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Cytokinin Metabolism in Nondividing and Auxin-Induced Dividing Explants of *Helianthus tuberosus* L. Tuber Tissue

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Abstract. Aqueous solutions of auxin (indole-3-acetic acid, α-naphthalene acetic acid, or 2,4-dichlorophenoxyacetic acid) were active in inducing DNA synthesis and mitosis in prewashed tissue explants of mature Jerusalem artichoke tubers. Explants did not respond in this way to aqueous solutions of cytokinin (zeatin, zeatin riboside, 6-benzylaminopurine, or kinetin). The metabolism of [8-^{3}H]zeatin riboside (ZR) was studied in nondividing and auxin-induced synchronously dividing explants over the first 36 h of culture. ZR was taken up rapidly and to the same extent by both tissues. Sequential analysis of tissue extracts by thin-layer and high-performance-liquid chromatography identified zeatin nucleotide(s) (ZN), O-glucosyl zeatin riboside (OGZR), adenosine, and adenine nucleotide(s) (AN) as the principal metabolites in both tissues. The proportion of radioactivity due to ZR declined steadily and OGZR accumulated steadily at similar rates in both tissues. ZN was the major metabolite in both tissues at 12 h; thereafter ZN continued to accumulate in nondividing tissue, but its level declined in dividing tissue, and a corresponding increase in the levels of AN and adenosine was observed. These treatment differences in cytokinin metabolism were apparent at least 6 h before the onset of mitosis.

The induction of cell division in excised tuber tissue of Jerusalem artichoke (*Helianthus tuberosus* L.) by applied auxin is well documented (Adamson 1962, Setterfield 1963, Yeoman and Mitchell 1970, Yeoman and Davidson 1971, Bennici et al. 1982). Growth by cell division is accompanied by cell expansion, and indeed under some conditions this has been recorded as the predominant

response to phytohormone treatment. The extent of induced cell division is dependent on a number of physiological and environmental factors, such as the length of the previous dormancy period, the extent of prewashing of excised tissue before treatment, the presence of light during the culture period, and the osmotic potential and composition of the nutrient medium. Synthetic cytokinins can act synergistically with auxin to promote both cell division and cell expansion in this system, but are apparently inactive in the absence of exogenous auxin (Adamson 1962, Setterfield 1963, Masuda 1965, Kamisaka et al. 1973, Minocha and Halperin 1974, Phillips and Dodds 1977). A reinvestigation of this system was prompted by its potential usefulness as a cytokinin-autonomous tissue in the study of the biosynthesis of cytokinins and their interaction with auxin in cell division. Unfortunately, it is difficult to make meaningful comparisons between previous studies of this system, since the different culture methods and pretreatments that have been used may have resulted in profound differences in the response to applied phytohormones. Particular care must be taken in the interpretation of earlier studies for which coconut milk was used in the culture media; this has now been shown to contain several potent growth substances, including cytokinins (Letham 1978).

In the present study the response of this tissue to auxins and cytokinins was first evaluated using aqueous solutions of phytohormones. Rather than increases in cell number and tissue mass, DNA synthesis and mitosis were taken as markers of cell division induction.

The timing of cytokinin action in this system may be reflected by changes in cytokinin metabolism during the culture period. Preliminary studies using bioassay indicated that zeatin riboside is the major endogenous cytokinin in this tissue (Palmer, unpublished). Accordingly, we have followed the metabolism of radiolabeled zeatin riboside at 6-h intervals over the first synchronous division of auxin-induced artichoke tissue, and in artichoke tissue cultured under identical conditions but in the absence of auxin.

In a more general context, this experimental system provides a rare opportunity for a detailed comparison of cytokinin metabolism in nondividing and synchronously dividing tissue of the same type.

Materials and Methods

Chemicals

Indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (K), and 6-benzylaminopurine (BAP) were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Unlabeled zeatin (Z) and zeatin riboside (ZR) were obtained from Calbiochem (La Jolla, California, USA). [8-^{3}H]ZR (244 mCi mmol^{-1}) and all other cytokinins were synthesized as described previously (Palni et al. 1984).

