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Regulation of cytokinin content in plant cells

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Cytokinin levels in plant cells are dependent on cytokinin biosynthesis and/or uptake from extracellular sources, metabolic interconversions, inactivation and degradation. Cytokinin conversion to compounds differing in polarity seems to be decisive for their entrapment within the cell and intracellular compartmentation, which affects their metabolic stability. Increase in cytokinin levels, resulting either from their uptake or intracellular biosynthesis, may promote further autoinductive accumulation of cytokinins which may function in the induction of cytokinin-initiated physiological processes. Accumulated cytokinins are capable of inducing cytokinin oxidase which consequently decreases cytokinin levels. This seems to be the mechanism of re-establishment and maintenance of cytokinin homeostasis required for further development of physiological events induced by transient cytokinin accumulation. Auxin may influence cytokinin levels by down regulation of cytokinin biosynthesis and/or by promotion of cytokinin degradation. A model of the regulation of cytokinin levels in plant cells based on these phenomena is presented and its physiological role(s) is discussed.

Key words – Auxin, cytokinin biosynthesis, cytokinin compartmentation, cytokinin content, cytokinin oxidase, cytokinin uptake, *ipt* gene.

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Introduction

The discovery of cytokinins and investigation of their roles in plants originated from testing the effects of exogenously applied substances promoting cell division in a number of plant systems. Exogenous application is associated with many well known limitations arising from differences in uptake (Laloue et al. 1981, Auer et al. 1992), metabolism (Kamínek 1992, McGaw and Burch 1995) and compartmentation (Jameson 1994) of applied substances in plant cells which complicate the interpretation of results. Therefore, it has been encouraging to learn that most of the biological effects described for exogenously applied cytokinins are also induced by elevated levels of endogenous cytokinins in transgenic plant cells expressing the cytokinin biosynthetic *ipt* gene coding for the enzyme Δ^2-isopentenyl pyrophosphate:5'-AMP Δ^2-isopentenyl transferase (see Klee and Romano 1994). These findings indicate that the regulation of particular physiological processes is really governed by changes in levels of endogenous cytokinins. It is obvious that both application of exogenous cytokinins and manipulated expression of the *ipt* gene in transgenic plant cells represent useful, if very crude tools for probing the actual role of cytokinins in plants.

Mechanisms of metabolic regulation of plant hormone levels should meet two basic requirements essential for hormonal control of plant development: (1) the ability to respond to hormonal or other signals and generate fast and significant changes in the concentration of a particular hormone or its concentration ratio with respect to other plant hormone(s) in particular cells; and (2) the capability to maintain hormonal homeostasis at certain stage(s) of cell development. Rapid changes in hormonal levels function in the initiation of major developmental processes (e.g. organ formation) while hormonal homeostasis is required for further development of initiated events (e.g. organ growth). Operation of

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these mechanisms is certainly dependent on cell hormonal status. Most plant cells and tissues are cytokinin-dependent when cultured in vitro, suggesting a strong dependence of their intracellular cytokinin levels on cytokinin supply from exogenous sources. However, this does not imply the cytokinin dependency of cells and tissues of intact plants, which exhibit limited cytokinin efflux and different 'cytokinin economy' as compared to isolated ones.

To understand the precise mechanisms controlling cytokinin levels and action we need to expand our knowledge of the uptake of different cytokinins and their compartmentation in plant cells, pathways and regulations of cytokinin biosynthesis, metabolic interconversions, inactivation and degradation and sensitivity of plant cells to so far poorly identified 'active forms' of cytokinins. The present contribution focuses on how cytokinin uptake, compartmentation, autoinductive accumulation and degradation regulate cytokinin levels in plant cells.

Abbreviations – BA3G, BA-3-glucoside; CKOX, cytokinin oxidase; DZ, dihydrozeatin; DZMP, dihydrozeatin nucleotide; DZOG, dihydrozeatin *O*-β-glucoside; DZR, dihydrozeatin riboside; DZROG, dihydrozeatin riboside *O*-β-glucoside; iP, N^6-(Δ^2-isopentenyl)adenine; iPA, N^6-(Δ^2-isopentenyl)adenosine; *ipt*, gene encoding isopentenyl transferase; IPT, isopentenyl transferase; NAA, 1-naphthaleneacetic acid; Z, zeatin; Z7G, zeatin-7-glucoside; ZMP, zeatin nucleotide; ZOG, zeatin *O*-β-glucoside, ZR, zeatin riboside; ZROG, zeatin riboside *O*-β-glucoside.

Cytokinin uptake

The differences in cytokinin uptake from extracellular solutes including xylem and phloem sap (see Letham 1994, Hoad 1995) by different plant cells may be decisive for long-distance delivery of hormonal signals and regulation of cytokinin levels in target plant cells. The existence of such signals and their possible physiological function have been supported by a number of reported correlations between changes in the cytokinin content in xylem sap and the developmental events that are under cytokinin control, such as leaf senescence of monocarpic plants (Sitton et al. 1967, Noodén et al. 1990), release of lateral buds from apical dominance in *Phaseolus vulgaris* and *Pisum sativum* (Bangerth 1994, Li et al. 1995) and bud burst of deciduous trees (e.g. Tromp and Ovaa 1990). Recently, Noodén et al. (1990) correlated the changes in cytokinin concentrations in xylem exudates with the response to pod removal in *Glycine max* plants. Depodding at full pod extension, which delayed leaf senescence, was accompanied by a dramatic increase of predominant cytokinins, *trans*-zeatin riboside (ZR) and dihydrozeatin riboside (DZR). Pod removal at late pod-filling did not delay senescence and had little effect on xylem cytokinins.

Concentration of cytokinins occurring in xylem exudates seems to be physiologically relevant. Application of cytokinins to derooted seedlings at multiples of their endogenous concentrations found in xylem sap was effective for the retention of leaf senescence in oats and regulation of transpiration in wheat seedlings (Badenoch-Jones et al. 1996). However, radioactivity from [^{3}H]ZR or [^{3}H]DZR supplied to *Nicotiana tabacum* plants via xylem was not preferentially accumulated in young leaves as compared to senescing ones, indicating that cytokinins in upper young leaves may also originate from other sources including their biosynthesis de novo (Singh at al. 1992).

Tobacco cells were found permeable to lipophilic cytokinin bases (N^6-benzyladenine [BA], N^6-[Δ^2-isopentenyl]adenine [iP]) and their respective ribosides, which become entrapped in the cells after their metabolic conversion to corresponding polar cytokinin nucleotides, *N*- and *O*-glucosides (Laloue et al. 1981, Laloue and Pethe 1982) (Fig. 1). Such conversion may be responsible for local accumulation of cytokinins in individual cells as described for *Nicotiana tabacum* leaf cells overexpressing the *ipt* gene (Estruch et al. 1991). The resulting shoot formation was restricted to individual cells and no morphological symptoms indicating an increase in cytokinin levels were recognised in neighbouring cells.

The primary role of cytokinin uptake in the regulation of cytokinin levels in cytokinin-dependent cells was clearly demonstrated by Palmer and Palni (1986). They found a similar percentage of [^{3}H]ZR uptake by cytokinin-dependent callus of *Glycine max* incubated for 20 h in solutions differing 100-fold in *trans*-zeatin (Z) concentration indicating that the growth response of this particular tissue to exogenous cytokinins over a broad range of concentrations corresponds to the cytokinin uptake.

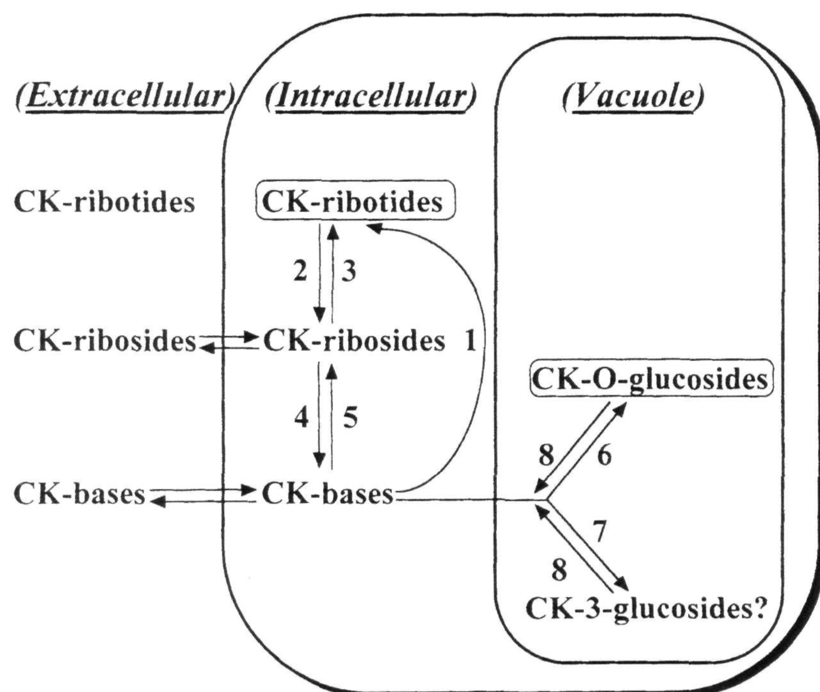


Fig. 1. Uptake, metabolic interconversions and compartmentation of cytokinins in plant cells. (1) Adenine phosphoribosyltransferase, (2) 5'-nucleotidase, (3) adenine kinase, (4) adenosine nucleosidase, (5) adenine phosphorylase, (6) *O*-glucosyltransferase, (7) 7(?)-glucosyltransferase, (8) β-glucosidase. Data compiled from Palmer et al. 1981, Laloue and Pethe 1982, McGaw et al. 1984, Fusseder and Ziegler 1988, Kamínek 1992 and Jameson 1994.

