Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6539–6543, July 1994
Plant Biology

Expression of an auxin- and cytokinin-regulated gene in cambial region in *Zinnia*

(tissue print hybridization/tracheary element/vascular differentiation)

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Contributed by Joseph E. Varner, March 23, 1994

ABSTRACT **The expression patterns of a cDNA clone, p48h-10, of an auxin-induced gene were examined in isolated mesophyll cells of *Zinnia* and in the organs of *Zinnia* plants. In the isolated mesophyll cells, the mRNA accumulates in 48 hr of culture with 1-naphthaleneacetic acid alone. Because the first cell division occurs before 36 hr of culture, the gene probably is not involved in cell division. Benzyladenine does not induce expression of this gene, but the combination of 1-naphthaleneacetic acid and benzyladenine induces the mRNA accumulation about 24 hr earlier than does 1-naphthaleneacetic acid alone. Tissue print hybridization shows that the mRNA is present predominantly in the cambial region in stems, leaves, and roots and in the vascular bundles in flower buds but does not occur in the apical regions of shoot or root. The characteristics of the gene expression, including auxin- and cytokinin-regulated induction and cambial region localization, encourage us to suggest that the gene is involved in the early process of vascular differentiation.**

The vascular cambium is the lateral meristem that gives rise to plant secondary vascular tissues, the secondary phloem, and the secondary xylem (1). The vascular systems are essential for support and conduction in land plants. Numerous reports show that vascular differentiation is mainly controlled by auxin and cytokinin. Auxin is the major control signal of vascular differentiation; cytokinin increases the sensitivity of the vascular cambium to the auxin stimulation (2, 3). Because of the difficulty of the isolation of pure cambial cells and of the inability to maintain cambial cells *in vitro*, a number of *in vivo* and *in vitro* systems other than cambial cells have been used to study the mechanism of vascular differentiation. Xylogenesis has been most studied because it is easily manipulated and xylem cells can be readily recognized (2, 4, 5). The *Zinnia*-isolated leaf mesophyll cell system is one of the best because auxin and cytokinin induce up to 60% of the cells to differentiate semisynchronously into tracheary elements (TEs) within 72 hr (4–6). Thus, the molecular dissection of the redifferentiation of *Zinnia* mesophyll cells into TEs should help us to understand the process of differentiation of cambial derivatives into the xylem elements *in vivo*.

The *Zinnia* system has been explored to study various physiological, biochemical, and molecular changes associated with TE formation (4–6). However, molecular changes that occur before onset of visible differentiation are not clearly understood. Recently, a number of differentially regulated genes associated with TE formation and not characterized before have been isolated in the *Zinnia* system (7, 8). Some of these genes are expressed before the onset of visible changes. These genes should provide useful tools for the dissection of the early process of xylogenesis.

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Two nearly identical cDNA clones, p48h-10 (7) and TED4 (8), were previously isolated from isolated *Zinnia* mesophyll cells treated with auxin and cytokinin. Their encoded proteins show high protein sequence identity to a barley aleurone-specific cDNA, B11E. That the encoded protein shares some features of plant nonspecific lipid transfer proteins (LTPs) aroused our interest for further analysis of this gene. In this paper we analyzed the regulation of expression of the p48h-10 gene by auxin and cytokinin in the *Zinnia* cultured cells. Furthermore, we examined developmental and spatial regulation of expression of the gene in the *Zinnia* organs. The relationship between the expression of this gene and vascular differentiation is discussed.

MATERIALS AND METHODS

Plant Materials. *Zinnia elegans* cv. Peter Pan was grown in the greenhouse. The internodes and nodes were arbitrarily numbered in order from young (top) to older (bottom).

Cell Isolation and Culture. Mesophyll cells from the first pair of leaves of 11-day-old *Zinnia* seedlings were isolated and cultured as described (7, 9).

RNA Isolation and Gel-Blot Analysis. The same procedures for total RNA isolation and gel-blot analysis were followed as described (7).

***In Vitro* Root Induction in *Zinnia* Stem.** The bottom ends of the stem segments (fourth internode) from 6-week-old *Zinnia* plants were treated with indole-3-butyric acid and transferred into potting mix for the induction of adventitious root formation. The adventitious root initials were formed near the cambial region of the treated stem 3 days after induction and the roots emerged through the epidermis 5 days after induction. The treated stems were taken for tissue printing 1–5 days after treatment.

Tissue Print Hybridization. Tissue printing and mRNA localization were performed as described (10). After tissue printing, the same sections were saved for toluidine blue staining to show the anatomy. Mature xylem walls stain blue with toluidine blue.