Tissue Culture

Helianthus tuberosus L. tubers were obtained locally and stored for up to 4 months at 4°C in moist vermiculite in the dark. Tubers were washed and sur-

face-sterilized with 10% sodium hypochlorite solution for 15 min, then washed twice with sterile water. All subsequent manipulations were performed under aseptic conditions. Tubers were sliced transversely using a razor blade, and the end slices discarded. One cylinder of pith tissue (c 2 mm × 10 mm) was removed from the centre of each slice, and discs (explants) c 1 mm thick were cut from each cylinder. For prewashing, c 200 explants were transferred to 50 cm^3 sterile distilled water at 4°C, and shaken gently in a conical flask at 4°C in the dark for 20 h. Explants were stored briefly on moist filter paper before being transferred to culture dishes. Explants (20 per dish) were cultured at 25°C in the dark in Petri dishes (6 cm × 1 cm) containing 3 cm^3 sterile distilled water and three discs of Whatman No. 2 filter paper. Test solutions of IAA, NAA, 2,4-D, Z, BAP, or K were added in 30 µl of 2% aqueous ethanol, to give a final concentration of 1.0 µg ml^{-1}.

In the study of the biological activity and metabolism of ZR, slightly larger (4 mm diameter) explants were used, and 4% sucrose was included in the medium. In four treatments, explants were cultured in the presence of either [8-^{3}H]ZR (0.13 µg ml^{-1}) or the same concentration of unlabeled ZR, with or without the addition of IAA (1.0 µg ml^{-1}) at the start of the culture period. Bioassay data indicate that this concentration of ZR is well in excess (c 10–50-fold) of the level of endogenous cytokinins in fresh artichoke tuber parenchyma tissue (Palmer, unpublished). Six culture dishes were used per treatment, and the contents were harvested at 6-h intervals between 6 h and 36 h from the start of culture.

Microscopy and Cytophotometry

Explants were fixed for 1 h in ethanol/glacial acetic acid (3:1) at 4°C, immediately after harvesting, then stored at 4°C in 70% ethanol for up to 24 h before staining. All subsequent procedures were carried out at room temperature; explants were rinsed once in distilled water between each step. Before staining, explants were partially digested for 15 min in a solution containing cellulysin, hemicellulase, and pectinase (Lin 1980), and then hydrolyzed in 5 M HCl for 45 min. Explants were then Feulgen-stained for 1 h, rinsed twice in a freshly prepared solution of 0.5% sodium metabisulphite in 0.1 M HCl, squashed and mounted in polyvinyl alcohol/glycerol/tris buffer, pH 8.5 (Heimer and Taylor 1974) for microscopy.

Estimates of mitotic frequency and DNA measurements of individual nuclei were made with a Leitz MPV 3 mirror-scanning microscope photometer, linked to a Digital DEC Datasystem computer, under control of the Leitz CELAN B program. Six explants were analyzed per treatment. Mitotic indices were determined by examining all the nuclei (c 1000) in each explant.

Recovery and Extraction of Radioactive Compounds

Explants were removed from culture dishes and rinsed first for 5 s in an aqueous solution of unlabeled ZR (10 µg ml^{-1}), then for 5 s in distilled water, and then dropped into a solution of chloroform/methanol/formic acid/water

Table 1. Characterization of [8-^{3}H]ZR and its metabolites by preparative TLC and HPLC.

Compound[a]	TLC zone	TLC Rf value	HPLC system used after TLC
Adenine nucleotides[b]	1	0	C
Z/DZ nucleotides[b]	1	0	C
Inosine marker	3	0.07	
O-glucosyl ZR[c]	3	0.07	B
O-glucosyl DZR[c]	3	0.08	B
N^7-glucosyl Z[c]	4	0.10	B
N^9-glucosyl Z[c]	4	0.10	B
Lupinic acid	4	0.10	B
O-glucosyl Z[c]	4	0.10	B
O-glucosyl DZ[c]	4	0.11	B
Congo Red (dye)	4	0.11	
Adenosine	6	0.23	A
ZR	8	0.30	C
DZR	8	0.30	C
Adenine	10	0.41	A
Z	12	0.50	C
DZ	12	0.50	C

[a] Abbreviations: Z, zeatin; DZ, dihydrozeatin; ZR, zeatin riboside; DZR, dihydrozeatin riboside.
[b] Identification also based on phosphatase treatment of TLC zone 1 compounds, followed by analysis of the hydrolysate by TLC and HPLC System C.
[c] Identification also based on glucosidase treatment of TLC zones 3 and 4, followed by analysis of the hydrolysates by TLC and HPLC System B.