Cytokinin compartmentation

Similarly to [^{3}H]ZR in tobacco, the [^{3}H]dihydrozeatin (DZ) supplied to photoautotrophically growing cell suspension cultures of *Chenopodium rubrum* was rapidly taken up and metabolised to [^{3}H]DZR, [^{3}H]dihydrozeatin *O*-glucoside (DZOG) and [^{3}H]dihydrozeatin riboside *O*-glucoside (DZROG). Gentle osmotic disruption and fractionation of protoplasts showed that both cytokinin-*O*-glucosides were localised within the vacuole whereas both [^{3}H]DZ and [^{3}H]DZR were localised outside the vacuole and part of [^{3}H]DZR was excreted into the medium (Fusseder and Ziegler 1988). In tomato crown gall cells ZR was localised by indirect immunogold labeling only in the cytoplasm (Eberle et al. 1987).

Several indirect data support compartmentation of cytokinin-*O*-glucosides separately from *β*-glucosidase activity. *O*-glucosides were the major metabolites of Z and DZ accumulated in detached senescing leaves of *Phaseolus vulgaris* (Palmer et al. 1981a,b). This is in contrast with their high activity in leaf senescence assays (Letham et al. 1983). Zeatin *O*-*β*-glucoside (ZOG) and DZOG were the two major cytokinins in transgenic tobacco callus tissues expressing the *ipt* gene (Redig et al. 1997) while zeatin riboside *O*-*β*-glucoside (ZROG) accumulated to large amounts in another transgenic tobacco tissue after derepression of the *ipt* gene by tetracycline (Tc) (Motyka et al. 1996). Comparing the incorporation of exogenously applied [^{3}H]adenine into cytokinins in suspension of tobacco BY-2 cells, most of the radioactivity was associated with ZROG beginning 1 h after [^{3}H]adenine application (Dobrev and M. Kamínek, unpublished results). Such accumulation seems to be possible if cytokinin-*O*-glucosides are not exposed to *β*-glucosidase. Although it is believed that *β*-glucosidases are ubiquitous, their precise localisation in tobacco cells is not known. High metabolic stability of exogenously applied [^{3}H]ZOG and [^{3}H]DZOG in *Phaseolus* leaves and *Raphanus* seedlings (McGaw and Horgan 1985) indicates that either these conjugates are protected against an attack by *β*-glucosidase during their translocation to the vacuole or the activity of this enzyme is very low in corresponding plant tissues.

Two different *β*-glucosidases capable of hydrolysing ZOG were recently found in *Zea mays* (Brzobohatý et al. 1993) and *Brassica napus* (Falk and Rask 1995). Both were preferentially expressed in young tissues undergoing intensive cell division and differentiation which are known to accumulate cytokinins (Dietrich et al. 1995). While the *B. napus β*-glucosidase was reported to be specific for ZOG, the maize enzyme uses ZOG and kinetin-3-glucoside as substrate; however, its potential ability to hydrolyse other non-cytokinin *β*-glucosides has not been characterised. Unfortunately, kinetic characteristics, which can help to elucidate the physiological relevance of these enzymes, have not been determined. Nevertheless, these reports represent the first indication that metabolic release of Z from ZOG may be specific and localised to certain plant tissues.

In contrast to cytokinin-*O*-glucosides, which are biologically active either themselves or after enzymatic hydrolysis, the cytokinin-*N*-glucosides are biologically inactive (see McGaw and Burch 1995). The exception might be (*N*)3-glucosides which were detected as metabolites of externally applied cytokinins (McGaw et al. 1984). N^6-benzyladenine-3-glucoside (BA3G) exhibited cytokinin activity in a root growth suppression bioassay and was hydrolysed in vivo in tobacco leaves (Faiss et al. 1996). If the (*N*)3-glucosides also represent a storage form of cytokinins and are hydrolysed in the same way as *O*-glucosides to yield active cytokinins, then their localisation in the vacuole should be considered. Compartmentation of cytokinins resulting from their metabolic interconversions to products differing in their mobility across the cell membranes is summarised in Fig. 1.

Regulatory effects of cytokinins and auxin on cytokinin levels

The complexity of hormonal control in plants is displayed by interactions among different classes of plant hormones in the regulation of different developmental and physiological processes. It is becoming increasingly evident that these interactions also involve alterations of one hormone level by another. In addition, the concentration of each hormone, including cytokinins, is under control of until now poorly understood metabolic regulations responding to its own levels in plant cells. In case of cytokinins these mechanisms may also include the ability of certain plant cells to switch on and off cytokinin biosynthesis and/or degradation.

Effects of cytokinins on their own accumulation

An interesting view on mechanisms controlling cytokinin levels in plant cells was presented by Meins (1989) who stated that the cell's capacity for cytokinin-autonomous growth is associated with elevated cytokinin levels. A biochemical-switch model for cytokinin habituation proposed that the habituated state is maintained by a positive feed-back loop in which cytokinins, or related cell division-promoting factors, either induce their own biosynthesis or inhibit their own degradation. Such a situation seems to exist in meristematic cells. It was reported that explants of tobacco pith derived from the mitotically active region of *Nicotiana tabacum* stem apex were cytokinin-autonomous and habituation frequency declined rapidly with increasing distance from the apex (Turgeon 1982). Accumulation of cytokinins is indeed correlated with the onset of cell division, as shown in partially synchronised tobacco cell cultures (Nishinari and Syôno 1986, Redig et al. 1996, Zažímalová et al. 1996).

Levels of endogenous isoprenoid cytokinins were increased in *Glycine max* (Thomas and Katterman 1986)

and *Nicotiana* (Hansen et al. 1987) callus tissues and in *Beta vulgaris* cell suspension (Vaňková et al. 1991) following exposition to exogenous urea-type cytokinin (thidiazuron) and to cytokinins bearing N^6 aromatic or heterocyclic side chains (BA and kinetin) which can be readily distinguished from and cannot be converted to isoprenoid cytokinins. The response of plant cells to exogenous cytokinin is very fast – a significant increase in total levels of endogenous cytokinins (preferentially ZR, DZR, zeatin nucleotide [ZMP], dihydrozeatin nucleotide [DZMP] and iP) in transgenic tobacco callus expressing *ipt* was recorded already 6 h after application of BA solution to the callus surface and corresponding cytokinin-*O*-glucosides accumulated during the following 6 h. However, an increase in endogenous isoprenoid cytokinin levels was not recorded in non-transformed tissue during this short-term experiment (Redig et al. 1997). Following the dynamics of cytokinin excretion from cytokinin-autonomous and cytokinin-dependent tobacco cells immobilised on solid support in a column-flow-through system, Vaňková et al. (1987) found that the former excreted a 4- to 5-fold higher amount of Z and ZR. Moreover, exposure of the cytokinin-dependent immobilised cells to a pulse of BA after 72 h of cytokinin starvation caused a 3- to 5-fold increase in the excretion rate of Z and ZR. The duration of excretion and the amount of excreted cytokinins indicated that the excreted cytokinins were at least partially synthesised de novo. Recent work (see Zhang et al. 1995) has indeed shown the promotion of *ipt* gene expression and promotion of cytokinin biosynthesis in response to BA which was detected by northern and western blotting in transformed tobacco callus.