RESULTS

Effects of Auxin and Cytokinin on Expression of the p48h-10 Gene in the Isolated *Zinnia* Mesophyll Cells. In the *Zinnia* system, isolated mesophyll cells cultured in the basal medium without hormone generally expand about 15–30%. With the addition of 0.5 μM 1-naphthaleneacetic acid (NAA) alone, about 30% of cells divide. Cells with the addition of benzyladenine alone elongate without division. Both NAA and BA are required for TE formation, and various auxins and cytokinins are effective for TE induction (11). We used different types of auxin at different concentrations to exam-

Abbreviations: LTP, lipid transfer protein; NAA, 1-naphthaleneacetic acid; TE, tracheary element.

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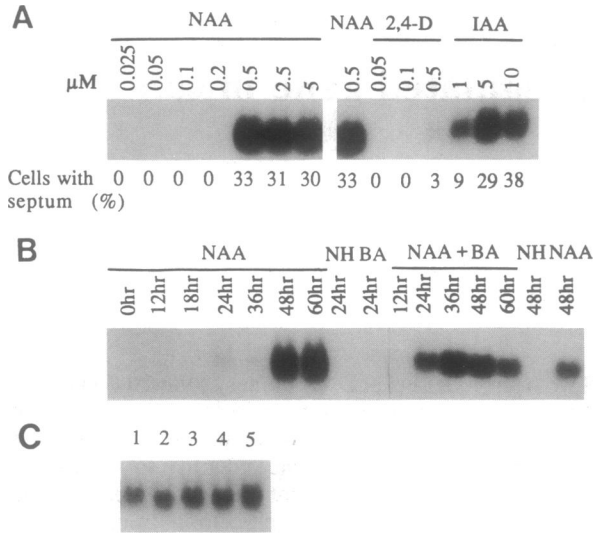

FIG. 1. RNA gel-blot analysis of the p48h-10 gene expression. Ten micrograms of total RNA were used for each lane. (*A*) Gene expression induced by NAA, 2,4-dichlorophenoxyacetic acid (2,4-D), and indoleacetic acid (IAA) in isolated *Zinnia* mesophyll cells. Cells were treated for 48 hr with different concentrations of auxins as indicated above each lane. The percent occurrence of cells with a septum is shown below each lane corresponding to different concentrations of auxin treatment. (*B*) Time courses of NAA- and/or benzyladenine (BA)-induced gene expression in isolated *Zinnia* mesophyll cells. Cells were treated with 0.5 μM NAA or 0.5 μM BA or both for different times as shown on the top of each lane. NH, no hormone. (*C*) Gene expression in 4-week-old *Zinnia* tissues. Lanes: 1, shoot tip including 2–3 mm of tissues below apical meristem; 2–5, first to fourth internodes, respectively.

ine whether expression of the p48h-10 gene is correlated with cell division. The dividing cells have septa, which are easily recognized under the microscope. The results in Fig. 1*A* indicate that NAA, 2,4-dichlorophenoxyacetic acid, and indoleacetic acid all induce expression of p48h-10 gene with different sensitivities to different auxins. When we compare in Fig. 1*A* the level of the mRNA accumulation with the percentage of the cells containing septa in the same treatment, we can see that the mRNA does not accumulate in those treatments without cell division, and the low level accumulation of the mRNA corresponds to the low frequency of cell division.

To clarify whether the gene is expressed during cell division or after cell division, we examined the time course of the mRNA accumulation induced by NAA alone. As shown in Fig. 1*B*, there was little mRNA accumulation until 48 hr after NAA treatment, while heavy accumulation of the mRNA occurred as early as 24 hr, when both NAA and benzyladenine were present (Fig. 1*B*). Because NAA-induced cell division of the isolated cells occurred between 24 hr and 36 hr under the culture conditions we used and because benzyladenine alone did not induce gene expression, these results indicate that expression of this gene occurs after cell division and that addition of benzyladenine changes the timing of the mRNA accumulation induced by NAA.