(5:12:1:2), at −10°C, using 5 cm^3 extraction solvent per 20 explants. Culture dishes were rinsed out three times with 50% aqueous methanol. Solutions from explant and dish rinses were combined for each treatment and dried down for estimation of radioactivity; [8-^{3}H]ZR uptake values were obtained by subtraction of these estimates from the known amounts of radioactivity supplied at the start of the experiment. Aliquots of staled culture medium from each 36-h incubation were analyzed directly by HPLC system B (see below), to check for the presence of [^{3}H-ZR] metabolites outside the tissue. Explants were left in extraction solvent at −10°C for 48 h, then homogenized. These extracts were centrifuged and the supernatants were decanted. The remaining pellets were resuspended in 80% aqueous methanol (3 ml), left at 4°C for 1 h, and centrifuged again. The two supernatants were combined for each treatment and stored at −10°C.

Purification and Identification of Metabolites

Aliquots from each extract were purified initially by thin-layer chromatography (TLC) on 0.3-mm-thick layers of Merck PF_{254} silica gel 60, developed in butan-1-ol/14 M ammonia/water (6:1:2, upper phase). The resolution of a range of cytokinins and cytokinin metabolites by this procedure is shown in Table 1. Extracts were chromatographed with unlabeled standards of Z, ZR, adenine, adenosine, inosine, and the marker dye Congo Red (Matheson, Coleman, and

Bell, Norwood, Ohio, USA). Each chromatogram was divided into 16 zones; inosine and Congo Red were used to delimit the "glucoside zones" of the chromatogram (Table 1). Each zone was scraped off and transferred to a scintillation vial containing 1.0 cm^3 methanol/water/acetic acid (10:89:1). Vials were shaken gently, then left to stand at room temperature for 6 h, with occasional shaking. Scintillation fluid was then added, and after a further 24 h in the dark at 4°C, the vials were analyzed for radioactivity using an LKB Wallac 1215 Rackbeta II liquid scintillation counter. Thus the radioactivity in each zone was quantified in relation to the total radioactivity extracted, and zones of interest were identified. In subsequent analyses, further aliquots of extracts were chromatographed in the same TLC system. Zones comprising major proportions of extracted radioactivity were eluted and further characterized by high-performance-liquid chromatography (HPLC) and, in some cases, enzymic degradation followed by TLC and/or HPLC of the hydrolysis product (Table 1).

Nucleotides were hydrolyzed by incubation with alkaline phosphatase (calf intestinal mucosa, 1140 units mg^{-1} protein; Sigma), at 37°C for 18 h in 0.5 cm^3 ethanolamine/HCl buffer (pH 9.5). Side-chain O-glucosides were hydrolyzed by incubation with β-glucosidase (almonds, 2 units mg^{-1}; Boehringer, Mannheim, FRG), at 25°C for 18 h in 0.5 cm^3 sodium acetate buffer (pH 5.0).

The three reversed-phase HPLC systems used in this study have been described in detail elsewhere (Palni et al. 1984, Badenoch-Jones et al. 1983). They comprised *System A:* an analytical μ-Bondapak phenyl column, eluted at 1.5 cm^3min^{-1} with a concave exponential gradient of 0 to 30% aqueous methanol for 20 min, and then isocratically with 30% methanol for a further 10 min; *System B:* a Waters C_8 RCM 100 column, eluted isocratically with 35% aqueous methanol at 2 cm^3min^{-1}; *System C:* a semi-preparative Zorbax C_8 column, eluted isocratically with 30% aqueous methanol at 4 cm^3min^{-1}. All solvents contained a constant concentration (0.1 M) of acetic acid.

