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Regulation of Tracheary Element Differentiation

Hideo Kuriyama* and Hiroo Fukuda

Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo 7-3-1, Tokyo 113-0033, Japan

Abstract

Tracheary elements (TEs) are highly specialized cells that play central roles in the development of vascular plants. TEs function as components of xylem vessels or tracheids to deliver water throughout plant parts and to confer mechanical strength on a plant body. As a result of distinctive cytological features, patterned secondary walls or the loss of the protoplast, a differentiated TE cell is easily recognized, and has long provided a simple analytical model system of plant cell differentiation. Evidence obtained from this simple system indicates, however, that the regulatory mechanisms underlying TE differentiation are quite complicated. Various factors such as plant hormones and unidentified offspring of cell–cell interactions are involved in the induction of TE differentiation. With the help of some signal transduction machinery, such developmental or environmental cues activate the gene expression cascades required for TE wall material synthesis and protoplast digestion. This review covers recent advances in physiological and genetic studies on this paradigm. The detailed mechanisms of the regulation of TE differentiation are discussed, especially analyses using *in vitro* culture systems for TE differentiation, transgenic plants, or mutants defective in leaf vascular pattern formation that are accelerating the expansion of our knowledge on the process of vascular cell fate specification.

Key words: Differentiation; Mutant; Tracheary element; Transgenic plant; Vascular development

Introduction

A vascular plant contains many cell types that work in a coordinated way to support growth, development, reproduction, and responses to environmental stresses. The expression of intrinsic cell differentiation programs during a life cycle leads to the production of various cell types. Among them, tracheary elements (TEs) are distinct from other cells in their ontogenic processes and behavior. During development, TEs commence construction of patterned secondary walls and undergo programmed cell death followed by drastic autolysis (Fukuda 1997ab, 2000; Groover and Jones 1999; Kuriyama 1999; McCann, 1997; Sugiyama and others 2000). These peculiar ontogenic processes result in a conspicuous morphotype (Barnett 1981), rendering TE differentiation particularly amenable to general physiological investigations. This has become one of the most studied cases of cell differentiation in plants (Sachs 2000). Apart from the numerous reports on the identification and characterization of substances, enzymes, or metabolic pathways required for TE-associated material synthesis, the mechanisms that integrate and control such machinery remain less well documented.

Anatomical and physiological studies have pro-

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Corresponding author; e-mail: kuriyama@jade.dti.ne.jp

vided important clues for understanding such mechanisms. TEs form within vascular tissues (Barnett 1981). There are strict developmental relationships between these vascular tissues and other tissues or organs. Stem vascular tissue formation is influenced by leaves and roots, which are well known as sources of certain plant hormones (Aloni 1987). Exogenous plant hormones can replace such organs and still induce or promote TE differentiation in plant tissue cultures *in vitro* (Aloni 1987; Clouse and Sasse 1998; Fukuda and Komamine 1985). These observations clearly indicate that plant hormones control vascular and TE cell differentiation. Analyses of hormonal actions, therefore, are crucial to elucidate the regulatory mechanisms of vascular cell differentiation leading to TE differentiation.

In addition to TEs, vascular tissues consist of various cell types including parenchyma cells, transfer cells, and fibers, all of which are arranged in a strikingly well ordered fashion within a narrow zone (reviewed, for example, by Aloni 1987). This organizational pattern allows TEs to form continuous cell files to conduct long-distance, inter-organ water transport. The preservation of this continuity is essential for plant growth, so that nascent TEs must arise from other vascular cells adjacent to the files. The presence of additional regulatory mechanisms involved in the fine control of vascular cell fate specification has, therefore, been postulated. According to Sachs (2000), the induction of TE or other vascular cell differentiation at the appropriate position seems to be mediated by local positional elements, such as the local gradient of plant hormone concentrations.

Analyses of these regulatory mechanisms at the molecular level began within the last decade. Several new strategies have been employed, such as gene characterization in a TE differentiation-inducible culture system (Fukuda 1996, 1997a; Roberts and McCann 2000), the production of transgenic plants (see for example, Scarpella and others 2000), and screening of mutants defective in vascular tissue patterning (Berleth and others 2000). These approaches are beginning to provide many important insights into mechanisms of vascular and TE cell development (Fukuda 2000). Here, recent studies of the genes and factors involved in the regulation of TE differentiation are reviewed to consider the molecular bases for generation of this distinct plant cell type.