Accumulation of isoprenoid cytokinins in response to application of exogenous cytokinin is capable of inducing habituation only in tissues which habituate readily. Exposure of cytokinin-dependent callus tissue of *Nicotiana tabacum* cv. Wisconsin 38 to exogenous kinetin did not induce habituation of the tissue for cytokinins. Fresh weight of tissue transferred at different time intervals from kinetin-containing to cytokinin-free medium was linearly proportional to the time of tissue preculture on kinetin-containing medium (Fig. 2). This indicates that either the number of 'inducible' cells in the tissue was too low or that the elevation of cytokinin levels in the cells remained under the threshold level required for habituation. Apparently, the effect of exogenous cytokinins on the accumulation of the endogenous ones is a widespread phenomenon in plants; however, the induction of habituation of cells for cytokinins depends on their competence for habituation as well as on genetic factors (Mok et al. 1980, Meins 1989).

It should be mentioned that exogenous auxins in contrast to cytokinins do not induce accumulation of indole-3-acetic acid (IAA). Application of 2,4-dichlorophenoxyacetic acid or naphthaleneacetic acid (NAA) to cultured hypocotyls of *Daucus carota* had minor effects on endogenous IAA concentration. Application of

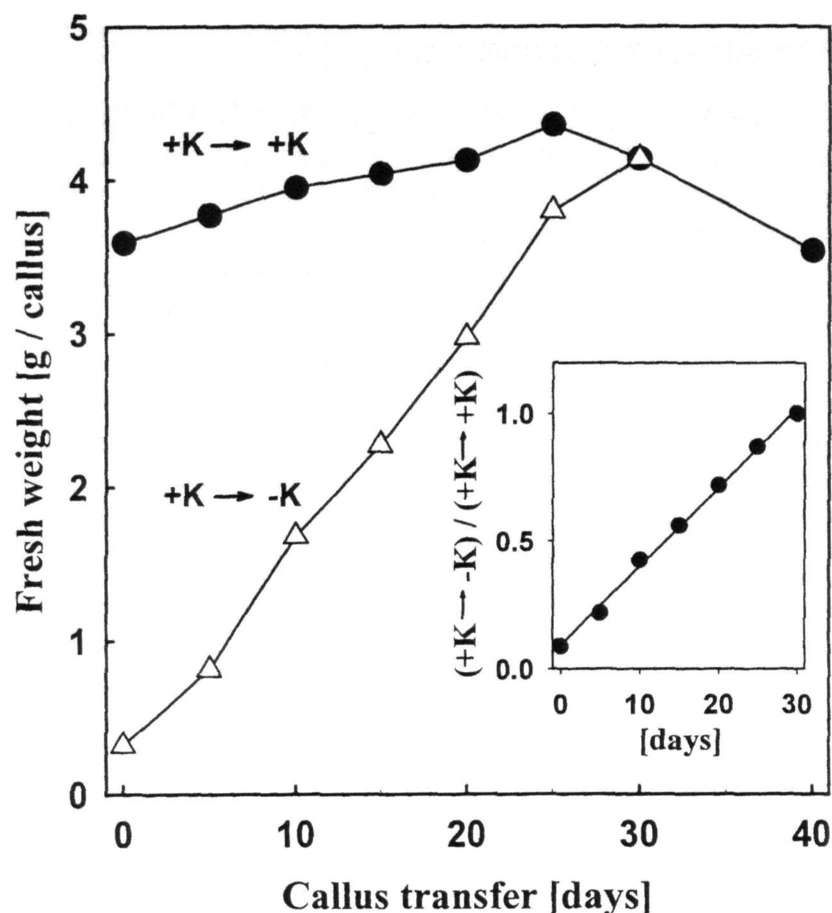


Fig. 2. The dependence of *Nicotiana tabacum* callus cv. Wisconsin 38 fresh weight yield on the period of tissue culture on kinetin-containing medium. Tissue was grown on Murashige-Skoog agar-solidified medium supplemented with NAA (5 μ*M*) and kinetin (0.2 μ*M*) at 26°C in darkness. At time intervals specified in the graph the tissue was transferred to the same medium (+K → +K) or to kinetin free medium (+K → –K) and harvested after 40 days of total culture.

[$^2H_4$]IAA promoted IAA conjugation and decreased levels of non-labelled IAA indicated its negative influence on de novo IAA biosynthesis, possibly by activation of a feedback mechanism for regulation of its own biosynthesis (Ribnicky et al. 1996).

Effects of cytokinins on cytokinin oxidase activity

Cytokinin oxidase (CKOX), which catalyses N^6 side chain cleavage of isoprenoid cytokinins, is the only cytokinin-specific enzyme known to inactivate cytokinins by their irreversible degradation. CKOXs from different plant materials vary in their biochemical properties (pH optima, molecular masses, Michaelis constants, glycosylation and stimulation of activity in the presence of imidazole-Cu^{2+} complexes; see Armstrong 1994 and Hare and van Staden 1994a). However, they are very conservative in their substrate specificity. Studies using different plant materials such as *Zea mays* kernels (Whitty and Hall 1974, McGaw and Horgan 1983), *Vinca rosea* crown gall (McGaw and Horgan 1983), wheat germ (Laloue and Fox 1989), *Phaseolus* calli (Chatfield and Armstrong 1986, Kamínek and Armstrong 1990), *Nicotiana tabacum* and *Populus* × *euroamericana* callus (Motyka and Kamínek 1992a, 1994) showed that iP is the most preferred substrate. N^6 side chain hydroxylation (forma-

tion of Z) reduces the affinity to CKOX from all plant materials but *Vinca rosea* crown gall and *O*-glucosylation protects the conjugates against enzyme attack. The affinity of CKOX to cytokinins is reduced but not removed by *N*-substitution of glucosyl or alanyl moieties on the substrate purine ring (McGaw and Horgan 1983). Cytokinins bearing a saturated N^6 side chain (DZ and DZR) and cytokinin nucleotides as well as cytokinins bearing aromatic side chains are not substrates of the CKOX. However, BA was reported to compete slightly with N^6-(Δ^2-isopentenyl)adenosine (iPA) as a substrate of CKOX from wheat germ (Laloue and Fox 1989).

If CKOX functions in the regulation of cytokinin levels it should respond to changes of cytokinin concentration in plant cells. This was first demonstrated by Terrine and Laloue (1980) who found that metabolic degradation of [^{14}C]iPA in vivo in cell suspensions of *Nicotiana tabacum* increased two- to threefold after exposure to exogenous iPA and BA. However, Palmer and Palni (1986) reported decreasing formation of degradative metabolites of ZR with increasing substrate concentration in cytokinin-dependent *Glycine max* callus; nevertheless, this effect was not significant at low ZR concentrations.

The promotion of CKOX activity assayed in vitro in response to in vivo application of exogenous cytokinins was reported for a number of plant systems (Tab. 1). Detailed analysis of *Phaseolus vulgaris* and *P. lunatus* callus tissues showed that the induction of enzyme activity is (1) relatively rapid, being detectable already 1 h after cytokinin application, (2) transient, lasting for not more than 8 h, and (3) significant, having increased up to threefold (Chatfield and Armstrong 1986, Kamínek and Armstrong 1990). RNA and protein synthesis seems to be required for the CKOX induction as indicated by its inhibition with cordycepin and cycloheximide (Chatfield and Armstrong 1986). Similar results were obtained with *Nicotiana tabacum* callus, where an increase in CKOX activity was recorded 2 h after application of 100 μ*M* Z solution to callus surface, culminating after 6 h and lasting for 24 h. The attenuation of enzyme activity was higher than that of total protein content (Motyka and Kamínek 1990). Increase in CKOX activity was also recorded in *Glycine max* callus after its long-term cultivation on media supplied with four different cytokinins, both substrates and non-substrates of CKOX (Motyka and Kamínek 1992b). All tested cytokinins, including cytokinin-active derivatives of urea, were effective as inducers of the enzyme. This indicates that the cytokinin effect on CKOX activity (at least in the case of non-substrate cytokinins) is not mediated by a simple induction of the enzyme by its substrates. Actually the non-substrate cytokinins exhibited very high inducing activity in *Phaseolus vulgaris* callus (Chatfield and Armstrong 1986, Kamínek and Armstrong 1990) not being degraded by the CKOX to inactive products (see Tab. 1).

Cytokinin oxidase activity is promoted (surprisingly) by both substrate and non-substrate cytokinins in vivo

It is an apparent paradox that cytokinins, that are not substrates of CKOX are capable of inducing two very contradictory processes, i.e. of accumulation of substrate cytokinins and CKOX activity.