Results

Biological Activity of Auxins and Cytokinins

Explants differed markedly in their response to auxin or cytokinin. By 48 h from the time of excision, (i.e. after 28 h culture), 30% to 60% of nuclei in auxin-treated explants had a 4C DNA level (Fig.1), and mitotic indices of up to 9.8% were recorded between 28 h and 36 h of culture (Table 2). In most cases, the response to IAA appeared to be greater than to the synthetic auxins NAA and 2,4-D (Fig. 1 and Table 2). In contrast, in control explants, or those treated with BAP or K alone, no response was observed up to 72 h from excision. 4C DNA levels were observed in a very small proportion of nuclei from Z-treated tissue (Fig. 1), and very low mitotic indices (0.1–0.5%) were recorded for two of six Z-treated explants after 28 h and 36 h of culture. No mitosis occurred in ZR-treated explants cultured in sucrose solution in the absence of IAA. In explants treated with both IAA and ZR, the first wave of mitosis began between 18 h and 24 h of culture, peaking between 24 h and 36 h (Table 2).

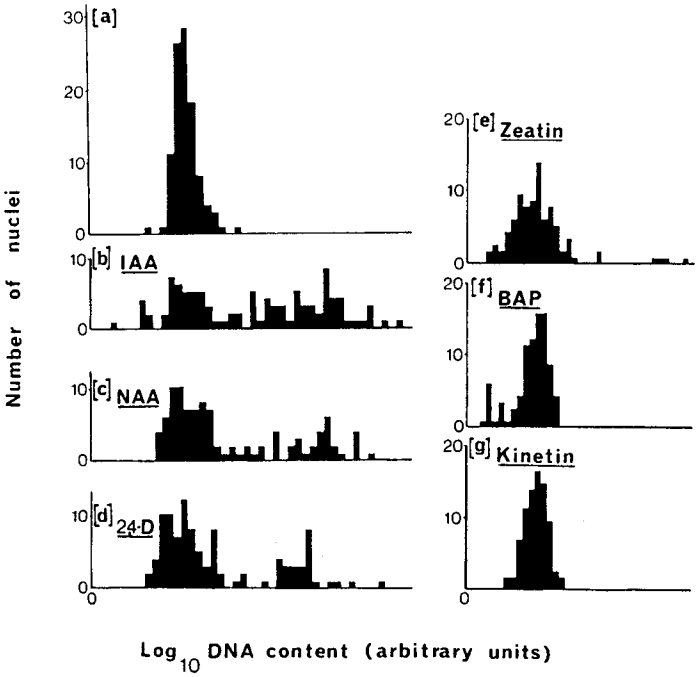

Fig. 1. Microdensitometric measurements of the DNA content of artichoke explants. Explants were prewashed for 20 h in water, then cultured for 28 h in water (a), or 1 mg l^{-1} aqueous solutions of auxins (b–d) or cytokinins (e–g).

Table 2. Mitotic indices (%) of cultured artichoke explants.

Hours of culture	18	24	30	36	42
2,4-D	0	nd	1.8	3.2	1.8
NAA	0	nd	1.0	3.0	0.9
IAA	0	nd	9.8	5.2	1.5
IAA + ZR + sucrose	0	5.1	9.3	5.9	nd
ZR + sucrose	0	0	0	0	nd

In three treatments, prewashed explants were cultured in aqueous solutions of 1 mg l^{-1} 2,4-D, NAA, or IAA. In two treatments ZR (0.13 mg l^{-1}) and sucrose (4 g l^{-1}) were included in the medium, as in the metabolism study, with or without IAA. No mitosis was observed in explants cultured for less than 18 h in any treatment.

nd = not determined.

Uptake and Metabolism of [8-^{3}H]ZR

Uptake of [8-^{3}H]ZR was very similar for both treatments (Fig. 2). Approximately half of the supplied radioactivity was taken up rapidly during the first 6 h of culture; thereafter the rate of uptake was apparently halved, resulting in a final tissue level of 70–75% of supplied radioactivity after 36 h of culture.

