Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 953–957, February 1993
Plant Biology

Cytolocalization of zeatin *O*-xylosyltransferase in *Phaseolus*

(cytokinins/phytohormones/legume/endosperm)

RUTH C. MARTIN, MACHTELD C. MOK, AND DAVID W. S. MOK*

Department of Horticulture and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR 97331

Communicated by S. J. Peloquin, October 19, 1992

ABSTRACT **Zeatin *O*-xylosyltransferase (EC 2.4.2.-) mediates the formation of *O*-xylosylzeatin from *trans*-zeatin and UDP-xylose in immature seeds of *Phaseolus vulgaris*. Tissue printing with a monoclonal antibody specific for the enzyme and a cDNA probe demonstrated that the enzyme was primarily localized and synthesized in the endosperm. Immunolocalization performed on monolayer endosperm at the free-nuclei stage and on EM sections demonstrated that the enzyme was associated with the nucleus as well as with the cytoplasm. Immunoanalysis of nuclear fractions revealed that the enzyme was retained in the nuclear pellet. Western analysis also showed that the enzyme was present in the nuclei of cotyledons and endosperm callus. The findings suggest that the enzyme may be involved in the nuclear–cytoplasmic transport of cytokinins and related molecules or, possibly, with chromatin of rapidly dividing cells.**

Cytokinins are plant hormones mediating cell division and differentiation (1). Zeatin is a naturally occurring cytokinin highly active in most plant systems (2). Genetic differences in cytokinin metabolism between species of *Phaseolus* were investigated by incubating immature seeds with radiolabeled zeatin (3). Exogenously supplied zeatin was rapidly converted to *O*-xylosylzeatin by immature seeds of *P. vulgaris*, while *O*-glucosylzeatin was formed in *P. lunatus*. The species-specific formation of zeatin metabolites was related to the occurrence of a single, distinct enzyme in immature seeds of each of the two species; zeatin *O*-xylosyltransferase in *P. vulgaris* and *O*-glucosyltransferase in *P. lunatus*. Both enzymes are highly specific for *trans*-zeatin but differ in specificity for the donor of the glycosyl moiety (4, 5). UDP-xylose (UDPX) serves as the donor for *O*-xylosyltransferase from *P. vulgaris*, whereas the *O*-glucosyltransferase of *P. lunatus* can utilize both UDP-glucose (UDPG) and UDPX to mediate the formation of *O*-glucosylzeatin and *O*-xylosylzeatin, respectively, but has 10 times higher affinity for UDPG. The two glycosyltransferases have a similar molecular size (M_r 50,000) but can be separated by ion exchange chromatography (4).

Two additional zeatin-metabolizing enzymes, a reductase and a cis–trans isomerase, have also been isolated from seeds of *Phaseolus* (6, 7). Interestingly, the activities of all four enzymes are particularly pronounced in immature seeds. We are interested in characterizing the developmental regulation of these enzymes. Part of this study is focused on localizing enzymes in the developing seed.

Zeatin *O*-xylosyltransferase was chosen as the first enzyme for localization studies because monoclonal antibodies (mAbs) specific for the enzyme have been generated (8) and immunopositive cDNA clones have been isolated from λgt11 expression libraries (9). In this paper we describe results obtained from tissue printing using a mAb (XZT-1) and a cDNA (pXZT82) as probes, immunolocalization performed on monolayer endosperm and EM sections, and Western analyses of cellular and nuclear fractions. The results indicate that the enzyme occurs predominantly in the endosperm nuclei and cytoplasm.

MATERIALS AND METHODS

Plant Materials. Immature seeds of *P. vulgaris* cv. Great Northern (GN) were used for all experiments. Endosperm callus tissues were established from abnormal, overgrown endosperm of *P. coccineus* × *P. vulgaris* hybrids (10, 11).

Tissue Printing. Protocols for tissue printing were those described by Cassab and Varner (12) with slight modifications. Nitrocellulose paper (pore size, 0.45 μm; Sigma) was soaked in 0.2 M $CaCl_2$ for 30 min and air-dried. Cross or vertical sections of seeds 3–6 mm in length were cut with a razor blade, carefully transferred to the membrane, covered with Kimwipes, and pressed against the membrane for 15–30 sec. The prints were air-dried overnight. For immunolocalization with the monospecific antibody (XZT-1), previously established protocols were followed (8). Secondary antibody was rabbit anti-mouse conjugated to alkaline phosphatase (Jackson Laboratories), and antigenic regions were visualized by using an alkaline phosphate substrate kit (Vector Laboratories). Control blots were treated in the same way, with the omission of mAb XZT-1. The location of proteins on tissue prints was detected by staining membranes with 0.05% Coomassie brilliant blue R (Sigma) dissolved in 12.5% trichloroacetic acid and destaining with 1% acetic acid in methanol. For RNA blots, nylon membranes (Hybond-N+, Amersham) were used without pretreatment (13). An insert (0.2 kbp) of a cDNA clone, pXZT82, containing the coding sequence of the epitope of the enzyme antigenic to mAb XZT-1 (9) was used as the probe. A multiprime labeling system (Amersham) was used to obtain radioactive DNA.