Approaches to the Study of Regulatory Mechanisms of TE Differentiation

Advances in the study of TE differentiation have been made using five distinct approaches. Until the early 1980s, TE differentiation was analyzed mainly by microscopic observations of vascular tissue sections of intact plants or those of cultured explants incubated under altered physiological conditions (Barnett 1981). This type of approach revealed the importance of plant hormones and nutrients for TE differentiation *in vitro* and *in vivo*. It is, however, not possible to address recent problems concerning molecular mechanisms. These classic methods are currently applied to the closer characterization of mutant phenotypes (for example, see Hobbie and others 1999), or to *in situ* gene expression analysis for the assessment of differential tissue or cell response to various external physiological stimuli (see for example Riou-Khamlichi and others 1999).

A significant breakthrough occurred approximately two decades ago. A suspension culture system that can induce frequent and synchronous TE differentiation from freshly isolated single mesophyll cells of *Zinnia elegans* was developed (Fukuda and Komamine 1980). The large proportion of TEs in such a suspension enabled us to obtain many TE differentiation-associated genes that are actually localized around TEs in plants. The synchronous progress of cell development in this system is particularly useful in developing an understanding of the exact order of related cytological, biochemical, and molecular biological events in the time course of vascular and TE cell differentiation (Fukuda 1996).

Mutants with vascular abnormalities indicate that the mutated genes play roles in the expression of normal phenotype. Screens for abnormal vascular patterning in cotyledons (Carland and others 1999; Deyholos and others 2000; Koizumi and others 2000) or stems (Zhong and others 1997, 1999) of *Arabidopsis thaliana* have been performed. However, mutations that abolish vascular bundle or TE formation are often accompanied by fatal damage to plant development or fertility. Therefore, efficient and successful isolation of such mutants requires a method that can circumvent the kind of difficulties described by Koizumi and others (2000).

The site of expression of a particular gene often points to the location where its product functions. The introduction of chimeric genes consisting of a promoter region fused with a GUS reporter gene has been used for *in vivo* expression analyses (Jefferson and others 1987). This method is very effective in localizing a gene transcript in a whole plant (Jefferson and others 1987). Genes expressed preferentially in vascular tissues, thus possibly working in vascular tissues, have been identified. A number of these are mentioned below. Similarly, the identification of *cis*-elements in promoter regions has been performed using deleted promoter-reporter gene

constructs. Results of such experiments provide valuable information about *trans*-factors that regulate the expression of such genes (see for example Grotewold and others 1994).

The production of transgenic plants is an important approach to the functional analysis of particular genes. Many genes that exhibit vascular- or xylem-associated expression patterns have been introduced to plants in a sense or antisense orientation driven by powerful promoters. Their functions are examined based on observations of anatomical features of transformants, such as aberrant vascular morphology or the alteration of the number or amount of vascular tissue components (for example, see Baima and others 2000). To avoid fatal or extremely deleterious effects of gene manipulation on the development of juvenile transgenic plants, *Drosophila heat shock protein 70* (*hsp70*) promoter (Schmülling and others 1989) or a dexamethasone (DEX)-inducible, glucocorticoid receptor-mediated system (Aoyama and Chua 1997; Steindler and others 1999) has often been employed. By regulating the timing of induction, tissue- or developmental stage-specific effects of exogenous gene overexpression or silencing can easily be assessed.

These methods have contributed much to the understanding of the fundamental framework of TE differentiation: inductive factors activate specific gene expression cascades leading to synthesis of the molecular apparatus for TE formation. Studies on detailed inductive factors and genes that participate in TE differentiation are described below.

Factors Involved in the Induction of TE Differentiation

Several classical plant hormones have been proven to regulate TE differentiation (Aloni 1987; Fukuda 1992). As the effects of every plant hormone are pleiotropic and their signaling pathways are often interconnected (McCourt 1999), all known plant hormones will need to be examined for regulatory roles in vascular and TE cell differentiation. Newly found peptide hormone-like factors (Matsubayashi and Sakagami 1996; Motose and others 2001; Ryan 2000) may also exhibit xylogenic activity. Here, recent developments in vascular and TE cell differentiation-inductive factors are described and their modes of regulatory action discussed.

Wound stimulus. Apart from TE differentiation in vascular tissues of intact plants, wound-induced regeneration of vascular tissues, which results in the restoration of vascular continuity, is well characterized (Aloni 1987; Fukuda 1992). If vessels or tracheids are disrupted by wounds, a group of TEs known as wound vessel members arises from neighboring parenchyma cells so that these conductive tissues can bypass lesions (Aloni 1987). In *Zinnia* culture, the initial mesophyll cell isolation process always generates a wound stimulus. This stimulus may be indispensable for the massive production of TEs, because it is probably impossible to induce frequent TE differentiation in cultures of leaf disks, where peeling of the epidermis elevates the frequency of TE differentiation from mesophyll cells (Fukuda 1992 and references therein).

