydrolase activity was purified 490-fold from female stem apices (Yang and others 1998). In assays, the enzyme from female plants converted *trans*-ZR to Z with 100% efficiency but did not use adenosine or *cis*-ZR as substrates. Male plants showed 25% conversion of *trans*-ZR to Z. Yang and colleagues (1998) claim this is the first report of a stereospecific *trans*-zeatin riboside β-ribosidase.

A delicate balance of cytokinin bases, ribosides, and ribotides seems to be tightly regulated in plant tissues and is likely to play a role in plant development. A recent study of white lupin (*Lupinus albus*) correlated the stages of seed and fruit development with the quantification of 18 cytokinins using an internal isotope dilution GC-MS method (Emery and others 2000). Riboside and ribotide conjugates of *trans*-Z, *cis*-Z and DHZ were the predominant forms of cytokinin in all tissues tested. During the first 10 days of ovary development, the riboside and ribotide forms accumulated to very high levels (up to 1.1 μmol/gfw), while little or no free base or O-glucosides were detected. Comparison of cytokinins in the embryonic axis, cotyledons, endosperm, seed coat, and pod wall showed patterns of riboside and ribotide accumulation specific to each tissue, with the lowest levels in the embryo axis and the highest levels in the endosperm. In addition, recent studies of tobacco and *Mercurialis annua* implicated the control of cytokinin riboside levels in the process of flower development (Dewitte and others 1999; Yang and others 1998). Examination of tobacco and wheat chloroplasts showed an accumulation of bases, ribosides, ribotides, and *N*–glucosides relative to the surrounding young or old leaf tissue (Benkova and others 1999). If constitutive expression of the housekeeping genes for adenine salvage were regulating levels of cytokinin ribotides and ribosides, it seems unlikely that distinct patterns of accumulation specific to plant organelles, tissues,

and stages of development would occur. Although the ADK and APT genes that have been discovered thus far are essential for adenine salvage, it is important to note that enzyme function *in vivo* does not depend on *in vitro* assay conditions but rather on enzyme abundance in cells, changes in enzyme level through time, compartmentation or localization within cells, and the availability of substrates (Allen and others 2001).

Discovery of Natural Cytokinin Conjugates

In the last few years, there have been few reports of novel cytokinins. It is impossible to say if this is due to a lack of research in this area, the technical difficulty in analyzing natural cytokinins in the large pool of adenine-related compounds, or the complete characterization of all of the natural cytokinins that occur in plants. When considering this question, it is important to note that relatively few plant species, primarily angiosperms, have been examined using the most rigorous analytical techniques (Auer 1997).

One example of cytokinin discovery was reported by Zhang and colleagues (2001) through their study of bud development in the gymnosperm *Pinus radiata.* While one novel conjugate had already been reported in this pine species (Taylor and others 1984), Zhang and co-workers (2001) identified three double sugar conjugates, including isopentenyladenine-9-(glucopyranosyl riboside), dihydro zeatin-9-(glucopyranosyl riboside), and zeatin-9-(glucopyranosyl riboside). In each conjugate, the glucosyl moiety was attached to one of the — OH groups of the N^9-linked ribosyl unit. Although the exact linkage between the two sugars was not determined, it is likely to be a β-linkage with a 3-linked ribosyl and 1-linked glucosyl unit. Phosphorylated forms of these new cytokinins were also detected. The double sugar conjugates of Z and DHZ were the most abundant cytokinins found in *P. radiata* mature buds, while O-glucosides were not detected. The authors speculate that conifers may have a different pathway of glucoside conjugation and that these novel conjugates may be active in bud morphogenesis (Zhang and others 2001).

The report of an cytokinin O-glucosylation enzyme specific to *cis*-zeatin (see above: Martin and others 2001b) suggests that the natural abundance and function of this cytokinin group may be important. Although *cis*-zeatin and its conjugates have been known for many years, they were primarily associated with tRNA-modified bases and have shown little or no biological activity in assays. It is not known if these cytokinins have a direct hormonal function or if they serve as a precursor for conversion to *trans*-Z compounds. A *cis–trans* Z isomerase protein has been reported in *Phaseolus* (Bassil and others 1993). However, the detection of *cis*-Z in plant tissues could be due to improper experimental controls (Suttle and Banowetz 2000; Dewitte and others 1999). Studies using antibodies to measure endogenous cytokinins or for immunolocalization should use tRNA as internal controls to check cross-reactivity; cytokinin quantitation studies should utilize extraction methods that preclude the measurement of tRNA degradation products (Suttle and Banowetz 2000; Dewitte and others 1999). A study of white lupin (*Lupinus albus*) correlated seed and fruit development with the accumulation and transport of 18 cytokinins (Emery and others 2000). *Cis*-isomers of Z, zeatin riboside, ribotides, and zeatin riboside-O-glucoside were detected along with the corresponding *trans*-Z conjugates. Developing ovaries (0–10 days dpa) had 37–85% of total cytokinins in the *cis*-isomer form. In developing fruits, the endosperm had a very high cytokinin concentration (0.6 μmol/gfw) but contained low levels of the *cis*-isomers. Xylem and phloem exudates collected at the base of the inflorescence contained primarily *cis*-isomers up to 10 dpa (67–97%), but the percentage of *cis*-isomers dropped dramatically as the fruit developed (1–3% at 77 dpa). Based on their calculations, most of the cytokinins in lupine fruit were not a result of transport and were derived from synthesis within seed tissues.

Although not the first study to document *cis*-Z cytokinins in potato, a recent report showed that *cis*-Z and *cis*-ZR may have a role in the termination of potato dormancy (Suttle and Banowetz 2000). Endogenous *cis*-Z levels increased during potato storage, *cis*-Z and *trans*-Z showed equal activity in promoting sprout development, and up to 9% of radiolabeled *cis*-Z was converted to *trans*-ZR. It is not known if both Z isomers have a function in the termination of potato tuber dormancy, or if one form is converted to the other. Further research is needed to determine the function of *cis*-Z and its relationship to *trans*-Z in different plant species.

Future Directions for Discovery

Contemporary research has produced both discoveries and dilemmas about the mechanisms involved in cytokinin regulation. Twenty years ago, researchers hoped to move toward a true under-

standing of the relationship between cytokinin metabolism, environmental stimuli, and the control of plant development (Letham and Palni 1983). This goal remains largely unrealized, especially with regard to the interaction of environment and cytokinin regulation. However, research that begins to integrate information about cytokinin metabolism and plant development can be found, such as the recent report on the action of the *Knotted1* class of homeobox genes affecting leaf shape (Frugis and others 2001). Transgenic lettuce leaves expressing a *Knotted1* homolog (*knat1*) showed a change in leaf shape, a large increase in iP-type cytokinins, and an inversion of the Z:iP ratio compared with control leaves. It was proposed that KNAT1 could change leaf shape through modification of cytokinin metabolism. It is likely that this type of research will lead to a better understanding of the relationship between cytokinins and plant development. However, while exciting new results have been obtained from genetic and molecular approaches, the research reviewed in this article makes it clear that a combination of molecular biology, enzymology, and analytical methods are essential to get an accurate picture of cytokinin pools and metabolic pathways in different plant species, tissues, and developmental events.

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