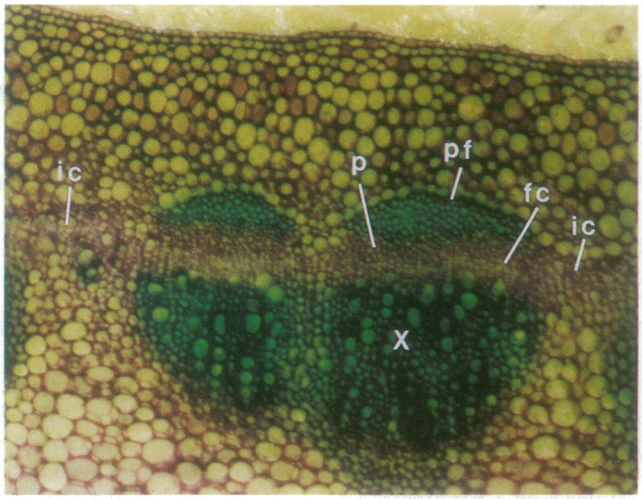

FIG. 2. Anatomy of the free-hand cross-section of second internode from a 6-week-old *Zinnia* plant. The section was stained with toluidine blue. fc, Fascicular cambial region; ic, interfascicular cambial region; p, phloem; pf, phloem fibers; x, xylem.

Expression of the p48h-10 gene in Developing Stems. Fig. 1*C* shows that the p48h-10 mRNA was present in the stems with a slight developmental gradient—i.e., the mRNA accumulation increased from shoot tip to the lower part of the stem.

Localization of the p48h-10 mRNA in *Zinnia* Tissues. As an aid in the interpretation of the anatomy, Fig. 2 shows vascular bundles and interfascicular cambia from the second internode of a 6-week-old *Zinnia* plant. Tissue print hybridization shown in Fig. 3*B* shows that the mRNA accumulates in the cambial region of a developing stem; this is different from the epidermal expression observed for some plant LTPs (12–15). Since a specific identification was not possible, we used the term "cambial region" to represent cambial initials and their derivatives containing phloem mother cells and xylem mother cells (1, 16). The localization of the signal was determined as follows. The mRNA signal is located between the xylem and phloem of the vascular bundles when the images between Fig. 3 *A* and *B* are superimposed. In addition, it is located between the bundles in the interfascicular cambia, which together with the fascicular cambia of the bundles, form a continuous ring of vascular cambium. The mRNA signal is well matched with the irregular cambial position. To rule out the possible continuity of newly differentiating xylem cells that have not shown differentiated features and hence are not visibly recognizable, we used a lignin-specific *O*-methyltransferase gene isolated from *Zinnia* (unpublished data) for its mRNA localization to

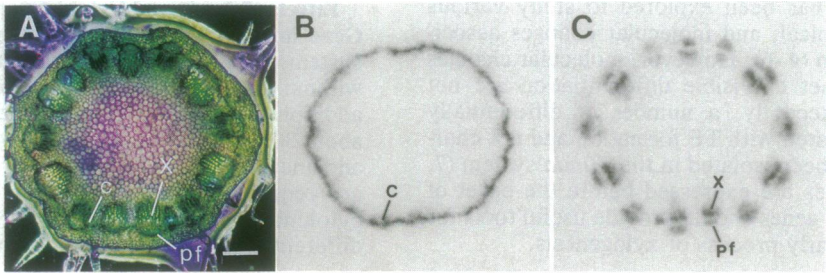

FIG. 3. Tissue print hybridization localization of mRNAs of the p48h-10 gene (*B*) and a lignin-specific *O*-methyltransferase (*C*) in the second internode of a 4-week-old *Zinnia* plant. (*A*) The anatomy of toluidine blue-stained section. c, Cambial region; pf, phloem fibers; x, xylem. (Bar = 0.4 mm.)

confirm further the discontinuity of vascular bundles. Fig. 3*C* shows that the *O*-methyltransferase mRNA localization is well matched with the xylem and phloem fiber bundles. The *O*-methyltransferase mRNA is present in differentiating xylem cells near the cambial region but not in the mature xylem cells. The discontinuity of the *O*-methyltransferase mRNA localization in the vascular region indicates that differentiating xylem cells are not present continuously in the interfascicular region. These comparisons indicate that the p48h-10 mRNA is present in the cambial region.

We determined whether there is a developmental regulation in tissue-specific gene expression. Fig. 4 shows that internodes at different developmental stages from different ages of plants possess the same cambial region-specific gene expression pattern. The mRNA signal in the first internode (the uppermost extended internode) of a 4-week-old plant is not continuous (Fig. 4 *A* and *B*). It is mainly localized in the fascicular cambial region, although the interfascicular regions can be recognized at this stage under higher magnification than the one shown in Fig. 4*A*. In the third internode of the 4-week-old plant (Fig. 4 *C* and *D*), the fascicular and interfascicular cambial regions are connected to form a cylinder; the mRNA signal forms a continuous ring. Interestingly, the mRNA signal in the first internode of the 6-week-old plant has already formed a continuous ring (Fig. 4*E*). Under higher magnification than the one shown in Fig. 4*E*, the interfascicular regions can be recognized. The mRNA is still present as a continuous ring in the fourth internode of the 6-week-old plant (Fig. 4 *G* and *H*). Differentiating xylem cells at this stage have not formed a cylinder since the mRNA localization of lignin-specific *O*-methyltransferase matches with the vascular bundles observed in Fig. 4*G* (data not shown).