Molecular details of wounding-mediated induction of TE differentiation are still unclear. In tomato plants, wounds that destroy plant tissues invoke a series of signaling reactions similar to inflammation responses in unwounded cells in animals (Ryan 2000). Cellular breakage allows the release of an 18-amino-acid peptide, systemin, which binds to a specific transmembrane receptor of neighboring cells and elevates Ca^{2+} influx, thereby inducing mitogen-activated protein kinase (MAP kinase) activity. The MAP kinase then activates phospholipase A_2, which releases linolenic acids from phospholipids of plasma membranes. Linolenic acids are metabolized through the octadecanoid pathway into jasmonates that induce the expression of a group of genes involved in plant defense responses. This plant peptide hormone molecule is initially synthesized as a precursor protein (prosystemin) with a much larger molecular weight and is posttranslationally cleaved to produce the mature form. Although it is unknown whether such signaling pathways occur in all vascular plants, some of the component molecules have been found in other plant species.

If the above machinery works for wound-induced vascular or TE development, transgenic tomato plants with a sense or antisense prosystemin sequence may provide important insights into the mechanisms of wound-induced TE differentiation. Although such plants can grow (Ryan 2000) and can probably form vascular tissues, detailed analyses concerning vascular development have not yet been performed.

Wound-inducible genes include those encoding proteinase inhibitors (PIs) (O'Donnell and others 1996). In tomato leaves, wound-inducible PI gene transcripts are supposed to emerge in mesophyll cells (Ryan 2000). In *Zinnia* culture, freshly isolated mesophyll cells express PI genes for the first 24–36 h (Figure 1; Fukuda 1997a). Once they differentiate into cells similar to those in vascular tissues (Fukuda 1996), the PI genes are down-regulated (Fukuda 1997a). These characteristic *Zinnia* PI gene expression patterns may reflect a change in the state of *Zinnia* cell differentiation (Fukuda 1996). It is of in-

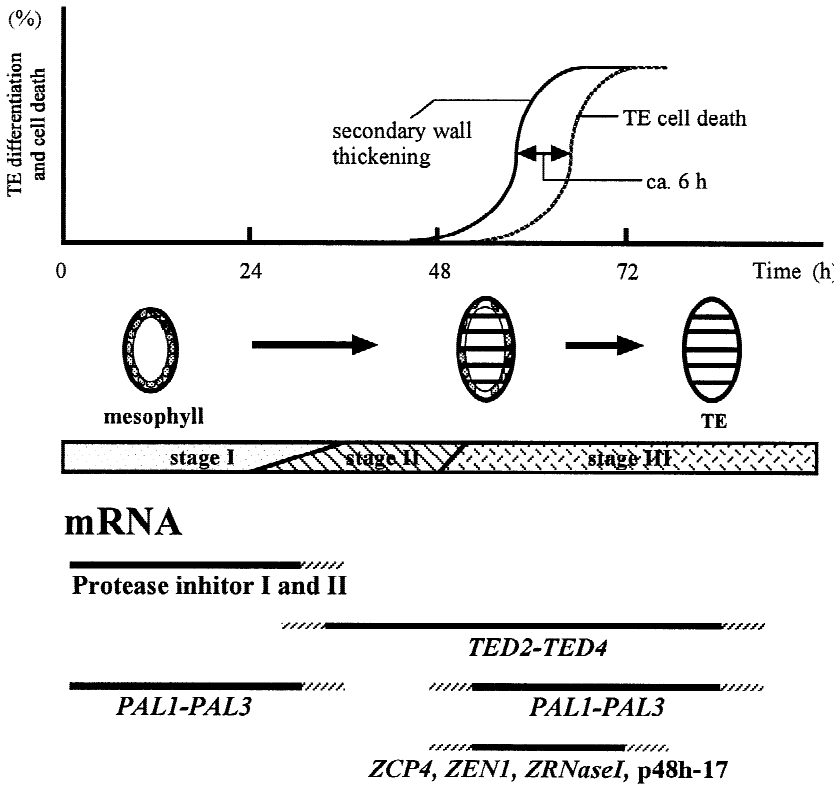

Figure 1. Time course of TE differentiation in *Zinnia* culture (a modified version of Fukuda 1997a). The process of transdifferentiation from mesophyll to TE cells can be divided into three stages (stage I–III). Dedifferentiation from mesophyll cells occurs at stage I. Then, these cells differentiate into procambial-, immature xylem-, or TE precursor-like cells during stage II. At stage III, secondary wall thickenings (corresponding to TE morphogenesis) become apparent, and then TE cell death occurs. Each stage is characterized by expression patterns of distinct sets of mRNAs. Only representative marker genes are shown here. *TED2* and *TED3* genes encode a protein homologous to pig lens ξ-crystallin and a possible cell wall protein, respectively (Demura and Fukuda 1994). *TED4* encodes a putative non-specific lipid transfer protein that acts as a proteasome inhibitor (Endo and others 2001). *ZEN1* and *ZCP4* encode a putative S1-type nuclease and a cysteine protease, respectively. *ZRNase I* and p48h-17 were shown to encode an RNase and a cysteine protease, respectively. PAL, phenylalanine ammonia-lyase.

terest to investigate expression patterns of wound signaling machinery component genes in *Zinnia* culture. In wounded tomato plants, such genes are supposed to be activated in vascular tissues in contrast to PIs (Ryan 2000). Wound signals may activate the vascular cell-associated expression of such genes to amplify themselves and promote vascular and TE cell development.