***In Situ* Immunolocalization Using Free-Nuclei Endosperm.** The monolayer endosperm was dissected from seeds 2 mm in length and transferred directly to a gelatin-coated microscope slide. The slides were fixed for 2 hr in Sorensen's phosphate buffer containing 4% glutaraldehyde. Rabbit anti-mouse antibody conjugated to alkaline phosphatase and goat anti-mouse antibody conjugated to colloidal gold (5 nm, BioCell Gold Conjugates, Redding, CA) were used to visualize the location of the primary antibody. Treatments using gold-labeled antibodies included silver enhancement according to the manufacturer's instructions (BioCell Research Laboratories). Control samples were incubated without the primary mAb XZT-1 or with an unrelated antibody (specific for viral coat proteins; a gift from R. Martin, Agricultural Canada).

Immunolocalization on EM Sections. Tissues were prepared essentially according to Spurr (14). Sections (0.1 μm) were prepared on a Sorvall Porter–Blum ultramicrotome. Grids were blocked with 0.5% bovine serum albumin in Tris-buffered saline overnight at 4°C and incubated for 2 hr at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: UDPG, UDP-glucose; UDPX, UDP-xylose; mAb, monoclonal antibody.

*To whom reprint requests should be addressed.

room temperature with the primary antibody, XZT-1. They were then treated with goat anti-mouse antibody (1:50 dilution) conjugated to colloidal gold (20 nm) for 2 hr at room temperature, poststained with Reynold's lead citrate (15), and examined with a Phillips CM12 scanning transmission electron microscope.

Western Analysis and Enzyme Assays. Procedures for enzyme extraction, protein determination, and immunodetection have been reported (4, 8). The extracts obtained from cotyledons were purified by Blue Sepharose 6B chromatography, whereas endosperm samples were used directly.

Isolation of Nuclei. Nuclei were isolated according to Cox and Goldberg (16). The nuclei were lysed with 0.5 M NaCl in Honda buffer (33 mM Tris·HCl pH 8.5/3.3% Ficoll/6.6% Dextran-T40/6.6 mM $MgCl_2$/3.3% Triton X-100) for 45 min at 4°C and centrifuged. The supernatant and pellet were analyzed by immunoblotting after SDS/PAGE (8).

RESULTS

Tissue Printing. Fig. 1 shows the direction of tissue sections and the location of endosperm (*Left*), the distribution of proteins (*Center*), and the distribution of *O*-xylosyltransferase (*Right*). In parallel sections (Fig. 1 *A* and *B*), the endosperm was located at the lower side distal to the radicle. A strong immunogenic response was visible in the same region. In a perpendicular section (Fig. 1*C*) of the seed, the enzyme was visualized as surrounding the developing cotyledons and the radicle, coinciding with the location of the endosperm. The results demonstrate that the enzyme zeatin *O*-xylosyltransferase is localized primarily in the endosperm.

To determine the location of *O*-xylosyltransferase mRNA, seed sections were pressed on nylon filters for Northern hybridization (13). The labeled cDNA probe (pXZT82) used for the hybridization contained the coding sequence of the enzyme epitope antigenic to mAb XZT-1 (9). Strong radioactivity (Fig. 2*B*) was detected in locations corresponding to the endosperm (Fig. 2*A*). This observation suggests that the complementary mRNA sequences also occur in the endosperm.

***In Situ* Immunolocalization Using Monolayer Endosperm.** Endosperm at the free-nuclei stage was used to determine the cellular location of *O*-xylosyltransferase. At this developmental stage, regions of the endosperm proximal to the radicle contain nuclei of varying sizes (as the result of endomitosis) sharing a cytoplasm. The nuclei are easily visualized with a light microscope (Fig. 3*A*). The presence of immunogenic proteins was determined with mAb XZT-1 and secondary antibodies conjugated with colloidal gold or alkaline phosphatase. The colloidal gold particles were concentrated in the nuclei (Fig. 3*B*). A sample of a nucleus in isolation (Fig. 3*C*) illustrates the contrast between nucleus and cytoplasm. Control samples with the omission of the primary antibody XZT-1 (Fig. 3*D*) or with unrelated antibodies showed only faint images of the nuclei against equally tinged cytoplasmic background. Treatment with secondary antibodies linked with alkaline phosphatase gave similar results. The nuclei were darkly stained in samples incubated with the primary antibody (Fig. 3*E*), whereas control samples (Fig. 3*F*) had similar coloration of nuclei and cytoplasm. These observations indicate that zeatin *O*-xylosyltransferase is associated with the nuclei as well as the cytoplasm of the endosperm.