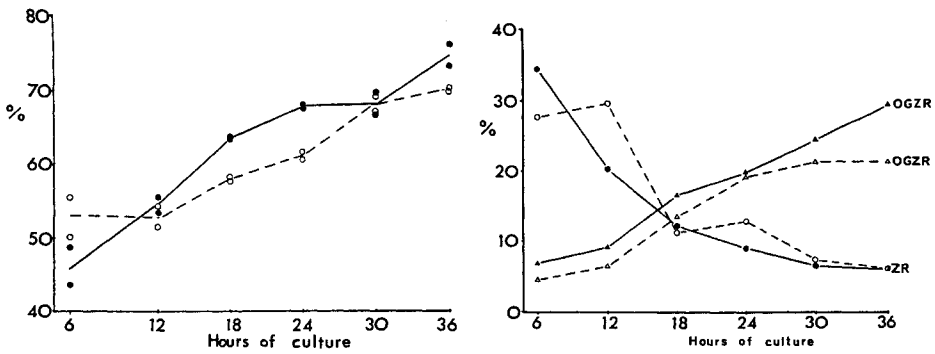

Fig. 2. Uptake of [8-^{3}H]ZR by artichoke explants in the presence (●—●—) or absence (O---O--) of IAA, expressed as % of supplied radioactivity.

Fig. 3. Levels of [8-^{3}H]ZR (●/O) and [8-^{3}H]OGZR (▲/△) in control (-----) and auxin-activated (——) artichoke explants, expressed as % of extracted radioactivity.

HPLC analysis indicated that [8-^{3}H]ZR accounted for 93% and 91.5% of the radioactivity present in staled medium from control and IAA-treated tissues, respectively, after 36 h incubation.

Extracted radioactivity accounted for 25% to 40% of the radiolabel taken up in each case. There were no marked or consistent differences in the recovery of radiolabel from treated and untreated tissues. The pattern of [8-^{3}H]ZR metabolism over 36 h appeared to be relatively simple. By selection of the appropriate HPLC systems (Table 1), zones of radioactivity from preparative TLC were almost completely resolved into residual [8-^{3}H]ZR and four major metabolites: O-glucosyl ZR (OGZR); adenosine, zeatin nucleotide(s) (ZN); and adenine nucleotide(s) (AN). Even after culture for 36 h, unidentified compounds comprised only a very small proportion of the extracted radioactivity. O-glucosyl dihydrozeatin riboside and O-glucosyl zeatin were present in all extracts in trace amounts, together comprising no more than 5% of the radioactivity due to OGZR. Free zeatin was not detected in any extract.

Changes in the proportions of residual ZR and OGZR were similar for both treatments throughout the culture period (Fig. 3). ZR declined steadily from c 30% of the extracted radioactivity after 6 h, to 6% at the end of culture. There was a corresponding increase in OGZR from c 6% after 6 h to a final value of c 25%. Slightly more OGZR appeared to be formed in IAA-treated explants than in control explants, particularly in the later stages of culture (Fig. 3). In contrast, marked treatment differences in the proportions of ZN, adenosine, and AN developed after the first 12 h of culture (Fig. 4). Thus, although ZN accumulated steadily in control explants from 23% to 50% at the end of the culture period, a parallel increase was observed in IAA-treated explants only during the first 12 h; thereafter ZN declined, eventually returning to a value slightly lower than that for 6 h (Fig. 4). Measurements of adenosine and AN indicated a corresponding accumulation of these degradative metabolites in auxin-treated explants, compared with a slight decline and generally lower levels of degradative metabolites in control explants. This was most evident in the final level of adenosine, which was 0.1% in control explants, compared

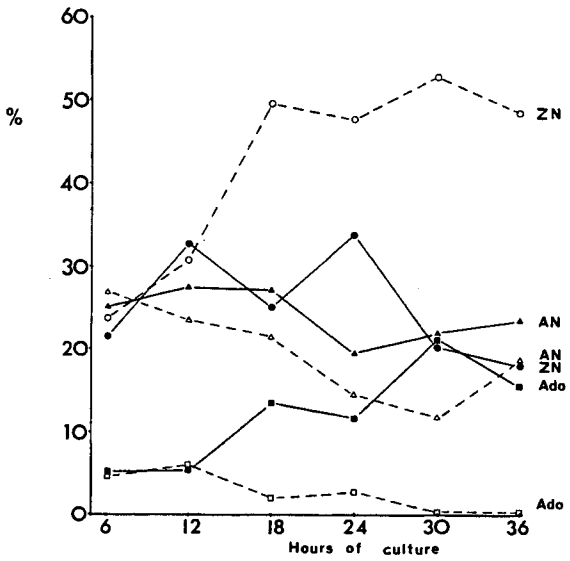

Fig. 4. Levels of [8-^{3}H]ZR metabolites in control (-----) and auxin-activated (——) artichoke explants, expressed as % of extracted radioactivity. ■/□ Ado, adenosine; ▲/△ AN, adenine nucleotide(s); ●/○ ZN, zeatin nucleotide(s).

to 16% in IAA-treated explants (Fig. 4). The accumulation of adenosine in IAA-treated explants appeared to reflect the depletion of ZN in the same tissue; treatment differences in the proportions of both of these metabolites were first apparent only after 12 h (Fig. 4).