Auxin. As a result of a wide range of physiological studies, it is generally accepted that auxin is one of the fundamental inducers of TE differentiation (Aloni 1987; Sachs 2000). *Zinnia* cells also require auxin during their transdifferentiation (Fukuda and Komamine 1985; Fukuda 1992). Modulating endogenous auxin levels by molecular genetics (Klee and others 1987; Romano and others 1991) affects the amount of xylem tissues and TEs. Because these findings strongly suggest that auxin signal transduction is implicated in vascular and TE cell differentiation, auxin transport and perception are now being intensively studied (Berleth and others 2000).

Vascular bundles form a complex, continuous network in cotyledons and leaves (Nelson and Dengler 1997). Fairly extensive physiological analyses on the effect of polar auxin flow on leaf vascular pattern formation were recently performed (Mattsson and others 1999; Sieburth 1999). When *Arabidopsis* seeds are germinated in a medium containing inhibitors of polar auxin transport, altered leaf vascular patterning occurs in an inhibitor dose-dependent manner. These inhibitors concentrate vascular bundles around the center of leaves. A higher concentration of inhibitor drastically increases vascular cell differentiation in the margin of the leaves. These results strongly suggest the inductive activity of polar auxin flow for the formation of (pro)vascular patterns, as explained by the auxin signal flow canalization hypothesis (Berleth and others 2000; Sachs 2000). According to these authors, auxin generated from the marginal region of leaves moves toward petioles and induces vascular cell differentiation on its way. Thus, relatively low concentrations of polar auxin transport inhibitor give rise to wider vascular bundles. The strong inhibition of auxin movement causes the accumulation of auxin in the marginal region, where more vascular cells develop.

Many *Arabidopsis* mutants with altered auxin physiology exhibit aberrant vascular patterning in their cotyledons and/or leaves (Berleth and others 2000). The *pin formed1* (*pin1*) mutants, which are characterized by pin-shaped inflorescence stems,

have increased leaf vascular tissues. This phenotype resembles that of wild type plant leaves treated with inhibitors of polar auxin transport. Therefore, the *PIN1* gene encoding putative auxin efflux carrier protein plays a role in vascular pattern formation regulating polar auxin transport. The *emb30/gnom* mutants exhibit severe disruption of vascular tissue patterning (Mayer and others 1991). The *EMB30/GNOM* encodes a protein similar to a guanine exchange factor that is involved in brefeldin A-sensitive vesicle transport in yeast (Steinmann and others 1999). The EMB30/GNOM protein works for the intracellular auxin-efflux carrier transport to localize them at only one side of an elongated cell. The disruption of polar auxin efflux carrier transport results in the disordered occurrence of TE cells in organs. Moreover, other mutants defective in auxin transport and/or perception, such as *lopped 1* (*lop1*), *monopteros* (*mp*), *auxin resistant 6* (*axr6*), and *bodenlos* (*bdl*) exhibit shortened or reduced veins in cotyledons and/or leaves (Berleth and Jürgens 1993; Carland and McHale 1996; Hamman and others 1999; Hobbie and others 1999). Gene cloning and characterization experiments indicate that *MP* encodes an auxin-responsive transcription factor (Hartdke and Berleth 1998). *ETTIN* and *PINOID,* the mutations of which disrupt vascular patterning in flower organs, encode genes homologous to auxin-responsive factor-like protein and serine-threonine protein kinase related to auxin actions, respectively (Christensen and others 2000; Sessions and others 1997). These mutations definitively mark auxin-induced vascular cell differentiation and vascular pattern formation.

What then, are the molecular mechanisms that mediate these actions of auxin? In addition to kinase cascades (Christensen and others 2000), Ca^{2+} and calmodulin are known to be implicated in the regulation of gene expression in response to auxin (Zielinski 1998). Promoter analysis of certain calmodulin genes indicated their vascular-associated expression patterns (Zielinski 1998). In the analysis carried out with *Zinnia* culture, the involvement of these factors has become apparent (Fukuda 1996). Ca^{2+} channel blockers and calmodulin antagonists suppress TE differentiation (Kobayashi and Fukuda 1994; Roberts and Haigler 1990). The amounts of calmodulin, calmodulin-binding proteins, and membrane-bound Ca^{2+} increase during TE differentiation.