Immunolocalization on EM Sections. The association of the enzyme with the nucleus was further examined by transmission electron microscopy. Regions of the endosperm distal to the radicle, containing highly mitotic cells, were used for

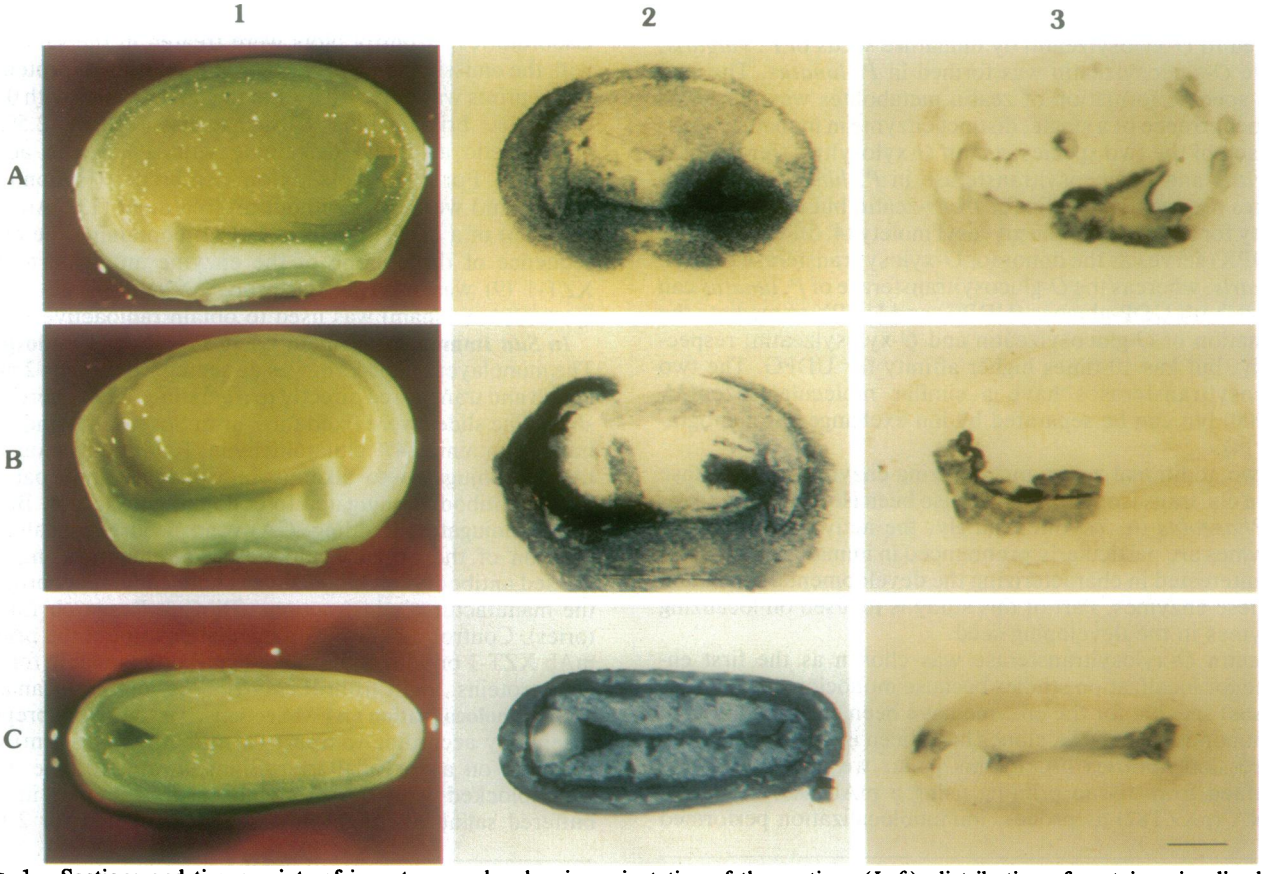

FIG. 1. Sections and tissue prints of immature seeds, showing orientation of the sections (*Left*), distribution of proteins visualized by Coomassie brilliant blue (*Center*), and distribution of zeatin *O*-xylosyltransferase detected by immunoblotting (*Right*). (*A* and *B*) Sections parallel to the plane of the cotyledons. (*C*) Section perpendicular to the plane of the cotyledons. (Bar = 1 mm.)