Several mechanisms of promotion of CKOX by non-substrate cytokinins may be considered. (1) Non-substrate cytokinins, similar to substrate cytokinins, may be capable of reacting with repressor(s) and derepress structural gene(s) encoding CKOX. (2) The CKOX may be an allosteric protein which is activated by both substrate and non-substrate cytokinins. (3) As described

Tab. 1. Effect of exogenously applied cytokinins to callus tissues of *Phaseolus vulgaris*, *P. lunatus* and *Nicotiana tabacum* and expression of *ipt* gene on cytokinin oxidase activity. Tissues were enriched in cytokinins either by application of cytokinin solutions to callus surface (100 μ*M*, 0.1 ml[a,b] or 0.2 ml[c] g^{-1} fresh weight) or by *ipt* gene expression prior to determination of cytokinin oxidase activity. NS, statistically not significant increase. References: [a]Chatfield and Armstrong 1986, [b]Kamínek and Armstrong 1990, [c]Motyka and Kamínek 1992a, [d]Redig et al. 1996, [e]Zhang et al. 1995, [f]Motyka et al. 1996, [g]Eklöf et al. 1996.

Cytokinin treatment/ *ipt* gene expression	Cytokinin oxidase activity		
	P. vulgaris (% of water control)	*P. lunatus* (% of time zero control)	*N. tabacum* (% of time zero control) or (% of *ipt*-non-transformed control)
Adenine	103[a]	105[b]	104[c]
iP	–	110[b]	–
DZ	281[a]	159[b]	–
Z	–	144[b]	201[c]
cis-Z	–	126[b]	–
BA	–	–	161[c]
BA	–	–	150–200[d]
Thidiazuron	328[a]	177[b]	156[c]
ipt expression	–	–	500[e]
ipt expression	–	–	150–300[d]
ipt expression	–	–	200–1 000[f]
ipt expression	–	–	NS[g]

above (in Effects of cytokinins on their own accumulation) cytokinins that are not substrates of CKOX are capable of promoting the accumulation of substrate cytokinins, which may subsequently induce CKOX. (4) Phenyl-urea-type cytokinins may promote CKOX activity by a distinct mechanism. These synthetic cytokinins are known to compete with substrate cytokinins for CKOX when assayed in vitro (Chatfield and Armstrong 1986, Kamínek and Armstrong 1990, Motyka and Kamínek 1992a, Hare and van Staden 1994b). In this way the urea-type cytokinins may protect endogenous substrate cytokinins which can accumulate and subsequently induce CKOX in vivo.

Nevertheless, there are strong indications that CKOX activity in plant cells responds to changes in substrate isoprenoid cytokinin levels. CKOX activity closely paralleled the content of isoprenoid cytokinins during the early stages of grain development of cereal plants. Transient peaks of ZR, Z and iP which appeared in *Zea mays* kernels during the intensive cell division phase between 6 and 10 days after pollination (Jones et al. 1992, Lur and Setter 1993, Dietrich et al. 1995) were accompanied by a corresponding dramatic increase of CKOX activity (Dietrich et al. 1995). Moreover, this culmination in CKOX activity was not recorded in aborting kernels (Dietrich et al. 1995) which contain low levels of cytokinins (M. Kamínek, unpublished results). Maize inbreds with high cytokinin levels had also high CKOX activity (Cheikh and Jones 1994). Similar correlation was found in developing grains of *Triticum aestivum* where transient accumulation of isoprenoid cytokinins 4–11 days after anthesis was accompanied by the increase in CKOX activity (Kamínek et al. 1994). Further, accumulation of a natural non-substrate cytokinin, N^6-(*meta*-hydroxybenzyl)adenosine, which was recorded in wheat kernels during grain filling when the substrate isoprenoid cytokinins remained at basal levels, had no effect on the CKOX activity (M. Kamínek and A. Gaudinová, unpublished results). Correlation between cytokinin levels and CKOX activity was also found in leaves of wild-type and genic male-sterile plants of *Brassica napus*. The latter contained lower levels of cytokinins and was less efficient in degradation of $[^{14}C]$Z to $[^{14}C]$adenine in vivo as well as in CKOX activity assayed in vitro (Shukla and Sawhney 1997). Induction of CKOX by its substrates may be involved in control of fluctuation of cytokinin levels at distinct stages of the plant cell cycle being induced by transient cytokinin accumulation at the end of the S phase and during mitosis (see Redig et al. 1996).

Regulation of cytokinin oxidase in transgenic plants expressing the *ipt* gene

Transgenic plants expressing the *ipt* gene represent a suitable tool for studies of the effects of changes in endogenous cytokinin levels on CKOX. The level, duration and site of the *ipt* gene expression can be chosen by fusion of the gene to constitutive, inducible or organ (tissue) specific promoters. As summarized in Tab. 1 promotion of CKOX activity in consequence of *ipt* gene expression in tobacco tissues was reported by several authors. According to Zhang et al. (1995) the constitutive expression of the *ipt* gene in *Nicotiana tabacum* callus grown on hormone-free medium resulted in a 2- to 4.7-fold increase in total cytokinins, which included about a 4-fold increase in cytokinins that are substrates of cytokinin oxidase (iP, iPA, Z, and ZR), and a 5-fold elevation of CKOX activity. A similar correlation between the accumulation of total and 'substrate' cytokinins and CKOX activity was found in another *Nicotiana tabacum* callus transformed with the *ipt* gene under the control of a light-inducible promoter. Analysis of 16 cytokinins showed that the transformed tissue contained 25-fold higher level of total cytokinins; however, only about 25% of the cytokinin pool in both tissues represented cytokinins that are substrates of CKOX. Enzyme preparations from *ipt*-transformed tissue exhibited 1.5-fold higher CKOX activity as compared with control tissues (Redig et al. 1997).

The sequence of processes leading to induction of CKOX activity was demonstrated by Motyka et al. (1996) who exploited the advantage of *Nicotiana tabacum* callus transformed with the *ipt* gene under the transcriptional control of the tetracycline-dependent 35S promoter (Gatz et al. 1992). The *ipt* gene transcript was detected by northern analysis in transformed callus already 2 h after tetracycline application. The levels of major cytokinins, ZR, ZROG and DZR, began to increase after the following 6 h and progressively increased for 8 days up to 100-fold as compared with non-transformed wild-type tissue. An increase in CKOX activity, as a consequence of elevated cytokinin levels, was recorded 16 to 20 h after gene derepression and was increased up to 10-fold after 13 days. The increase in CKOX activity in cytokinin-overproducing tissue was associated with induction of the glycosylated form of the enzyme, indicating induction of transcription of a specific gene. A similar increase in CKOX activity was also found in detached leaves (8-fold after 4 days) and roots (4-fold after 3 days) of transformed plants in response to tetracycline treatment.

Overproduction of cytokinins in transgenic plants expressing the *ipt* gene is often too high to be physiological. Eklöf et al. (1996) analysed tobacco plants with moderately altered phenotypes expressing the *ipt* gene at low levels. As compared to wild-type plants the transgenic ones accumulated preferentially zeatin-7-glucoside (Z7G), which is less favorable substrate of CKOX as compared to Z (McGaw and Horgan 1983), and CKOX resistant ZMP. Unfortunately, determination of ZR and iPA together with the corresponding nucleotides does not allow us to relate levels of the cytokinin ribosides to CKOX activity. Both the concentration of Z and CKOX activity were similar in leaves of wild-type and transformed plants. However, a higher content of cytoki-

nins in young leaves, as compared to the old ones, correlated with CKOX activity in both types of plants.

Regulation of cytokinin levels by auxin

Cytokinins and auxins interact in the control of many developmental processes in plants such as cell division and differentiation, organ formation in cultured tissues, apical dominance and leaf senescence (see Thimann 1992). Their involvement in initiation and regulation of cell division and cell elongation underlines the central role of both auxins and cytokinins in plant development and responses of plants to environmental stimuli (see Hobbie et al. 1994). Using transgenic plants expressing the *ipt* gene and improved plant hormone analysis techniques significant progress has been made during the last decade in understanding the molecular basis of auxin-cytokinin interactions.

As expected the expression of the *ipt* gene from *Agrobacterium tumefaciens* in plants of several genera including *Nicotiana*, *Arabidopsis*, *Asparagus*, *Petunia* and *Kalanchoë* resulted in physiological responses known to be induced by exogenous cytokinins. These effects included cytokinin-autotrophy of transformed tissues cultured in vitro (Beinsberger et al. 1991, McKenzie et al. 1994), release of axillary buds and formation of shooty phenotype (Medford et al. 1989, Smigocki 1991, Van Loven et al. 1993), inhibition of root growth and development (Medford et al. 1989, Van Loven et al. 1993, Hewelt et al. 1994) and retardation of leaf senescence (Smart et al. 1991, Hewelt et al. 1994). An increase in cytokinin content up to 600-fold was reported in all these plant systems as a consequence of *ipt* gene expression.