Cytokinin. Cytokinin is known to promote the induction of TE differentiation in many tissue culture systems (Aloni 1987; Fukuda and Komamine 1985). It is needed for the induction of TE formation even in the *Zinnia* cell culture system in which mesophyll cells differentiate into TEs without intervening cell division. This suggests that cytokinin itself is a prerequisite for the induction of TE differentiation.

Arabidopsis altered meristem programming1 (*amp1*) carries a mutation that causes the overproduction of endogenous cytokinin (Chaudhury and others 1993). Currently available mutants related to altered cytokinin content are all allelic to *AMP1.* Although *amp1* mutants show pleiotropic phenotypes (such as excess cotyledons, short hypocotyls, short primary roots, excess branching, enlarged shoot apical meristem, and early flower formation), their vascular- or TE-related aspects have not been reported. Even the *amp1* extra cotyledons appear to have normal vascular patterns (Conway and Poethig 1997), but the more detailed anatomical analysis of *amp1* might aid in understanding of the mechanism of cytokinin-mediated vascular or TE cell differentiation.

The overexpression of bacterial genes involved in cytokinin production (isopentenyltransferase; *ipt*) in *Arabidopsis* plants results in the acceleration of pith parenchyma and vascular bundle development accompanied by the circumferential expansion of hypocotyls (Rupp and others 1999). Thus, cytokinin seems to promote vascular development in plants. It remains undetermined, however, whether cytokinin directly induces differentiation of vascular cells or simply promotes cell proliferation and thus provides room for the formation of larger or increased vascular bundles. The enlargement of vascular systems by increased cambial cell division is also observed in other cases (Steindler and others 1999). Therefore, the specific role of cytokinin needs to be examined at the cellular level with appropriate transgenic systems.

Brassinosteroid. Brassinosteroids are plant steroid hormones involved in several aspects of plant growth and development (Clouse and Sasse 1998). Physiological studies have suggested the implications of this hormone in TE differentiation (Fukuda 1997a; Fukuda and others 1998). Exogenously applied brassinosteroids at nanomolar concentrations can increase and hasten TE differentiation in Jerusalem artichoke explants (Clouse and Sasse 1998). Uniconazole, which inhibits the synthesis of brassinosteroids and gibberellin, suppresses TE differentiation in *Zinnia* culture (Iwasaki and Shibaoka 1991). Brassinazole, a newly synthesized, specific inhibitor of brassinosteroid synthesis (Asami and others 2000), has a similar effect (Yamamoto and others 2001). Exogenous brassinosteroids, but not gibberellins, can reverse these effects. Gas chromatographic and mass spectroscopic analyses verified the presence of several brassinolide intermediates in both cultured *Zinnia* cells and culture media (Yamamoto and others 2001). Levels of some intermedi-

ates increased rapidly before TE morphogenesis. This fact indicates that initial hormonal composition in the inductive medium (auxin and cytokinin) should be sufficient to invoke brassinosteroid synthesis in *Zinnia* cells. The final stage of TE differentiation, including secondary wall formation and cell death, must be triggered by such newly synthesized endogenous brassinosteroids. Castasterone, an active brassinosteroid, is secreted preferentially out of the cell, suggesting that it may function at the outer surface of the cell as an intercellular signal (Yamamoto and others 2001).

Arabidopsis mutants defective in brassinosteroid synthesis show aberrant vascular bundle structures. The *constitutive photomorphogenesis and dwarfism* (*cpd*) mutants, which cannot synthesize active brassinosteroids because they lack a fully functional cytochrome P450 (CYP90) enzyme, exhibit skotomorphogenic developmental defects and aberrant vascular bundle organization (Szekeres and others 1996). In their hypocotyls, the number of xylem cells is decreased, whereas that of phloem is increased compared with those of wild-type plants. Similarly, *dwarf7* mutants, in which brassinosteroid biosynthesis is also blocked due to another enzyme defect, contain decreased xylem tissues, reduced or missing interfascicular parenchyma, and normal or increased phloem tissues (Choe and others 1999). These findings strongly suggest that brassinosteroids can initiate tissue specification in the vasculature. The balance between amounts of xylem and phloem cells may also be regulated by brassinosteroids.

In addition, brassinosteroids regulate the expression of soybean *BRU1* gene, which encodes xyloglucan endotransglycosylase and is possibly involved in the cell wall modification of xylem cells surrounding TEs (Oh and others 1998). This gene may control the coordinate expansion of such cells and possibly TEs during xylem development in response to the elevation of endogenous brassinosteroid levels.