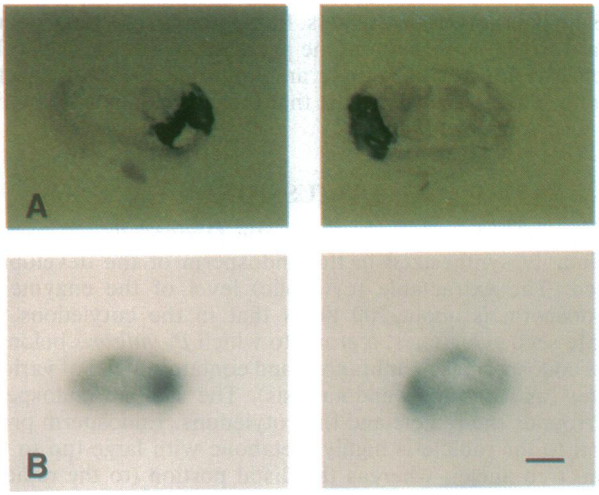

FIG. 2. Immunoblots and Northern blots of tissue prints. (*A*) Immunolocation of *O*-xylosyltransferase in parallel sections. (*B*) Northern hybridization of corresponding sections. (Bar = 2 mm.)

immunolocalization on ultrathin sections. After treatment with mAb XZT-1, the location of the enzyme was visualized with rabbit anti-mouse antibody conjugated to colloidal gold. In these samples, the gold particles were deposited on both the nucleus and the cytosol (Fig. 4 *A* and *B*). The gold particles in the cytoplasm did not appear to be concentrated in particular locations. No deposits were observed in structurally intact organelles such as the vacuoles. Gold particles were associated with electron-dense regions such as the condensed chromatin (Fig. 4*C*). A similar pattern was observed in endomitotic cells with more than one nucleus (Fig. 4*D*). The nuclear envelope did not have higher concentrations of the gold particles (Fig. 4*E*). A control sample (Fig. 4*F*) incubated with unrelated primary antibody did not contain any colloidal gold. Similar results were obtained from sections of cotyledon tissues.

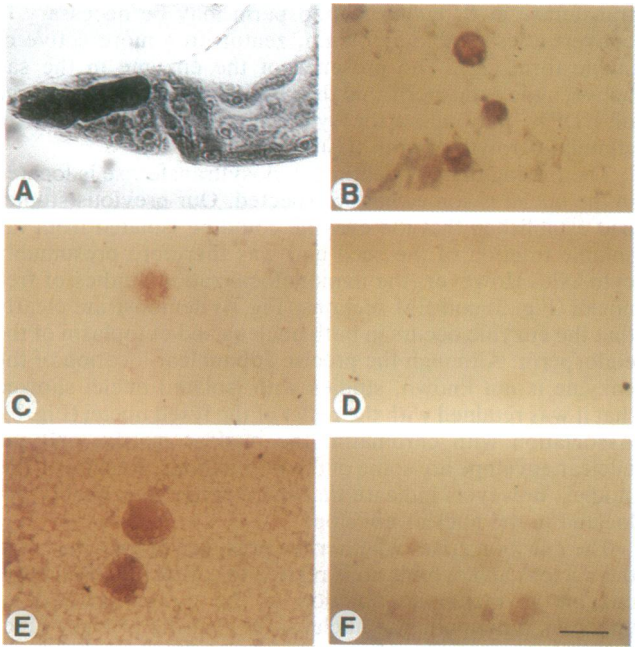

FIG. 3. Representative sample of developing endosperm and *in situ* immunolocalization on endosperm at free-nuclei stage. (*A*) Embryo surrounded by sac-like endosperm at free-nuclei stage, 72 hr after fertilization. (*B*) Localization of *O*-xylosyltransferase in nuclei detected with goat anti-mouse antibodies conjugated to colloidal gold and silver enhancement. (*C*) Same treatment as *B*, showing a single nucleus and cytoplasm. (*D*) Control sample of treatment *B*, incubated without primary antibody. (*E*) Localization of *O*-xylosyltransferase in nuclei detected with rabbit anti-mouse antibodies conjugated to alkaline phosphatase. (*F*) Control sample of treatment *E*, incubated without primary antibody. (Bar = 0.5 μm.)

Western Analyses of Nuclear Fractions from Reproductive and Vegetative Tissues. To confirm the association of the enzyme with the nucleus, nuclei were isolated from endosperm and cotyledons. After lysis, the soluble nuclear proteins and those remaining with the pellet were analyzed by immunoblotting after SDS/PAGE. Immunogenic bands were detected by the antibody in the pellet of lysed nuclei (Fig. 5, lanes 1 and 2) but not in the supernatant containing nuclear proteins (lanes 4 and 5), suggesting that the enzyme may be associated with chromatin, nuclear matrix, or the nuclear envelope.