Surprisingly, a dramatic increase in cytokinin content was found in tumors incited on *Nicotiana tabacum* stems by *Agrobacterium tumefaciens* plasmids with mutated auxin-biosynthesis genes, while mutation in the *ipt* gene had little effect on auxin levels (Akiyoshi et al. 1983). Changes in the auxin/cytokinin ratio due to the expression of T-DNA mutated in different genes affected tumour morphological patterns in the same way as described by Skoog and Miller (1957) for exogenously applied auxin and cytokinin. Similar changes in cytokinin and auxin content in tobacco crown gall tissues carrying inactivated or deleted auxin and/or cytokinin biosynthesis T-DNA genes were reported by McGaw et al. (1988) and Smigocki and Owens (1989). However, levels of both auxin and cytokinins in non-morphogenic carrot tumours induced by Ti plasmids lacking active auxin biosynthetic genes were similar to those found in tumours transformed with wild-type Ti plasmids (Ishikawa et al. 1988). The inhibiting effect of auxin on cytokinin levels may be responsible for the reported lethal effect of expression of gene *1*, coding for tryptophan-2-monooxygenase, catalyzing the first step of IAA biosynthesis in *Asparagus* crown gall tissues (Prinsen et al. 1990). On the other hand, *Nicotiana tabacum* plants exhibited increased tolerance to exogenous auxins and auxin transport inhibitors due to the expression of the *ipt* gene under an auxin-inducible promoter (Li et al. 1994).

Auxin applied exogenously to plant tissues had a similar effect on the lowering of cytokinin levels as that produced endogenously in transformed plants expressing auxin biosynthetic genes. Hansen et al. (1987) did not detect any ZR in cytokinin-requiring and cytokinin-autotrophic tobacco callus lines when cultured on NAA+kinetin-containing medium, however, both lines accumulated ZR and iPA on the same medium lacking auxin. A similar lowering of cytokinin content due to the exposure of tobacco cells to exogenous NAA was reported by Beinsberger et al. (1991). Application of a 24-h pulse of NAA to immobilised cytokinin-dependent tobacco cells in a column-flow-through system allowed Vaňková et al. (1992) to monitor the dynamics of excretion of cytokinins in response to NAA. The levels of predominant cytokinins ZR and iPA decreased 3- and 4-fold, respectively, during the first 6 h of cell exposure to NAA. Interestingly, the effect of NAA was transient culminating 6 h after NAA application and the rate of cytokinin excretion returned to the original levels during the next 18 h when the cells were still exposed to NAA. This dynamics of cytokinin excretion corresponds to the above proposed ability of cells to generate fast changes in hormonal levels in response to extracellular signal(s) inducing certain developmental process(es) and subsequently to maintain hormonal homeostasis essential for progress of initiated events. By following dynamics of endogenous cytokinins in an auxin-dependent and cytokinin-autonomous *Nicotiana tabacum* cell suspension culture, Zažímalová et al. (1996) found striking effects of exogenous auxin on the cytokinin levels. Culture of the cells in medium containing one-tenth of the optimum auxin concentration for growth resulted in a 10-fold increase in cytokinin levels per single cell. Moreover, cytokinin bases (iP, Z), which are physiologically more active than corresponding ribosides, were preferentially accumulated.

Auxin seems to regulate cytokinin levels also in intact plants (Tab. 2). Removal of apical buds as a source of auxin or complete disbudding induced rapid and significant increase of cytokinin content and efflux in xylem exudates in several plant species. This effect was accompanied with physiological and morphological responses, such as reduction of apical dominance and retention of leaf senescence, known to be induced by exogenous cytokinins. Detailed studies of Bangerth's group showed that cytokinin content (Z+ZR and iP+iPA) in xylem exudate of *Phaseolus vulgaris* and *Pisum sativum* seedlings was increased 25- and 6- to 10-fold, respectively, within 12–16 h after decapitation. The effect of decapitation was almost completely reversed after restoration of auxin supply by application of NAA on decapitated shoot (Bangerth 1994, Li et al. 1995). The dynamics of the response indicated fast delivery of the auxin signal to distant sites of cytokinin synthesis or translocation.

Tab. 2. Enhancement of cytokinin content and stimulation of cytokinin-induced physiological processes by reduction of auxin formation or removal of auxin sources in plant tissues and intact plants.

Way of reduction of auxin levels	Increase in cytokinin content (fold)	Physiological effect	Reference
Inactivation of auxin biosynthetic genes in tobacco crown gall tumors	20	Shoot formation	Akiyoshi et al. 1983
Inactivation of auxin biosynthetic gene in tobacco crown gall tumors	13	Shoot formation (not in all tumors)	McGaw et al. 1988
Disbudding of tobacco and tomato plants	4–30	Delay of senescence	Colbert and Beever 1981
Decapitation of bean plants	25–40	Reduction of apical dominance	Bangerth 1994
Decapitation of pea plants	6–10	Reduction of apical dominance	Guevara et al. 1995
Partial auxin deprivation in culture medium for tobacco cell suspension	10	Maintenance of high rate of cell division	Zažímalová et al. 1996

Interaction of auxin and cytokinins is further complicated by auxin-induced production of ethylene making it difficult to separate effects of auxin on cytokinin and ethylene levels (Yang and Hoffman 1984). Using transgenic *Nicotiana* and *Arabidopsis* plants overproducing auxin and expressing an ethylene synthesis-inhibiting 1-aminocyclopropane-1-carboxylate deaminase Romano et al. (1993) uncoupled overproduction of auxin from accumulation of ethylene. This allowed them to demonstrate that lateral bud growth in *Nicotiana* and *Arabidopsis* and leaf epinasty in *Arabidopsis* are under control of the cytokinin/auxin ratio.

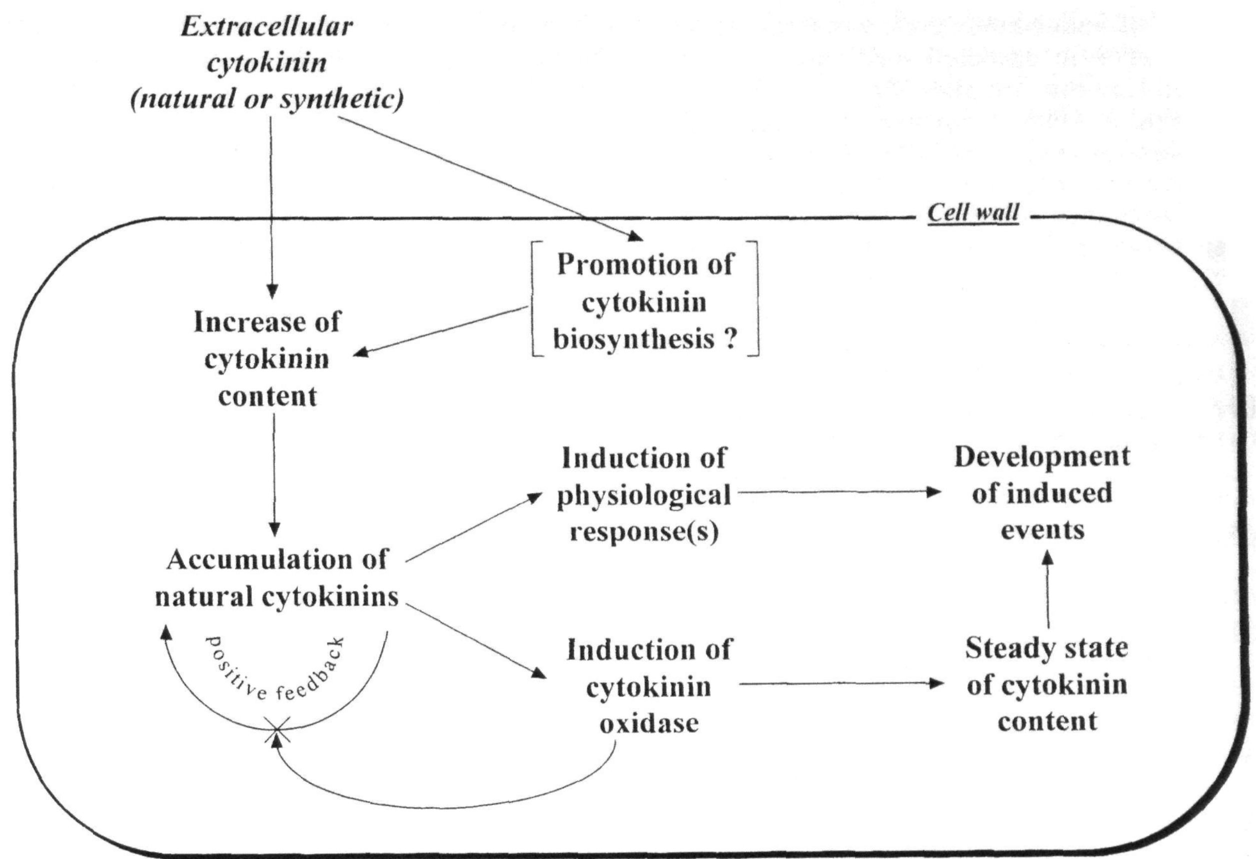
How does auxin regulate the cytokinin levels?