Discussion

The observation that cytokinins are inactive in inducing cell division in prewashed artichoke tuber tissue is in accord with the majority of published data on this system (Adamson 1962, Setterfield 1963, Kamisaka and Masuda 1970, Minocha 1979), although this is the first time that the effect of naturally occurring cytokinins (Z and ZR) has been examined.

Measurements made between 6 h and 36 h after the start of culture indicated that IAA had no effect on the uptake of [8-^{3}H]ZR by artichoke explants. ZR was taken up at the same rate by tissue comprising predominantly dividing cells as by tissue comprising only quiescent or expanding cells. This is surprising, not only because of the physiological differences between the two tissues, but also because of the differences in ZR metabolism that were detected in the later stages of culture. Previous studies of the uptake and metabolism of cytokinins by plant cells have indicated that these processes are closely related (Laloue et al. 1981, Laloue and Pethe 1982). A possible explanation of the artichoke results is that the level of ZN in the tissue was sufficiently high to buffer the system against degradative metabolism during cell division, and thus negated any effect on cytokinin uptake.

The physiological significance of nucleotide and glucoside conjugates of cytokinins is not clear, although there is some evidence that side-chain O-glucosides are storage and inactivation forms, while nucleotides have been as-

sociated with storage, uptake, and biosynthesis of cytokinins (Letham and Palni 1983). In the present study, the steady accumulation of OGZR in both dividing and nondividing tissue suggests that this metabolite is an inactivation or sequestered form of ZR, but the nucleotide, which was labile in dividing tissue, may function as a readily available storage metabolite and perhaps plays a role in hormonal homeostasis.

A direct comparison of cytokinin metabolism in dividing and nondividing tissue of the same type has not been reported before. However, interesting parallels can be drawn between these results and data from a number of studies of the metabolism of radiolabeled BAP, isopentenyladenine and their ribosides in cytokinin-dependent tobacco cell cultures (Laloue et al. 1981, Laloue and Pethe 1982). In that system the formation of N^7-glucosides was associated with inactivation, whereas the nucleotides appeared to be involved in steady-state maintenance of base and riboside levels. However, in contrast to artichoke tissue, in which free-base cytokinins were not detected in the present study, free bases were found as metabolites in dividing tobacco cells (Laloue and Pethe 1982) and were proposed as the active forms. If biological action of cytokinins is linked to degradative metabolism, the accumulation of adenosine and AN, rather than ZR, that was associated with the depletion of ZN in dividing artichoke tissue could be a consequence of the action of the nucleotide *per se*. The very high levels of ZN in artichoke tissues shortly after the start of culture are consistent with the hypothesis that these conjugates are involved in cytokinin uptake. The first recorded differences in ZR metabolism between nondividing and synchronously dividing artichoke tissues occurred at least 6 h before the onset of mitosis; subsequently, during the final 18 h of culture, changes in the relative levels of AN, ZN, and adenosine were much less apparent (Fig. 4). The timing of these differences in ZR metabolism suggests that cytokinins may act in cell division before mitosis occurs; some observations that have been made using other plant systems also support this proposal (Fosket 1977).

The marked effect of IAA on ZR metabolism reported here is a rare example of the modification of cytokinin metabolism by auxin. The only precedents for this are the reported effects of IAA on BAP metabolism in stem cuttings of *Solanum andigena* (Woolley and Wareing 1972), and the inhibition of α-(6-alkylaminopurine-9-yl)alanine synthase, *in vitro*, by IAA and 4-chloro-IAA (Letham et al. 1982). Plant cell division and morphogenesis are very sensitive to, and probably controlled by, relative levels of auxin and cytokinin in the tissue (Foskett 1977, Greshoff 1978, Morris et al. 1982). The possibility that cytokinin metabolism is regulated by auxin would thus be of great significance in developmental botany.