We have established in previous work (17) that *O*-xylosyltransferase occurs in soluble fractions of seeds but is not detectable in comparable fractions of vegetative tissues. In light of the findings described above, this difference could be the result of nuclear localization rather than the absence of the enzyme from vegetative tissues. To evaluate this possibility, cytosolic and nuclear fractions of roots, leaves, shoot meristems, and endosperm-derived callus were examined. As observed in earlier work, the enzyme was not present in the cytosolic or nuclear fractions of vegetative tissues. However, the enzyme was detected in the nuclear fraction of endosperm-derived callus (data not shown). These observations suggest that the expression of genes encoding the enzyme is tissue-specific and apparent only in reproductive tissues. Endosperm-derived callus represents a unique intermediate between reproductive and vegetative states.

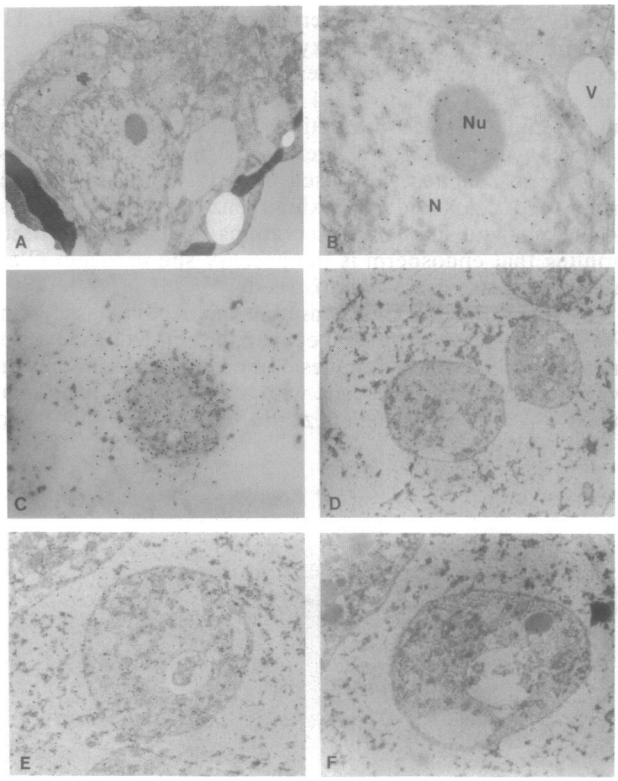

FIG. 4. Immunolocalization of *O*-xylosyltransferase on EM sections of endosperm. (*A*) Section showing deposit of gold particles in cytoplasm and nucleus. (×4200.) (*B*) Close-up of *A* showing nucleus (N), nucleolus (Nu), and vacuole (V). (×14,800.) (*C*) Gold particles on condensed chromatin. (×9000.) (*D*) Distribution of gold particles in cell containing two nuclei. (×4200.) (*E*) Close-up of a nucleus with intact nuclear envelope. (×5300.) (*F*) Control section treated with an unrelated primary antibody. (×5300.)

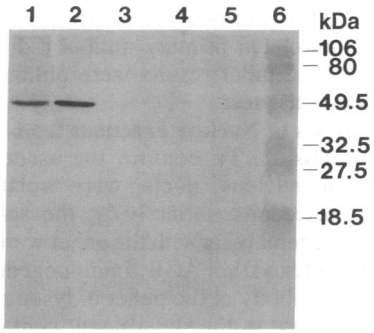

FIG. 5. Immunoblot of nuclear fractions after SDS/PAGE. Lanes 1 and 2, proteins from pellet of lysed nuclei isolated from seeds and endosperm, respectively; lane 3, blank; lanes 4 and 5, proteins from supernatant (nuclear protein) of lysed nuclei obtained from seeds and endosperm, respectively. Samples were treated with the mAb XZT-1 and visualized with goat anti-mouse secondary antibodies conjugated to alkaline phosphatase.

Enzyme Levels in Endosperm and Cotyledons. The relative levels of the enzyme in the cytosolic fractions of the endosperm and cotyledon were determined by Western blotting and activity assays. For immunoblotting, equal amounts (5 μg) of proteins (Fig. 6, lanes 3 and 4) extracted from endosperm and cotyledons were separated by SDS/PAGE and detected with the mAb (lanes 1 and 2). A single antigenic band of 50 kDa (which is the size of purified zeatin *O*-xylosyltransferase) occurred in both samples. However, the intensity of the antigenic band derived from the endosperm samples was much higher. Immunoblots of PAGE samples (on 10% gel at pH 8.5) also detected only a single band with the correct R_f of 0.5 in each sample (results not shown).