Down regulation of cytokinin levels by auxin may occur at the level of inhibition of cytokinin biosynthesis, or promotion of cytokinin metabolic inactivation by *N*-glucosylation or degradation by CKOX. Palni et al. (1988) reported that metabolic stability of [^{3}H]Z supplied to tobacco stem pith is inversely related to NAA concentration in incubation medium and proposed that the effect of auxin on cytokinin metabolism is mediated, at least in part, through CKOX. Zhang et al. (1995) arrived at the same conclusion when testing the effect of exogenous NAA on cytokinin levels and CKOX activity in *Nicotiana tabacum* callus expressing the *ipt* gene. In their experiments addition of NAA to the culture medium decreased the total cytokinin content by 84% in transformed and by 67% in non-transformed tissue and promoted in vivo conversion of Z-type cytokinins to adenine derivatives. They did not test the effect of NAA on CKOX activity extracted from treated tissues. However, the effect of NAA added to the assay solution at relatively high concentrations (4- to 10-fold higher than that of the substrate) on the CKOX activity was dependent on the type of assay buffer. The CKOX activity was increased in Tris-HCl buffer (by 90% maximally at 50 μM NAA) and decreased in imidazole-$CuCl_2$ buffer (by 33% maximally at 25 μM NAA). This contradiction may be due to the performance of the assay at the same pH (6.5) while the pH optimum of tobacco CKOX in imidazole buffer is very different from that in other buffers (Kamínek and Armstrong 1990, Motyka and Kamínek 1994). When tested in Tris-HCl buffer activity of both glycosylated and non-glycosylated forms of purified CKOX increased by about 50% in the presence of 50 μM NAA. However, the action of auxins on CKOX activity in vivo may be different as indicated by the application of solutions of IAA, NAA and indole-3-butyric acid to surface of tobacco callus (20 μM final concentration). None of these auxins affected CKOX activity assayed in vitro (Motyka and Kamínek 1992a).

Two reports support the view that auxin regulates cytokinin levels by influencing cytokinin biosynthesis. Investigating the effect of the strong synthetic auxin, picloram, on cytokinin levels in crown gall single cell clones of *Phaseolus vulgaris* induced by *Agrobacterium tumefaciens* Song et al. (1995) detected *ipt* transcripts in tumour tissue cultured on hormone-free medium. However, *ipt* RNA was undetectable by northern blot hybridisation in clones grown on picloram-supplemented medium. In spite of considerable clonal variation in the cytokinin contents of crown gall single cell clones, auxin significantly reduced both the cytokinin content and *ipt* mRNA accumulation in all three clones tested. Similar effects of auxin on the reduction of cytokinin, *ipt* mRNA and also IPT protein levels in *ipt*-transgenic tobacco callus were reported by Zhang et al. (1996). Levels of cytokinins, *ipt* RNA and IPT were decreased by auxin within 4 days, before the occurrence of any change in tissue morphology. Exogenous BA antagonised the effect of auxin on cytokinin, *ipt* mRNA and IPT levels although it did not directly affect *ipt* gene expression.

It seems unlikely that auxin regulates cytokinin levels by affecting both CKOX and cytokinin biosynthesis in vivo. Data concerning auxin-induced inhibition of *ipt* gene expression are less controversial, however, *ipt* does

Fig. 3. Proposed model illustrating co-operation of different regulatory mechanisms in control of cytokinin levels in plant cells and their physiological role(s).



not seem to be the gene coding for cytokinin biosynthesis in non-transformed plant cells (see McGaw and Burch 1995). Nevertheless, inhibition of cytokinin accumulation in normal plant cells by auxin indicates that the same or similar regulatory mechanism(s) operates in both non-transformed and *ipt*-transformed plant cells.

Proposed model of regulation of cytokinin content in plant cells

Taking into account the metabolic and physiological responses to changes in cytokinin supply and/or intracellular accumulation described, we propose a model for the regulation of cytokinin content in plant cells (Fig. 3). According to this scheme the increase in content of endogenous cytokinins due to their supply from extracellular sources (xylem or phloem sap, exogenous application) may induce further cytokinin accumulation by activation of a positive feedback loop. Having little effect on auxin levels, the accumulation of cytokinins may also increase the cytokinin/auxin ratio. Under these conditions accumulated cytokinins may induce in competent plant cells certain physiological and/or structural processes such as cytokinin-autonomy and shoot formation. However, accumulated cytokinins also act as substrate inducers of CKOX and in this way the cytokinin level may be subsequently reduced. This seems to be a mechanism of reestablishment and/or maintenance of cytokinin homeostasis required for further development of events initiated by transient cytokinin accumulation. There is also another developmental alternative: Cells exhibiting strong positive feedback or weak induction of CKOX may become cytokinin-autonomous and/or meristematic. If the positive feedback is based on autoinduction of cytokinin biosynthesis then such cells may become new sites of cytokinin biosynthesis.

Auxin may affect cytokinin levels by down regulation of cytokinin biosynthesis and/or by promotion of CKOX. In this way auxin may not only decrease the cytokinin levels but also dramatically decrease cytokinin/auxin ratio and induce certain physiological events, such as root formation. Cytokinins may reduce the auxin effect on their own levels, however, their effect on auxin levels in plant cells seems to be limited. Nevertheless, the actual operation of these mechanisms in plant cells may be expected to differ depending on the cell's developmental reponse to plant hormones.

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References

Akiyoshi, D. E., Morris, R. O., Hinz, R., Mischke, B. S., Kosuge, T., Garfinkel, D. J., Gordon, M. P. & Nester, E. W. 1983. Cytokinin/auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. – Proc. Natl. Acad. Sci. USA 80: 407–411.

Armstrong, D. J. 1994. Cytokinin oxidase and the regulation of cytokinin degradation. – *In* Cytokinins: Chemistry, Activity, and Function (D. W. S. Mok and M. C. Mok, eds), pp. 139–154. CRC Press, Boca Raton, FL. ISBN 0-8493-6252-0.

Auer, C. A., Cohen, J. D., Laloue, M. & Cooke, T. J. 1992. Comparison of benzyladenine metabolism in two *Petunia hybrida* lines differing in shoot organogenesis. – Plant Physiol. 98: 1035–1041.

Badenoch-Jones, J., Parker, C. W., Letham, D. S. & Singh, S. 1996. Effect of cytokinins supplied via the xylem at multi-