We then determined whether the mRNA is present throughout the cambial region of the node and whether it is also expressed in the cambial regions of leaves and branch stem. Fig. 5 *A*, *C*, and *E* show how leaf and branch stem vascular bundles are connected with the main stem. Because the conditions for tissue prints, hybridization, and exposure time are the same, the intensity of the signal among them can be compared semiquantitatively. The results show that (*i*) the gene is expressed in cambial regions of all six leaf vascular bundles, but with lower intensity compared with stem cambial region; (*ii*) the mRNA can also be detected in the procambial regions in newly emerging branch stems, but at a low level (Fig. 5 *A–D*); (*iii*) a higher mRNA level is found in the nodal cambial region (Fig. 5 *E* and *F*) compared with the internodal one (Fig. 5 *A–D*); (*iv*) Fig. 5 *D*, *F*, and *H* show that the mRNA does not form a continuous ring; the nodal cambial cylinder is disrupted by the development of stem and/or leaf vascular bundles; and (*v*) from Fig. 5 *B* and *D* we see that a stronger signal is present in the fascicular cambial region than in the interfascicular one.

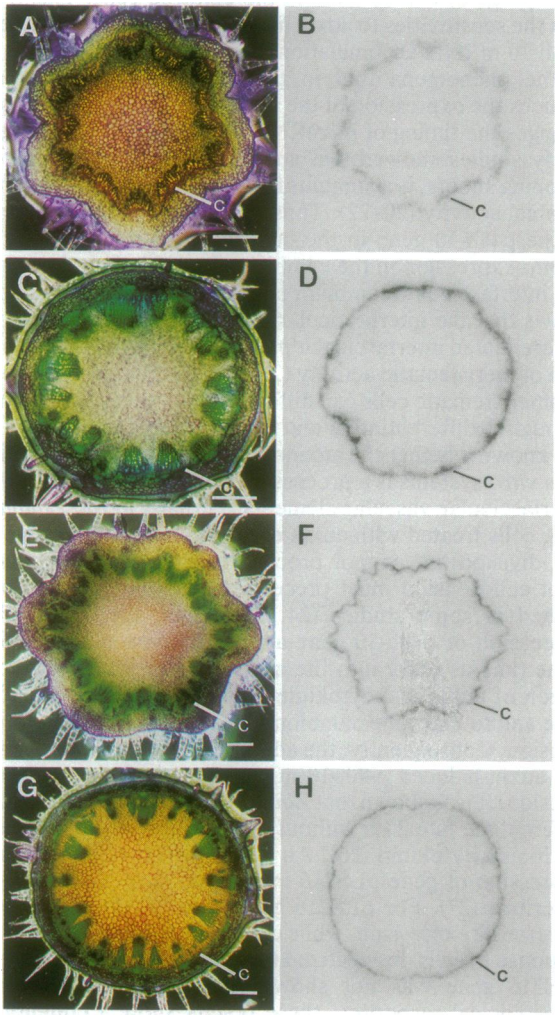

FIG. 4. Tissue print hybridization localization of the p48h-10 mRNA in the internodes. (*Left*) Anatomy of toluidine blue-stained sections. (*Right*) Corresponding mRNA localization. (*A* and *C*) First and third internodes of a 4-week-old *Zinnia* plant, respectively. (*E* and *G*) First and fourth internodes of a 6-week-old *Zinnia* plant, respectively. c, Cambial region. (Bars = 0.6 mm.)