Enzyme activity was determined as described (4). The specific activity (cpm of *O*-xylosylzeatin formed per μg of protein) of the enzyme in the endosperm was about 200 times that in the cotyledons (7264 vs. 39). This is probably an underestimate, since the activity assays of the cotyledon samples were performed after partial purification whereas endosperm samples were used directly. Samples obtained from seed coats did not exhibit enzyme activity. These results also substantiate observations obtained from tissue printing that endosperm is the primary site of zeatin *O*-xylosyltransferase in *P. vulgaris* seeds.

Enzyme Activity of the Endosperm Sap. Activity assays and Western analysis of the sap between the cotyledons revealed high enzyme activity. Samples of the liquid endosperm were withdrawn from intact seeds with a microsyringe and incubated directly with labeled zeatin, zeatin plus UDPX, or zeatin plus cotyledon extracts. Interestingly, *O*-xylosylzeatin was formed with zeatin in the presence of UDPX or cotyledon extract, but only trace amounts occurred with zeatin alone. These results suggest that the cotyledons may be the *in vivo* source of UDPX.

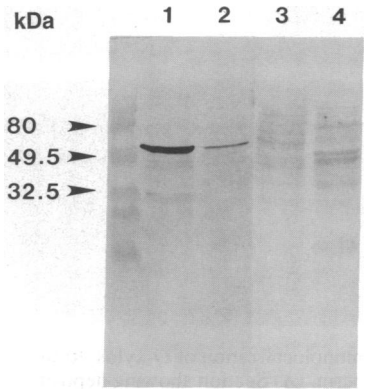

FIG. 6. Immunoblots of zeatin *O*-xylosyltransferase and proteins after SDS/PAGE. Lanes 1 and 2, endosperm and cotyledon samples (5 μg of protein), respectively, detected with mAb. Lanes 3 and 4, endosperm and cotyledon samples, respectively, stained with Coomassie blue.

DISCUSSION

The results demonstrate that zeatin *O*-xylosyltransferase is primarily synthesized in the endosperm of the developing seed. The extractable (cytosolic) level of the enzyme in endosperm is about 200 times that in the cotyledons. In large-seeded legumes, a group to which *P. vulgaris* belongs, the endosperm is membranous and contains nuclei of various sizes (as a result of endomitosis). The sac-like endosperm surrounds the radicle and the cotyledons. Endosperm proximal to the radicle is highly metabolic with large (up to 0.5 μm) free nuclei, whereas the distal portion (to the radicle) consists of mitotic cells. At later stages of seed development, coincident with rapid expansion of the cotyledon, the endosperm gradually ceases to divide, desiccates, and remains as a dry membrane between the seed coat and the cotyledons. The localization of the enzyme in the endosperm agrees with the tests of enzyme activity and with the earlier finding that enzyme activity decreases with seed maturation (17). Using immunoassays, we have detected antigenic proteins in seeds of other plants including *Zea mays*, *Vicia faba*, and lupin (9). However, the proteins differ in mass from the *O*-glycosyltransferases of *Phaseolus*. We have not determined whether the immunoreactive proteins in seeds of other species are also located in the endosperm.

In general, hormone activity is high during active seed growth (18). Elevated levels of cytokinins were found in endosperms of maize, rice, and wheat during the highly mitotic phase (19–21). The high concentration of *O*-xylosyltransferase in *P. vulgaris* endosperm may be necessary to convert a less stable cytokinin, zeatin, to a more active or stable form (22). The presence of the enzyme in the sap between the endosperm and the cotyledons, the likely source of UDPX, may allow the synthesis of *O*-xylosylzeatin to stimulate growth of both tissues.

The finding that the zeatin *O*-xylosyltransferase is located in the nuclei is somewhat unexpected. Our previous studies had established that the enzyme could be isolated from the soluble fraction of the seed and was therefore presumably cytosolic. However, the immunolocalization studies of free nuclei (Fig. 3) and EM sections (Fig. 4) demonstrate clearly that the enzyme occurs in both nucleus and cytoplasm of the endosperm. Although the precise subnuclear location of the enzyme is not known, studies with isolated nuclei showed that it was retained with the pellet of the lysed nuclei (Fig. 5). Association with chromatin, the nuclear matrix, and the nuclear envelope are some of the possibilities. Results of EM studies, however, indicate that the enzyme is probably not located in the nuclear envelope.