Gibberellin. Earlier studies yielded contradictory results about the effect of gibberellic acids (GAs) on the promotion of TE differentiation (reviewed by Fukuda and Komamine 1985). Kalev and Aloni (1998) examined the roles of GAs by applying GA_3 to pine hypocotyl slices. This alone did not induce TE differentiation but, in the presence of auxin, substantial TE cell elongation was induced. Exogenous GAs are not necessary for massive TE production in *Zinnia* culture, but elongation of TEs is promoted by GAs. Although it is not currently assumed that GAs are involved in regulation of TE differentiation-specific genes, GAs likely participate in regulation of TE cell elongation to build up the well-ordered bundle architecture of vascular tissues in plants.

Although a large number of review articles describing gibberellin-related mutants are available (see for example Hedden and Kamiya 1997; Sun 2000), detailed analyses concerning vascular tissue development in these mutants are scarce. Recently, Eriksson and others (2000) generated transgenic hybrid aspen plants in which a gene encoding GA_{20} oxidase, a key enzyme in GA biosynthesis, is ectopically overexpressed. These plants exhibit marked increases in levels of active GAs, shoot length, shoot diameter, and the number of cells in fully elongated internodes. Interestingly, the number and length of xylem fiber cells is also increased, indicating that GAs are involved not only in cell elongation, but also in xylem cell differentiation accompanied with secondary growth (also see Aloni 1987). Results that will be obtained from these plants may facilitate an understanding of the roles of GAs in vascular development.

Ethylene. The roles of ethylene in TE development also require further investigation. Eklund and Tiltu (1999) observed ethylene-mediated promotion of TE differentiation in stems of spruce trees. These results were consistent with previous reports on other species (reviewed by Aloni 1987), but Kalev and Aloni (1999) showed that this hormone stimulated TE differentiation in pine hypocotyl slices only when applied with the appropriate amount of auxin. They also found that inhibitors of ethylene synthesis and perception abolish the promotive effect of auxin on TE differentiation. Thus, upon TE differentiation induction, ethylene exerts its effects through a signaling pathway that partially overlaps that of auxin. Interestingly, ethylene (together with auxin) treatment produced many non-polar, isodiametric TEs, which are distinct from those generated under the influence of gibberellin (Kalev and Aloni 1998, 1999). Ethylene and gibberellin might be able to modulate the establishment of TE cell polarity, which is thought to be important for proper TE morphogenesis in continuous vascular tissues.

Morphological and anatomical features of xylem or TE cells in ethylene-signaling mutants (Kieber 1997) have not yet been reported. However, several lines of evidence point to ethylene having roles in biochemical aspects of TE development. Ethylene induces enzyme activity for xylem lignification (Hennion and others 1992) and modifies wall polysaccharide composition for xylogenesis (Eklund 1991; Ingemarsson and others 1991). Ethylene can also regulate the expression of wound-inducible genes (O'Donnell and others 1996), implying that this hormone has a role in the transduction of some developmental signals corresponding to those acti-

vated by a wound stimulus, which is required for TE differentiation (Fukuda 1992).

Abscisic acid (ABA). ABA is a hormone that regulates seed development, germination, and responses to various environmental stresses. This hormone suppresses TE differentiation in tissue cultures (Fukuda and Komamine 1985), but its detailed actions have been little studied.

Although phenotypes showing abnormal vascular development have not been documented so far in ABA-biosynthetic or -signaling mutants (for example, see Leung and Giraudat 1998), the involvement of ABA in vascular development was suggested from the characterization of an *Arabidopsis* ABA-inducible homeobox gene (*ATHB6;* Söderman and others 1999). *ATHB6* gene expression occurs in developing guard cells, the root meristem, and leaf primordia at the early stage of seedling development, but later converges in vascular bundles. The *ATHB6* gene may be involved in cell division and/or cell differentiation in such regions under the control of ABA. In addition to the production and characterization of *ATHB6* sense and antisense transgenic plants, the anatomical analysis of ABA-insensitive mutants, *abi1* and *abi2,* may help to explore the role of ABA and *ATHB6* in vascular bundle development, as these mutants block the ABA-mediated induction of *ATHB6* transcription.

It is well known that ABA regulates the expression of late embryogenesis abundant (*LEA*) genes during seed development. Some so-called Em proteins, a type of LEA protein, are expressed transiently in embryo procambial tissues of carrot (Wurtele and others 1993) or embryo vascular tissues of *Arabidopsis* (Vicient and others 2000). In particular, in the case of *Arabidopsis AtEm1* gene, exogenous ABA invokes the vascular bundle-associated expression of this gene even in adult plants (Vicient and others 2000). These characteristics imply some role of ABA in the regulation of unknown aspects of vascular cell development.