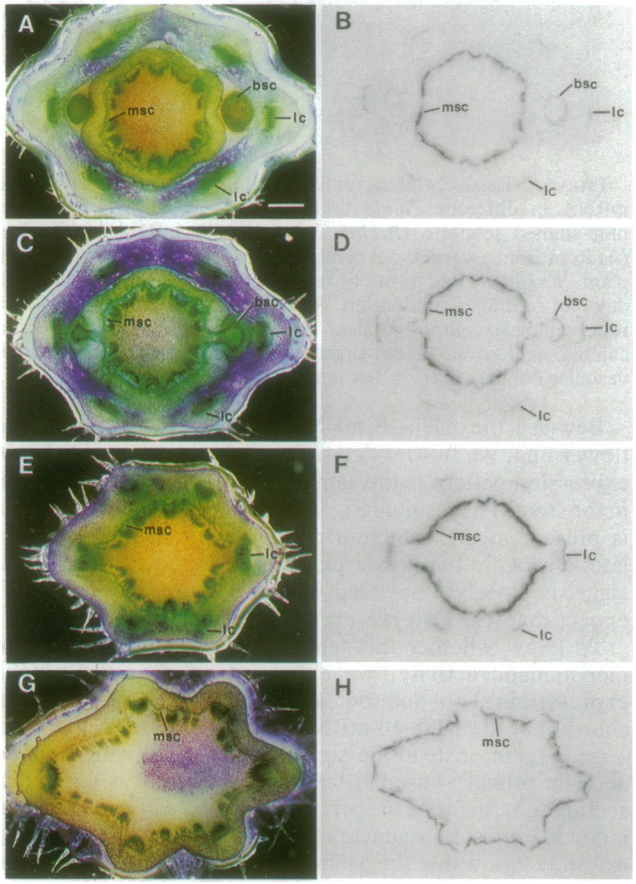

FIG. 5. Tissue print hybridization localization of the p48h-10 mRNA in the nodes. (*Left*) Anatomy of toluidine blue-stained sections. (*Right*) Corresponding mRNA localization. (*A*, *C*, and *E*) Three successive sections from top to bottom of the second node region of the 4-week-old *Zinnia* plant. (*G*) Second node of the 6-week-old *Zinnia* plant. bsc, Branch stem procambial region; lc, leaf cambial region; msc, main stem cambial region. (Bar = 1 mm.)

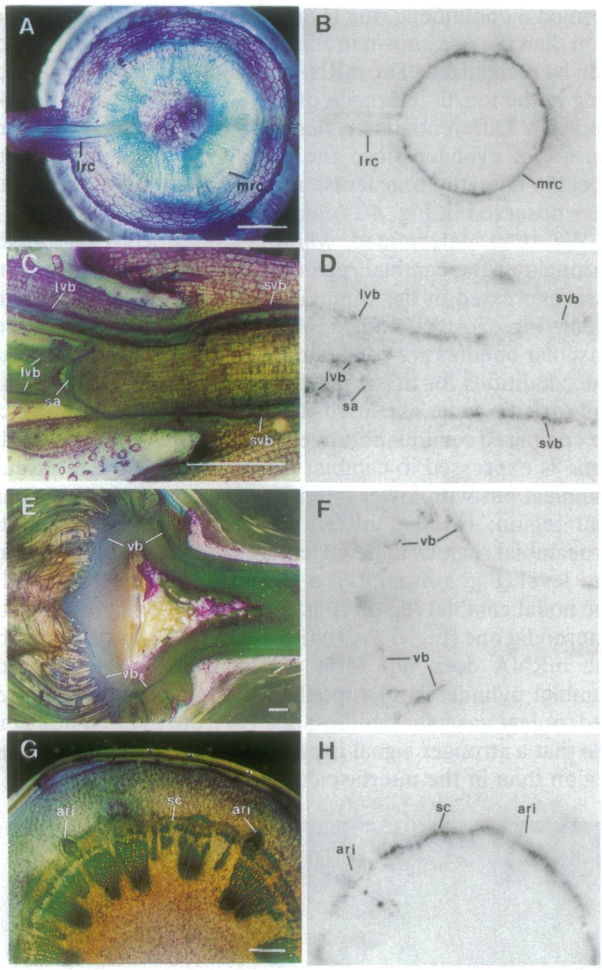

FIG. 6. Tissue print hybridization localization of the p48h-10 mRNA in different *Zinnia* organs. (*Left*) Anatomy of toluidine blue-stained sections. (*Right*) Corresponding mRNA localization. (*A*) Root from a 4-week-old plant. (*C*) Shoot apex from a 2-week-old plant. (*E*) Flower bud from a 6-week-old plant. (*G*) Stem section after day 3 of rooting induction. ari, Adventitious root initial; lrc, lateral root cambial region; lvb, leaf vascular bundle; mrc, main root cambial region; sa, shoot apex; svb, stem vascular bundle; vb, vascular bundle. (Bars = 0.8 mm.)