The question arises whether the presence of the enzyme in the nucleus and cytoplasm is related to a dual function of the enzyme—a metabolic function in the cytoplasm and an unknown function in the nucleus—or that the primary function is in the nucleus and the occurrence of the enzyme in the cytoplasm is due to the rapid nuclear divisions and endomitotic nature of the endosperm. Proteins associated with the nucleus, such as transcription factors, chromosomal proteins, and components of the nuclear envelope, are dispersed in the cytoplasm during cell division and reassembled after the reformation of the nucleus. Examples of such proteins include transcription activators (23, 24), the mitotic regulator protein RCC1 (25, 26), and the lamin kinase (27). In slowly dividing tissues, the amount of nuclear proteins present in the cytoplasm is expected to be low and transient. However, in

bean endosperm, the presence of such proteins may persist due to the rapid succession of dissolution and reassembly of the nuclei. This would lead to detection of the *O*-xylosyltransferase in the cytoplasm, but not in structurally intact bodies such as the vacuoles, which is in agreement with the results of the EM studies. The presence of the enzyme in the soluble fraction of the cotyledons (Fig. 6), a rapidly dividing tissue, and its absence from the soluble fraction of endosperm-derived callus, a slowly dividing tissue, also support this interpretation. Although it is uncertain to what extent the presence of the enzyme in the endosperm cytoplasm is related to the effect of nuclear division, it is clear that the presence of the enzyme in the nucleus is not the result of the reverse effect—i.e., inclusion in the nucleus of enzyme normally present in the cytoplasm due to rapid nuclear division. In that case the enzyme should have been found in the soluble nuclear protein fraction, whereas our results demonstrate that the protein is associated with the nuclear pellet.

Association of the enzyme with the nucleus implies that either the enzyme, or its substrate, or its product may interact directly with nuclear components. Possibly, the enzyme is involved in the transport and targeting of cytokinins or cytokinin-related molecules between the nucleus and cytoplasm. Alternatively, the enzyme may be part of the chromatin complex as structural protein or may have DNA-binding properties. Cellular proteins such as Ras and nuclear lamins contain isoprenyl groups (farnesyl and geranylgeranyl), and attention has been directed at the possible role of isoprenoid derivatives in mammalian systems (28). Recently, antibodies against isopentenyladenosine (a plant cytokinin) were used to identify a protein (i^6A26) from Chinese hamster cell cultures, the level of which correlated with the rate of cell division (29). It was suggested that the i^6A26 protein may mediate DNA synthesis. As most naturally occurring cytokinins (e.g., isopentenyladenine and zeatin) are N^6-isoprenoid derivatives, it is conceivable that plant proteins with high affinity to cytokinins such as the *O*-xylosyltransferase may also interact with nuclear components related to the regulation of cell cycle.

As the protein is intermediate in size (50 kDa), it is likely that an active transport mechanism is required to localize the protein to the nucleus (30). Studies of nuclear targeting in mammals, yeast, and amphibians revealed active transport mechanisms for larger molecules (≥40 kDa) which have a nuclear localization signal (NLS) (31–33). Several plant proteins, including the opaque-2 gene product and auxin-binding protein, were reported to be localized in the nucleus (34, 35), and the amino acid sequence responsible for nuclear targeting of plant transcription factors is being investigated (36). As the N-terminal sequence of the *Agrobacterium* VirD2 protein was able to target a fusion protein to the plant nucleus (37), the NLS is believed to operate across species boundaries. Sequence analyses of the enzyme and comparisons with known NLSs and DNA-binding proteins may provide additional clues to the mechanism of nuclear targeting and the significance of nuclear localization of zeatin *O*-xylosyltransferase.

Genes involved in cytokinin biosynthesis and metabolism have not yet been isolated from higher plants. In prokaryotic systems, the only example is the *ipt* gene identified in *Agrobacterium* (38, 39). Zeatin-specific enzymes of *Phaseolus* may be exploited for genetic and molecular studies of cytokinin metabolism in plant systems. Even for the zeatin *O*-glycosyltransferases, we have identified multiple but related transcripts in *P. vulgaris* immature embryos (unpublished results). The analyses of gene sequences, their expression, and localization of their products in relation to the function of the respective enzymes should further the understanding of the genetic regulation of cytokinin metabolism in higher plants.

The research was supported by grants from the National Science Foundation (DCB-8818620 and DCB-9116490) and the Oregon Agricultural Experiment Station. This is technical paper no. 9702.