ples of endogenous concentrations on transpiration and senescence in derooted seedlings of oat and wheat. – Plant Cell Environ. 19: 504–516.
Bangerth, F. 1994. Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment and relationship to apical dominance. – Planta 194: 439–442.
Beinsberger, S. E. I., Valcke, R. L. M., Deblaere, R. Y., Clijsters, H. M. M., De Greef, R. & Van Onckelen, H. A. 1991. Effects of the introduction of *Agrobacterium tumefaciens* T-DNA *ipt* gene in *Nicotiana tabacum* L. cv. Petit Havana SR1 plant cells. – Plant Cell Physiol. 32: 489–496.
Brzobohatý, B., Moore, I., Kristoffersen, P., Bako, L., Campos, N., Schell, J. & Palme, K. 1993. Release of active cytokinin by a β-glucosidase localized to the maize root meristem. – Science 262: 1051–1054.
Chatfield, J. M. & Armstrong, D. J. 1986. Regulation of cytokinin oxidase activity in callus tissues of *Phaseolus vulgaris* L. cv. Great Northern. – Plant Physiol. 80: 493–499.
Cheikh, N. & Jones, R. J. 1994. Disruption of maize kernel growth and development by heat stress. – Plant Physiol. 106: 45–51.
Colbert, C. A. & Beever, J. E. 1981. Effect of disbudding on root cytokinin export and leaf senescence in tomato and tobacco. – J. Exp. Bot. 32: 121–127.
Dietrich, J. T., Kamínek, M., Blevins, D. G., Reinbott, T. M. & Morris, R. O. 1995. Changes in cytokinins and cytokinin oxidase activity in developing maize kernels and the effects of exogenous cytokinin on kernel development. – Plant Physiol. Biochem. 33: 327–336.
Eberle, J., Wang, T. L., Cook, S., Wells, B. & Weiler, E. W. 1987. Immunoassay and ultrastructural localization of isopentenyladenine and related cytokinins using monoclonal antibodies. – Planta 172: 289–297.
Eklöf, S., Åstot, C., Moritz, T., Blackwell, J., Olsson, O. & Sandberg, G. 1996. Cytokinin metabolites and gradients in wild type and transgenic tobacco with moderate cytokinin over-production. – Physiol. Plant. 98: 333–344.
Estruch, J. J., Prinsen, E., Van Onckelen, H., Schell, J. & Spena, A. 1991. Viviparous leaves produced by somatic activation of an inactive cytokinin-synthesising gene. – Science 254: 1364–1367.
Faiss, M., Strnad, M., Redig, P., Doležal, K., Hanuš, J., Van Onckelen, H. & Schmülling, T. 1996. Chemically induced expression of the *rolC*-encoded β-glucosidase in transgenic tobacco plants and analysis of cytokinin metabolism: rolC does not hydrolyze endogenous cytokinin glucosides. – Plant J. 10: 33–46.
Falk, A. & Rask, L. 1995. Expression of a zeatin-*O*-glucoside-degrading β-glucosidase in *Brassica napus*. – Plant Physiol. 108: 1369–1377.
Fusseder, A. & Ziegler, P. 1988. Metabolism and compartmentation of dihydrozeatin exogenously supplied to photoautotrophic suspension cultures of *Chenopodium rubrum*. – Planta 173: 104–109.
Gatz, C., Frohberg, C. & Wendenburg, R. 1992. Stringent repression and homogenous de-repression by tetracycline of a modified CaMV 35S promoter in intact tobacco plants. – Plant J. 2: 397–404.
Hansen, C. E., Meins, F. Jr & Aebi, R. 1987. Hormonal regulation of zeatin-riboside accumulation by cultured tobacco cells. – Planta 172: 520–525.
Hare, P. D. & van Staden, J. 1994a. Cytokinin oxidase: Biochemical features and physiological significance. – Physiol. Plant. 91: 128–136.
– & van Staden, J. 1994b. Inhibitory effect of thidiazuron on the activity of cytokinin oxidase isolated from soybean callus. – Plant Cell Physiol. 35: 1121–1125.
Hewelt, A., Prinsen, E., Schell, J., Van Onckelen, H. & Schmülling, T. 1994. Promoter tagging with a promoterless *ipt* gene leads to cytokinin-induced phenotypic variability in transgenic tobacco plants: Implications of gene dosage effects. – Plant J. 6: 879–891.
Hoad, G. V. 1995. Transport of hormones in the phloem of higher plants. – Plant Growth Regul. 16: 173–182.
Hobbie, L., Timpte, C. & Estelle, M. 1994. Molecular genetics of auxin and cytokinin. – Plant Mol. Biol. 26: 1499–1519.
Ishikawa, K., Kamada, H., Yamaguchi, I., Takahashi, N. & Harada, H. 1988. Morphology and hormone levels of tobacco and carrot tissues transformed by *Agrobacterium tumefaciens*. I. Auxin and cytokinin contents of cultured tissues transformed with wild-type and mutant Ti plasmids. – Plant Cell Physiol. 29: 461–466.
Jameson, P. E. 1994. Cytokinin metabolism and compartmentation. – *In* Cytokinins: Chemistry, Activity, and Function (D. W. S. Mok and M. C. Mok, eds), pp. 113–128. CRC Press, Boca Raton, FL. ISBN 0-8493-6252-0.
Jones, R. J., Schreiber, B. M., McNeil, K., Brenner, M. L. & Foxon, G. 1992. Cytokinin levels and oxidase activity during maize kernel development. – *In* Progress in Plant Cellular and Molecular Biology. Proceedings of the 7th International Congress on Plant Tissue and Cell Culture (H. J. J. Nijkamp, L. H. Van der Plas and J. van Aartrijk, eds), pp. 235–239. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-0873-5.
Kamínek, M. 1992. Progress in cytokinin research. – Trends Biotechnol. 10: 159–164.
– & Armstrong, D. J. 1990. Genotypic variation in cytokinin oxidase from *Phaseolus* callus cultures. – Plant Physiol. 93: 1530–1538.
– , Trčková, M., Motyka, V. & Gaudinová, A. 1994. Role of cytokinins in control of wheat grain development and utilization of nutrients. – *Biol. Plant. 36(Suppl.): 15.*
Klee, H. J. & Romano, C. P. 1994. The roles of phytohormones in development as studied in transgenic plants. – Crit. Rev. Plant Sci. 13: 311–324.
Laloue, M. & Fox, E. 1989. Cytokinin oxidase from wheat: Partial purification and general properties. – Plant Physiol. 90: 899–906.
– & Pethe, C. 1982. Dynamics of cytokinin metabolism in tobacco cells. – *In* Plant Growth Substances 1982 (P. F. Wareing, ed.), pp. 186–195. Academic Press, London. ISBN 0-12-735380-1.
– , Pethe-Terrine, C. & Guern, J. 1981. Uptake and metabolism of cytokinins in tobacco cells: Studies in relation to the expression of their biological activities. – *In* Metabolism and Molecular Activities of Cytokinins (J. Guern and C. Péaud-Lenoël, eds), pp. 80–96. Springer-Verlag, Berlin. ISBN 3-540-10711-8.
Letham, D. S. 1994. Cytokinins as phytohormones – sites of biosynthesis. – *In* Cytokinins: Chemistry, Activity, and Function (D. W. S. Mok and M. C. Mok, eds), pp. 57–80. CRC Press, Boca Raton, FL. ISBN 0-8493-6252-0.
– , Palni, L. M. S., Tao, G.-O., Gollnow, B. I. & Bates, C. M. 1983. The activities of cytokinin glucosides and alanine conjugates in cytokinin bioassays. – J. Plant Growth Regul. 2: 103–115.
Li, C.-J., Guevara, E., Herrera, J. & Bangerth, F. 1995. Effect of apex excision and replacement by 1-naphthylacetic acid on cytokinin concentration and apical dominance in pea plants. – Physiol. Plant. 94: 465–469.
Li, Y., Shi, X., Strabala, T. J., Hagen, G. & Guilfoye, T. J. 1994. Transgenic tobacco plants that overproduce cytokinins show increased tolerance to exogenous auxin and auxin transport inhibitors. – Plant Sci. 100: 9–14.
Lur, H. & Setter, T. L. 1993. Role of auxin in maize endosperm development. – Plant Physiol. 103: 273–280.
McGaw, B. A. & Burch, L. R. 1995. Cytokinin biosynthesis and metabolism. – *In* Plant Hormones, Physiology, Biochemistry and Molecular Biology (P. J. Davies, ed.), pp. 96–117. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-2984-8.
– & Horgan, R. 1983. Cytokinin oxidase from *Zea mays* kernels and *Vinca rosea* crown gall tissue. – Planta 159: 30–37.
– & Horgan, R. 1985. Cytokinin metabolism and the control of cytokinin activity. – Biol. Plant. 27: 180–187.
– , Heald, J. K. & Horgan, R. 1984. Dihydrozeatin metabolism in radish seedlings. – Phytochemistry 23: 1373–1377.

– , Horgan, R., Heald, J. K., Wullems, G. J. & Schilperoort, R. A. 1988. Mass-spectrometric quantitation of cytokinins in tobacco crown-gall tumors induced by mutated octopine Ti plasmids of *Agrobacterium tumefaciens*. – Planta 176: 230–234.

McKenzie, M. J., Jameson, P. & Poulter, R. T. M. 1994. Cloning of *ipt* gene from *Agrobacterium tumefaciens*: Characterisation of cytokinins in derivative transgenic plant tissue. – Plant Growth Regul. 14: 217–228.

Medford, J., Horgan, R., El-Sawi, Z. & Klee, H. 1989. Alternations in endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. – Plant Cell 1: 403–413.

Meins, F. Jr. 1989. Habituation: Heritable variation in the requirement of cultured plant cells for hormones. – Annu. Rev. Genet. 23: 395–408.

Mok, M. C., Mok, D. W. S., Armstrong, D. J., Rabakoarihanta, A. & Kim, S.-G. 1980. Cytokinin autonomy in tissue cultures of *Phaseolus*: A genotype-specific and heritable trait. – Genetics 94: 675–686.