Because the p48h-10 mRNA is present in the root and flower bud, we further determined whether the spatial gene expression pattern is the same in the root and flower bud as in the stem. The result in Fig. 6 *A* and *B* shows that the mRNA is present in the cambium regions of both main root and lateral root. In the flower bud (Fig. 6 *E* and *F*), the mRNA signal is closely associated with the vascular bundles. We expect that it is also present in the cambial region.

To know whether the gene expression is related to the meristematic activity, we examined whether the gene is expressed in shoot and root apical meristems. Fig. 6 *C* and *D* shows that the p48h-10 mRNA is not detected in the shoot apical region; instead the signal is associated with the stem and leaf primary vascular bundles. Because it is difficult to section root tip for tissue printing, we used adventitious roots induced from stem segments for the mRNA localization. Fig. 6 *G* and *H* show that the p48h-10 mRNA is not detected in the apical region of the adventitious root. The mRNA is present in the stem cambial region 3 days after the stem is cut and transplanted in the soil.

DISCUSSION

Our results indicate that the p48h-10 gene probably is not involved in the cell division process because, with auxin treatment only, its mRNA accumulates after cell division. The expression of another gene, TED4, was also found not to be associated directly with cell division (8). The p48h-10 and TED4 cDNAs share almost identical sequence in the coding and the 3′ untranslated regions. However, the expression of the TED4 gene was not induced by NAA alone. This suggests that a family of this type of gene might exist in the *Zinnia* genome, and expression of these genes might be differentially regulated. The absence of p48h-10 mRNA signal in shoot and root apical meristems further confirms that the gene expression is not correlated with meristematic activity. This is consistent with the functional differences between the vascular cambium and the apical meristem. The vascular cambium initiates specific tissues, while the apical meristems produce complete organs (1). Consequently, a number of shoot apical meristem-specific genes have been characterized from different plant species (17–19). Because expression of the p48h-10 gene is not correlated with meristematic activity, this, in combination with the cambial region localization in *Zinnia* organs, suggests that the p48h-10 protein is involved in a process unique to cambial activity, such as the process of formation of cambial derivatives and/or the process of differentiation of cambial derivatives into vascular elements. Although with auxin treatment only the mRNA accumulates after cell division, its level of accumulation corresponds to the percentage of cell division in the cultured cells. This implies that the sensitivities to auxin induction are similar between the p48h-10 mRNA accumulation and cell division.

The phenomena that in the cultured cells auxin alone induces the expression of the p48h-10 gene and that cytokinin changes the timing of its mRNA accumulation are intriguing. Early studies showed that auxin, produced in buds and developing shoots, is a stimulus for initiation and maintenance of cambial activity (20–22). Thus, the auxin-induced expression of the p48h-10 gene in the cultured cells may reflect the *in planta* expression in the cambial cells. Although the origin of the interfascicular cambium is controversial, much evidence shows that the interfascicular cambium is a derivative of the differentiated interfascicular parenchyma through the resumption of meristematic activity (23). This indicates that *in planta* nonmeristematic cells can differentiate into vascular elements through dedifferentiation and formation of cambial cells. It is not known whether the auxin-induced vascular differentiation in *in vitro* systems is a process similar to the differentiation of interfascicular vascular elements. Perhaps in the *Zinnia* system, cells treated with auxin enter a cambium-like state after cell division but cannot proceed further without cytokinin. That cell division must precede differentiation was demonstrated in some studies (24, 25). Thus, the auxin-induced expression of p48h-10 gene after cell division occurs at the same time as entry into the cambium-like state. The combination of auxin and cytokinin may cause cells to dedifferentiate and to enter the cambium-like state at a time before cell division. Consequently, the addition of cytokinin brings about the auxin-induced p48h-10 mRNA accumulation before cell division. It was shown that *Zinnia*-isolated mesophyll cells can differentiate into TEs without cell division in the presence of auxin and cytokinin (26). An auxin- and cytokinin-regulated expression of gene pLS216 in tobacco suspension cells was described (27). For pLS216 gene, both auxin and cytokinin increase its expression, while cytokinin produces a heightened response in cells desensitized to auxin. Although activation of pLS216 gene was not shown to be associated with any physiological responses, these results show a common feature—i.e., cytokinin can change an auxin-induced gene expression pattern.