Jasmonic acid (JA) and methyl jasmonate (MeJ). Actions of this hormone on vascular tissue development remain elusive. Among vegetative tissues, relatively high levels of endogenous JA accumulate in paraveinal mesophyll cells or bundle sheath cells surrounding vascular bundles (Creelman and Mullet 1997). JA accumulation in these tissues implies a role for this hormone in developmental events occurring in cells of these regions. The expression of allene oxide synthase and allene oxide cyclase, which are key enzymes for JA synthesis, occurs in vascular bundle and/or neighboring parenchyma cells (Hause and others 2000; Kubigsteltig and others 1999; Maucher and others 2000). These findings also suggest a special developmental role for JA related to these cells.

As mentioned above, a wound stimulus induces JA production (Ryan 2000), and JA amplifies wound signals by inducing the expression of genes encoding prosystemin. The expression of genes of other wound-signaling machinery components is also invoked by JA. In tomato leaves, the prosystemin gene is expressed in vascular cells (Ryan 2000). MeJ enhances prosystemin mRNA expression selectively in vascular tissues. Such regions may be able to cover the area where parenchyma-derived vessel regeneration occurs after vascular breakage (Aloni 1987). JA or MeJ might amplify wound signals in vascular tissues, thereby inducing the expression of genes involved in vascular and TE cell development.

*Phytosulfokine-**α**(PSK-**α**).* A sulfated pentapeptide H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH, called PSK-α, was originally isolated as a novel cell proliferation regulator from a conditioned medium of cultured asparagus cells (Matsubayashi and Sakagami 1996). In cultures, asparagus mesophyll cells enter a cell division cycle only after they produce and excrete an adequate amount of PSK-α in the presence of appropriate amounts of auxin and cytokinin (Yang and others 2000). Although asparagus cells cannot divide in cultures at low cell density, exogenously added PSK-α allows these cells to overcome such suppressive effects and even to shorten the period required for DNA replication.

Recently, effects of PSK-α on developmental aspects were examined using the *Zinnia* culture system (Matsubayashi and others 1999). In *Zinnia* culture, lowered cell density suppresses both cell division and TE differentiation. Although a cell density 20 times lower than optimal suppresses TE differentiation, cells fed with PSK-α can overcome such a suppressive effect and differentiate into TEs as frequently as those cultured at the optimal cell density (Matsubayashi and others 1999). Conditioned medium of *Zinnia* cell culture can also compromise the suppressive effect of lowered cell density. PSK-α or conditioned medium-activated TE differentiation is independent from cell viability changes or cell proliferation. Chromatographic analysis revealed that *Zinnia* cells also produce and excrete PSK-α. These findings suggest that PSK-α can promote TE differentiation. However, it is unknown whether PSK-α directly stimulates the progression of gene expression cascades for TE differentiation, or works for the enhancement of cellular metabolic, transcriptional, and/or translational activities that are commonly important both in cell division and in TE differentiation. Expression analyses of both TE differentia-

tion- and cell division-related genes in PSK-α-containing *Zinnia* culture will answer this question.

In rice plants, the expression of a gene encoding PSK-α precursor protein occurs in virtually all tissues showing higher levels in meristematic regions (Yang and others 2000). It is also important to know how PSK-α works for cell division and TE differentiation in plants.

Xylogen. Motose and others (2001) noticed that TEs often form clusters in *Zinnia* suspension culture. Careful statistical examination using elaborately modified *Zinnia* culture systems indicated that the frequency of TE differentiation depends on local cell density. This finding led to the notion that some conditioning factor(s), called "xylogen", promotes TE differentiation in such cultures. To characterize this hypothetical substance, effects of medium conditioning factors were assayed using low cell density cultures, which normally do not produce TE cells at all. Although the supplement of crude samples of total conditioning factors enables frequent TE differentiation even in such cultures, the selective elimination of their high molecular weight (>50 kDa) fractions inhibits their TE differentiation-inductive activity at low cell density without affecting cytokinesis and viability. The results demonstrated that TE differentiation can be promoted by a novel conditioning factor(s) that is distinct from those required for cell division. This activity was abolished by protease treatments, showing that xylogen consists of proteinaceous molecules. During the time course of TE transdifferentiation, this xylogen activity appeared in the medium concurrently with the appearance of TEs. Thus, xylogen may be produced by living TE or TE precursor cells. In tissues, xylogen may be involved in propagating positional information to stimulate TE differentiation. A further study of this local signaling factor(s) will shed new light not only on the mechanisms of TE differentiation, but also on those of flexible, continuous TE cell file patterning in plant organs.

Sucrose and other factors. *In vitro* culture systems for TE and vascular cell differentiation are very useful for examining the effects of various physical and chemical factors on TE and vascular cell differentiation. Related experiments have revealed that sucrose, forms of inorganic nitrogen source, red/far-red light, temperature, and pH of the medium can influence TE differentiation (reviewed by Fukuda and Komamine 1985).