Motyka, V. & Kamínek, M. 1990. Regulation of cytokinin oxidase catabolism in tobacco callus cultures. – *In* Progress in Plant Cellular and Molecular Biology. Proceedings of the 7th International Congress on Plant Tissue and Cell Culture (H. J. J. Nijkamp, L. H. Van der Plas and J. van Aartrijk, eds), pp. 492–497. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-0873-5.

– & Kamínek, M. 1992a. Characterization of cytokinin oxidase from tobacco and poplar callus cultures. – *In* Physiology and Biochemistry of Cytokinins in Plants (M. Kamínek, D. W. S. Mok and E. Zažímalová, eds), pp. 33–39. SPB Academic Publishing, The Hague. ISBN 90-5103-066-5.

– & Kamínek, M. 1992b. The effect of exogenous cytokinins in the culture medium on cytokinin oxidase activity in soybean callus culture. – Biol. Plant. 34(Suppl.): 581–582.

– & Kamínek, M. 1994. Cytokinin oxidase from auxin- and cytokinin-dependent callus cultures of tobacco (*Nicotiana tabacum* L.). – J. Plant Growth Regul. 13: 1–9.

– , Faiss, M., Strnad, M., Kamínek, M. & Schmülling, T. 1996. Changes in cytokinin content and cytokinin oxidase activity in response to derepression of *ipt* gene transcription in transgenic tobacco calli and plants. – Plant Physiol. 112: 1035–1043.

Nishinari, N. & Syôno, K. 1986. Induction of cell division synchrony and variation of cytokinin contents through the cell cycle in tobacco cultured cells. – Plant Cell Physiol. 27: 147–153.

Noodén, L. D., Singh, S. & Letham, D. S. 1990. Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. – Plant Physiol. 93: 33–39.

Palmer, M. V. & Palni, L. M. S. 1986. Substrate effects on cytokinin metabolism in soybean callus tissue. – J. Plant Physiol. 126: 365–371.

– , Horgan, R. & Wareing, P. F. 1981a. Cytokinin metabolism in *Phaseolus vulgaris* L. I. Variation in cytokinin levels in leaves of decapitated plants in relation to lateral bud outgrowth. – J. Exp. Bot. 32: 1231–1241.

– , Scott, I. M. & Horgan, R. 1981b. Cytokinin metabolism in *Phaseolus vulgaris* L. II. Comparative metabolism of exogenous cytokinin by detached leaves. – Plant Sci. Lett. 22: 187–195.

Palni, L. M. S., Burch, L. & Horgan, R. 1988. The effect of auxin concentration on cytokinin stability and metabolism. – Planta 174: 231–234.

Prinsen, E., Bytebier, B., Hernalsteens, J.-P., De Greef, J. & Van Onckelen, H. 1990. Functional expression of *Agrobacterium tumefaciens* T-DNA *onc*-genes in *Asparagus* crown gall tissues. – Plant Cell Biol. 31: 69–75.

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

– , Motyka, V., Van Onckelen, H. A. & Kamínek, M. 1997. Regulation of cytokinin oxidase activity in tobacco callus expressing the T-DNA *ipt* gene. – Physiol. Plant. 99: 89–96.

Ribnicky, D. M., Ilíc, N., Cohen, J. D. & Cooke, T. J. 1996. The effects of exogenous auxins on endogenous indole-3-acetic acid metabolism. – Plant Physiol. 112: 549–558.

Romano, C. P., Cooper, M. L. & Klee, H. J. 1993. Uncoupling auxin and ethylene effects in transgenic tobacco and *Arabidopsis* plants. – Plant Cell 5: 181–189.

Shukla, A. & Sawhney, V. K. 1997. Cytokinin metabolism and cytokinin oxidase and adenine phosphoribosyltransferase activity in male sterile *Brassica napus* leaves. – Phytochemistry 44: 377–381.

Singh, S., Palni, L. M. S. & Letham, S. 1992. Cytokinin biochemistry in relation to leaf senescence. V. Endogenous cytokinin levels and metabolism of zeatin riboside in leaf discs from green and senescent tobacco (*Nicotiana rustica*) leaves. – J. Plant Physiol. 139: 279–283.

Sitton, D., Ithai, C. & Kende, H. 1967. Decreased cytokinin production in the roots as a factor of shoot senescence. – Planta 73: 296–300.

Skoog, F. & Miller, C. O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. – Symp. Soc. Exp. Biol. 11: 118–131.

Smart, C. M., Scofield, S. R., Bevan, M. V. & Dyer, T. A. 1991. Delayed senescence in tobacco plants transformed with *tmr*, a gene for cytokinin production in *Agrobacterium*. – Plant Cell 3: 647–656.

Smigocki, A. C. 1991. Cytokinin content and tissue distribution in plants transformed by a reconstructed isopentenyl transferase gene. – Plant Mol. Biol. 16: 105–115.

– & Owens, L. D. 1989. Cytokinin-to-auxin ratios and morphology of shoots and tissues transformed by a chimeric isopentenyl transferase gene. – Plant Physiol. 91: 808–811.

Song, J. Y., Choi, E. Y., Lee, H. S., Choi, D.-W., Oh, M.-H. & Kim, S.-G. 1995. Effect of auxin on expression of the isopentenyl transferase gene (*ipt*) in transformed bean (*Phaseolus vulgaris* L.) single-cell clones induced by *Agrobacterium tumefaciens* C58. – J. Plant Physiol. 146: 148–154.

Terrine, C. & Laloue, M. 1980. Kinetics of N6-(2-isopentenyl)adenosine degradation in tobacco cells. – Plant Physiol. 65: 1090–1095.

Thimann, K. V. 1992. Antagonisms and similarities between cytokinins, abscisic acid and auxin (minireview). – *In* Physiology and Biochemistry of Cytokinins in Plants (M. Kamínek, D. W. S. Mok and E. Zažímalová, eds), pp. 395–400. SPB Academic Publishing, The Hague. ISBN 90-5103-066-5.

Thomas, J. C. & Katterman, F. R. 1986. Cytokinin activity induced by thidiazuron. – Plant Physiol. 81: 681–683.

Tromp, J. & Ovaa, J. 1990. Seasonal changes in the cytokinin composition of xylem sap of apple. – J. Plant Physiol. 136: 606–614.

Turgeon, R. 1982. Cytokinesis, cell expansion, and the potential for cytokinin-autonomous growth of tobacco pith. – Plant Physiol. 70: 1071–1074.

Van Loven, K., Beinsberger, S. E. I., Valcke, R. L. M., Van Onckelen, H. A. & Clijsters, H. M. M. 1993. Morphometric analysis of the growth of Phsp70-ipt transgenic tobacco plants. – J. Exp. Bot. 44: 1671–1678.

Vaňková, R., Hsiao, K.-C., Bornman, C. H. & Gaudinová, A. 1991. Effects of synthetic cytokinins on levels of endogenous cytokinins and respiration patterns of *Beta vulgaris* cells in suspension. – J. Plant Growth Regul. 10: 197–199.

– , Kamínek, M., Eder, J. & Vaněk, T. 1987. Dynamics of production of *trans*-zeatin and *trans*-zeatin riboside by immobilised cytokinin-autonomous and cytokinin-dependent tobacco cells. – J. Plant Growth Regul. 6: 147–157.

– , Gaudinová, A., Kamínek, M. & Eder, J. 1992. The effect of interaction of synthetic cytokinin and auxin on production of natural cytokinins by immobilised tobacco cells. – *In* Physiology and Biochemistry of Cytokinins in Plants (M. Kamínek, D. W. S. Mok and E. Zažímalová, eds), pp. 47–51. SPB Academic Publishing, The Hague. ISBN 90-5103-066-5.

Whitty, C. D. & Hall, R. H. 1974. A cytokinin oxidase from *Zea mays*. – Can. J. Biochem. 52: 789–799.

Yang, S. F. & Hoffman, N. E. 1984. Ethylene biosynthesis and

its regulation in higher plants. – Annu. Rev. Plant Physiol. 35: 155–189.

Zažímalová, E., Březinová, A., Holík, J. & Opatrný, Z. 1996. Partial auxin deprivation affects endogenous cytokinins in an auxin-dependent, cytokinin-independent tobacco cell strain. – Plant Cell Rep. 16: 76–79.

Zhang, R., Zhang, X., Wang, J., Letham, D. S., McKinney, S. A. & Higgins, T. J. V. 1995. The effect of auxin on cytokinin levels and metabolism in transgenic tobacco tissue expressing an *ipt* gene. – Planta 196: 84–94.

Zhang, X. D., Letham, D. S., Zhang, R. & Higgins, T. J. V. 1996. Expression of the isopentenyl transferase gene is regulated by auxin in transgenic tobacco callus. – Transgen. Res. 5: 57–65.

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