It is well known that auxin and/or cytokinin are important for vascular differentiation (2). Numerous experiments using organs and tissues show that auxin can induce vascular differentiation; cytokinin alone has no effect (2). Reduction of

auxin levels *in vivo* in transgenic tobacco by the introduction of indoleacetic acid-lysine synthetase gene resulted in inhibition of vascular differentiation (28). However, cytokinin increases the sensitivity of tissues to auxin and thereby increases auxin-induced vascular differentiation (3). The *in vivo* effect of cytokinin on xylem formation has been demonstrated with transgenic plants (29–31). The auxin- and cytokinin-regulated expression pattern of the p48h-10 gene correlates with this phenomenon of auxin- and cytokinin-induced vascular differentiation.

Different time courses of mRNA accumulation of p48h-10 and TED4 were observed with respect to the time course of TE formation. In the presence of NAA and BA, the p48h-10 mRNA heavily accumulates as early as 24 hr after culture. This is about 12–24 hr before the initiation of secondary wall thickening can be detected. But TED4 mRNA heavily accumulates between 48 hr and 72 hr (8). Thus, the heavy accumulation of TED4 mRNA seems to correlate with the process of secondary wall thickening and lignification which occurs between 52 hr and 72 hr (8). These results indicate that the p48h-10 and TED4 proteins may function at different stages during the progression of TE formation. p48h-10 is involved in a very early stage of TE formation, which is consistent with its mRNA localization in the cambial region of *Zinnia* organs, while TED4 participates in a later stage.

p48h-10-encoded protein shows some features similar to those of plant nonspecific LTPs, which usually show epidermal cell accumulation. A similar protein was found in barley aleurone (32). This indicates that these genes have various tissue-specific expression. Thus, if they were LTPs, these gene products might have functions unique to specific processes or represent different isoforms performing general functions in different tissues. In yeast, it was shown that a phosphatidylinositol transfer protein has a specific role essential for constitutive secretion (33). It was suggested that as with yeast phosphatidylinositol transfer protein, plant nonspecific LTPs may also have restricted functions *in vivo* (13–15, 34–36). Membrane biogenesis and vesicle secretion are active processes in cambial cells (37). It is possible that LTPs may be involved. Different time courses of expression of the p48h-10 and TED4 genes indicate that they may perform these activities at different stages. Although the p48h-10 mRNA is predominantly present in the cambial region, the gene product may be stable and function at later stages.

Xylem differentiation in nodes of *Coleus* was shown to be at a more advanced state than in adjacent internodes. It was suggested that the increased xylogenesis in the nodal regions resulted from locally enhanced auxin levels, which was the result of transport from the local leaf pair (38). Since auxin is a stimulus for cambial initiation and activity (20–22), it seems likely that nodal cambium is more active than internodal cambium at early stages of stem development. Consequently, the p48h-10 mRNA signal is more intense in the cambial region of the node than in that of the internode in the upper part of young *Zinnia* stem (Fig. 5). Within the same upper internode of plants of different ages, the mRNA signal is localized in the fascicular cambial region only in the young plant, while it forms a continuous ring in the older one (Fig. 4). Because the interfascicular regions can be recognized at both internodes, we infer that cambial derivatives may be formed in the internode from the older plant but not in the internode from the young one. In the different organs and different developmental stages examined, the p48h-10 mRNA is predominantly present in the cambial regions (Figs. 3–6). Taken together, the data encourage us to suggest that the p48h-10 gene is a molecular marker for cambial distribution and the degree of cambial activity. Further analysis of the function of the p48h-10-encoded protein should help us to understand more about the early process of vascular differentiation.

We thank Ray F. Evert and Candace H. Haigler for constructive comments on the manuscript and Mike Dyer for assistance on *in vitro* root induction experiment. This work was supported by a grant from the U.S. Department of Energy (DE-FG 0284ER13255) to J.E.V. Z.-H.Y. was supported in part by Monsanto Predoctoral Fellowship.