1. Skoog, F. (1973) in *Genes, Enzymes and Populations*, ed. Srb, A. M. (Plenum, New York), pp. 47–184.
2. Skoog, F. & Armstrong, D. J. (1970) *Annu. Rev. Plant Physiol.* **21**, 359–384.
3. Lee, Y. H., Mok, M. C., Mok, D. W. S. & Griffin, D. A. (1985) *Plant Physiol.* **77**, 635–641.
4. Dixon, S. C., Martin, R. C., Mok, M. C., Shaw, G. & Mok, D. W. S. (1989) *Plant Physiol.* **90**, 1316–1321.
5. Turner, J. E., Mok, D. W. S., Mok, M. C. & Shaw, G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3714–3717.
6. Martin, R. C., Mok, M. C., Shaw, G. & Mok, D. W. S. (1989) *Plant Physiol.* **90**, 1630–1635.
7. Mok, M. C., Martin, R. C., Mok, D. W. S. & Shaw, G. (1992) in *Physiology and Biochemistry of Cytokinins in Plants*, eds. Kaminek, M., Mok, D. W. S. & Zazimalova, E. (SPB, The Hague, The Netherlands), pp. 41–46.
8. Martin, R. C., Martin, R. R., Mok, M. C. & Mok, D. W. S. (1990) *Plant Physiol.* **94**, 1290–1294.
9. Mok, D. W. S., Mok, M. C., Martin, R. C., Bassil, N. V. & Lightfoot, D. A. (1992) in *Progress in Plant Growth Regulation*, eds. Karssen, C. M., van Loon, L. C. & Vreugdenhil, D. (Kluwer, Dordrecht, The Netherlands), pp. 597–606.
10. Shii, C. T., Rabakoarihanta, A., Mok, M. C. & Mok, D. W. S. (1982) *Theor. Appl. Genet.* **62**, 59–64.
11. Mok, M. C., Mok, D. W. S. & Armstrong, D. J. (1978) *Plant Physiol.* **61**, 72–75.
12. Cassab, G. I. & Varner, J. E. (1987) *J. Cell. Biol.* **105**, 2581–2588.
13. McClure, B. A. & Guilfoyle, T. J. (1989) *Plant Mol. Biol.* **12**, 517–524.
14. Spurr, A. R. (1969) *J. Ultrastructure Res.* **26**, 31–43.
15. Reynolds, E. S. (1963) *J. Cell Biol.* **17**, 208–213.
16. Cox, K. H. & Goldberg, R. B. (1988) in *Plant Molecular Biology, A Practical Approach*, ed. Shaw, C. H. (IRL, Oxford), pp. 1–34.
17. Mok, D. W. S., Mok, M. C., Martin, R. C., Bassil, N. & Shaw, G. (1992) in *Physiology and Biochemistry of Cytokinins in Plants*, eds. Kaminek, M., Mok, D. W. S. & Zazimalova, E. (SPB, The Hague, The Netherlands), pp. 17–24.
18. Quatrano, R. S. (1987) in *Plant Hormones and Their Role in Plant Growth and Development*, ed. Davies, P. J. (Nijhoff, The Hague, The Netherlands), pp. 494–514.
19. Tollenaar, M. (1977) *Maydica* **22**, 49–75.
20. Saha, S., Nagar, P. K. & Sircar, P. K. (1986) *Can. J. Bot.* **64**, 2068–2072.
21. Lenton, J. R. & Appleford, N. E. J. (1987) in *Cytokinins: Plant Hormones in Search of a Role*, eds. Horgan, R. & Jeffcoat, B. (Bri. Plant Growth Reg. Group, Bristol, U.K.), pp. 99–113.
22. Mok, M. C., Mok, D. W. S., Marsden, K. E. & Shaw, G. (1987) *J. Plant Physiol.* **130**, 423–431.
23. Kornberg, R. D. & Lorch, Y. (1991) *Cell* **67**, 833–836.
24. Meisterernst, M. & Roeder, R. G. (1991) *Cell* **67**, 557–567.
25. Bischoff, F. R. & Ponstingl, H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10830–10834.
26. Ohtsubo, M., Okazaki, H. & Nishimoto, T. (1989) *J. Cell Biol.* **109**, 1389–1397.
27. Tamar, E., Peter, M., Nurse, P. & Erich, A. N. (1991) *J. Cell Biol.* **112**, 797–807.
28. Casey, P. J., Thissen, J. A. & Moomaw, J. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8631–8635.
29. Faust, J. R. & Dice, J. F. (1991) *J. Biol. Chem.* **15**, 9961–9970.
30. Nigg, E. A., Baeuerle, P. A. & Luhrmann, R. (1991) *Cell* **66**, 15–22.
31. Dingwall, C. & Laskey, R. A. (1986) *Annu. Rev. Cell. Biol.* **2**, 367–390.
32. Goldfarb, D. S. (1989) *Curr. Op. Cell Biol.* **1**, 441–446.
33. Silver, P. A. (1991) *Cell* **64**, 489–497.
34. Varagona, M. J., Schmidt, R. J. & Raikhel, N. V. (1991) *Plant Cell* **3**, 105–113.
35. Prasad, P. & Jones, A. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5479–5483.
36. Van der Krol, A. R. & Chua, N. H. (1991) *Plant Cell* **3**, 667–675.
37. Herrera-Estrella, A., Van Montagu, M. & Wang, K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9534–9537.
38. Akiyoshi, D. E., Klee, H., Amasino, R. M., Nester, E. W. & Gordon, M. P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5994–5998.
39. Barry, G. F., Rogers, S. G., Fraley, R. T. & Brand, L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4776–4780.