References

Adamson D (1962) Expansion and division in auxin-treated plant cells. Can J Bot 40:719–744

Badenoch-Jones J, Rolfe B, Letham DS (1983) Cytokinin metabolism in effective and ineffective pea root nodules. Plant Physiol, in press

Bennici A, Cionini PG, Genna D, Cionini G (1982) Cell cycle in *Helianthus tuberosus* tuber tissue in relation to dormancy. Protoplasma 112:133–137

Fosket DE (1977) Regulation of the plant cell cycle by cytokinin. In: Rost TL, Gifford EM (eds) Mechanism and control of cell division. Dowden, Hutchison and Ross, New York, pp 66–91

Greshoff PM (1978) Phytohormones and growth and differentiation of cells and tissues cultured *in vitro*. In: Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds: A comprehensive treatise. Elsevier/North Holland, Amsterdam, vol 2, pp 1–29

Heimer GV, Taylor CED (1974) Improved mountant for immunofluorescence preparations. J Clin Pathol 27:254–256

Kamisaka S, Masuda Y (1970) Auxin-induced growth of *Helianthus* tuber tissue. V. Role of DNA synthesis in hormonal control of cell growth. Physiol Plant 23:343–350

Kamisaka S, Sakurai N, Masuda Y (1973) Auxin-induced growth of tuber tissue of Jerusalem artichoke. VIII. Role of cyclic AMP in the action of auxin, cytokinin and gibberellic acid. Plant Cell Physiol 14:183–193

Laloue M, Pethe-Terrine C, Guern J (1981) Uptake and metabolism of cytokinins in tobacco cells. In: Guern J, Péaud-Lenoël C (eds) Metabolism and molecular activities of cytokinins. Springer-Verlag, Berlin Heidelberg New York, pp 80–96

Laloue M, Pethe C (1982) Dynamics of cytokinin metabolism. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, London, pp 185–195

Letham DS (1978) Cytokinins. In: Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds: A comprehensive treatise. Elsevier/North Holland, Amsterdam, vol 1, pp 205–263

Letham DS, Palni LMS (1983) The biosynthesis and metabolism of cytokinins. Annual Rev Plant Physiol 34:163–197

Letham DS, Tao G-Q, Parker CW (1982) An overview of cytokinin metabolism. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, London, pp 143–153

Lin W (1980) Corn root protoplasts. Plant Physiol 66:550–554

Masuda Y (1965) RNA in relation to the effect of auxin, kinetin and gibberellic acid on the tuber tissue of Jerusalem artichoke. Physiol Plant 18:15–23

Minocha SC (1979) The role of auxin and abscisic acid in the induction of cell division in Jerusalem artichoke tuber tissue cultured *in vitro*. Z Pflanzenphysiol 92:431–441

Minocha SC, Halperin W (1974) Hormones and metabolites which control tracheid differentiation, with or without concomitant effects on growth, in cultured tuber tissue of *Helianthus tuberosus* L. Planta 116:321–333

Morris RO, Akiyoshi DE, MacDonald EMS, Morris JW, Regier DA, Zaerr JB (1982) Cytokinin metabolism in relation to tumour induction by *Agrobacterium tumefaciens*. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, London, pp 175–183

Palni LMS, Palmer MV, Letham DS (1984) The stability and biological activity of cytokinin metabolites in soybean callus tissue. Planta, in press

Phillips R, Dodds JH (1977) Rapid differentiation of tracheary elements in cultured explants of Jerusalem artichoke. Planta 135:207–212

Setterfield G (1963) Growth regulation in excised slices of Jerusalem artichoke tissue. Symp Soc Exp Biol 17:98–126

Woolley DJ, Wareing PF (1972) Hormonal interaction, movement and metabolism of a cytokinin in rootless cuttings. New Phytol 71:781–793

Yeoman MM, Davidson AW (1971) Effect of light on cell division in developing callus cultures. Ann Bot 35:1085–1092

Yeoman MM, Mitchell LP (1970) Changes accompanying the addition of 2,4-D to excised Jerusalem artichoke tissue. Ann Bot 34:799–810