1. Steeves, T. A. & Sussex, I. M. (1989) *Patterns in Plant Development* (Cambridge Univ. Press, Cambridge).
2. Aloni, R. (1987) *Annu. Rev. Plant Physiol.* **38**, 179–204.
3. Aloni, R. (1993) *Aust. J. Plant Physiol.* **20**, 601–608.
4. Fukuda, H. (1992) *Int. Rev. Cytol.* **136**, 289–332.
5. Fukuda, H. & Komamine, A. (1985) *Cell Cult. Somatic Cell Genet. Plants* **2**, 149–212.
6. Church, D. L. (1993) *Plant Growth Regul.* **12**, 179–188.
7. Ye, Z.-H. & Varner, J. E. (1993) *Plant Physiol.* **103**, 805–813.
8. Demura, T. & Fukuda, H. (1993) *Plant Physiol.* **103**, 815–821.
9. Fukuda, H. & Komamine, A. (1980) *Plant Physiol.* **65**, 57–60.
10. Ye, Z.-H. & Varner, J. E. (1991) *Plant Cell* **3**, 23–37.
11. Church, D. L. & Galston, A. W. (1988) *Phytochemistry* **27**, 2435–2439.
12. Fleming, A. J., Mandel, T., Hofmann, S., Sterk, P., de Vries, S. C. & Kuhlemeier, C. (1992) *Plant J.* **2**, 855–862.
13. Sossountzov, L., Ruiz-Avila, L., Vignols, F., Jolliot, A., Arondel, V., Tchang, F., Grosbois, M., Guerbette, F., Miginiac, E., Delseny, M., Puigdomenèch, P. & Kader, J.-C. (1991) *Plant Cell* **3**, 923–933.
14. Sterk, P., Booij, H., Schellekens, G. A., Kammen, A. V. & Vries, S. C. D. (1991) *Plant Cell* **3**, 907–921.
15. Thoma, S., Kaneko, Y. & Somerville, C. (1993) *Plant J.* **3**, 427–436.
16. Iqbal, M. & Ghouse, A. K. M. (1990) in *The Vascular Cambium*, ed. Iqbal, M. (Research Studies Press, Taunton, Somerset, England), pp. 1–36.
17. Fleming, A. J., Mandel, T., Roth, I. & Kuhlemeier, C. (1993) *Plant Cell* **5**, 297–309.
18. Kelly, A. J., Zagotta, M. T., White, R. A., Chang, C. & Meeks-Wagner, D. R. (1990) *Plant Cell* **2**, 963–972.
19. Medford, J. I., Elmer, J. S. & Klee, H. J. (1991) *Plant Cell* **3**, 359–370.
20. Fahn, A. & Werker, E. (1990) in *The Vascular Cambium*, ed. Iqbal, M. (Research Studies Press, Taunton, Somerset, England), pp. 139–157.
21. Creber, G. T. & Chaloner, W. G. (1990) in *The Vascular Cambium*, ed. Iqbal, M. (Research Studies Press, Taunton, Somerset, England), pp. 159–199.
22. Savidge, R. A. (1983) *Histochem. J.* **15**, 447–466.
23. Soh, W. Y. (1990) in *The Vascular Cambium*, ed. Iqbal, M. (Research Studies Press, Taunton, Somerset, England), pp. 37–62.
24. Fosket, D. E. (1970) *Plant Physiol.* **46**, 64–68.
25. Shininger, T. L. (1975) *Dev. Biol.* **45**, 137–150.
26. Fukuda, H. & Komamine, A. (1980) *Plant Physiol.* **65**, 61–64.
27. Dominov, J. A., Stenzler, L., Lee, S., Schwarz, J. J., Leisner, S. & Howell, S. H. (1992) *Plant Cell* **4**, 451–461.
28. Romano, C. P., Hein, M. B. & Klee, H. J. (1991) *Genes Dev.* **5**, 438–446.
29. Ainley, W. M., McNeil, K. J., Hill, J. W., Lingle, W. L., Simpson, R. B., Brenner, M. L., Nagao, R. T. & Key, J. L. (1993) *Plant Mol. Biol.* **22**, 13–23.
30. Li, Y., Hagen, G. & Guilfoyle, T. J. (1992) *Dev. Biol.* **153**, 386–395.
31. Medford, J. L., Horgan, R., El-Sawi, Z. & Klee, H. J. (1989) *Plant Cell* **1**, 403–413.
32. Jakobson, K., Klemsdal, S. S., Aalen, R. B., Bosnes, M., Alexander, D. & Olsen, O.-A. (1989) *Plant Mol. Biol.* **12**, 285–293.
33. Bankaitis, V. A., Aitken, J. R., Cleves, A. E. & Dowhan, W. (1990) *Nature (London)* **347**, 561–562.
34. Chasan, R. (1991) *Plant Cell* **3**, 842–843.
35. Molina, A., Segura, A. & Garcia-Olmedo, F. (1993) *FEBS Lett.* **316**, 119–122.
36. Tsuboi, S., Osafune, T., Tsugeki, R., Nishimura, M. & Yamada, M. (1992) *J. Biochem. (Tokyo)* **111**, 500–508.
37. Catesson, A. M. (1990) in *The Vascular Cambium*, ed. Iqbal, M. (Research Studies Press, Taunton, Somerset, England), pp. 63–112.
38. Bruck, D. K. & Paolillo, D. J. (1984) *Am. J. Bot.* **71**, 151–157.