Roberts and Haigler (1994) investigated in detail the effect of medium pH on the frequency of TE differentiation in *Zinnia* culture. Usually, the pH of the medium is adjusted to 5.5 before the initiation of culture. As the culture process proceeds, the pH oscillates between 4.8 and 5.6 pH. In particular, the pH declines to the lowest level just before the onset of secondary wall thickening. If the initial pH of the medium is increased to 6.0, the ultimate number of TEs can increase nearly to that of the control. However, more neutral pH levels suppress TE differentiation and cause the substantial expansion of cultured cells. These results suggest that extracellular pH needs to be controlled upon TE differentiation, but the process by which this is affected by high pH of the medium is currently unknown.

Although these various factors are involved (possibly by acting in concert) in the induction or regulation of vascular or TE differentiation, their modes of action seem to differ. Notably, in most cases, auxin needs to be exogenously supplied to induce vascular and/or TE differentiation *in vitro* (Fukuda and Komamine 1980, 1985; Aloni 1987). Auxin's mode of action is distinct from that of brassinosteroids, PSK-α, and xylogen, all of which can be endogenously and adequately produced, and then induce TE differentiation within an *in vitro* system (Matsubayashi and others 1999; Motose and others 2001; Yamamoto and others 2001). Auxin would act as a non-autonomous regulator of vascular and TE cell differentiation, and thereby can control the formation of proper vascular tissue patterning in plant organs. In contrast, some of the other factors have characteristics of autonomous regulators, or otherwise local signaling factor-like molecules. They may arise and work via a kind of local cell–cell communication and may have roles mainly in cell (or tissue) specification of the vasculature.

Regulation of Gene Expression in the Course of TE Differentiation

Gene expression cascades. The induction of cell differentiation occurs via the activation of a set of specific genes. Extensive studies on the *Zinnia* culture system showed that the progression of TE development is governed by a cascade of gene expression (Fukuda 1996). The isolation and characterization of many genes that are up-regulated during TE differentiation in *Zinnia* culture revealed several characteristic gene expression patterns (Yamamoto and others 1997). Based on these results, Fukuda (1997a) proposed a model that describes the stepwise transition of cell differentiation state toward TE formation. The course of TE differentiation in *Zinnia* culture can be divided into three stages that correspond to three distinct cell differentiation states (Figure 1). Soon after culture initiation, mesophyll cells enter the dedifferentiation process expressing wound-inducible genes (Figure 1, protease inhibitors I and II), and become dedifferentiated cells (Fig-

ure 1, stage I). After 24–36 h, cells begin to express a distinct set of genes (Figure 1, *TED2–TED4*), most of which are associated with procambial, immature xylem, or TE precursor cells in stems and roots (Fukuda 1996). Thus, dedifferentiated cells change their differentiation state to that of procambial-, immature xylem-, or TE precursor-like cells during stage II. After 48–60 h of culture, TEs with thickened secondary walls appear (Figure 1, stage III). At this period, cultured cells express other sets of genes involved directly in TE morphogenesis and autolysis (Figure 1, *ZCP4, ZEN1, ZRNase1,* p48h-17) (Fukuda 2000). Interestingly, there are several genes that are expressed at stage I, down-regulated at stage II, and again activated at stage III (Figure 1, *PAL1–PAL3*). Because transcripts of these genes remain present after the majority of TEs have undergone cell death, at least a part of non-TE cell population participates in the synthesis of these mRNAs. Endogenous brassinosteroids are involved in the activation of these stage III genes (Yamamoto and others 1997).

The characterization of various other TE-associated enzymes and their genes are extensively reviewed elsewhere (Fukuda 1996, 1997a). In this review, we focus mainly on the mechanisms regulating the expression of such genes.

Unfortunately, in the *Zinnia* culture system, the cloning and characterization of genes encoding transcription factors have not yet been documented. The only case of *Zinnia* gene promoter analysis is the report on characteristics of *TED3* gene 5′-upstream regions (Igarashi and others 1998). The promoter region of the *TED3* gene can confer a TE-associated expression pattern even in *Arabidopsis,* as revealed with transgenic plants introduced with chimeric *TED3* promoter-reporter (GUS) genes (Igarashi and others 1998). *TED3* gene expression may be mediated by tissue-specific transcription machinery common to *A. thaliana* and *Z. elegans,* but *trans*-factors of such machinery have not been identified.

Regulation of lignin precursor synthesis. As noted previously (Fukuda 1996), phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL), enzymes of the phenylpropanoid pathway involved in lignin precursor synthesis, are expressed in vascular bundles of other plant species. Promoter analyses of bean *PAL2* and parsley *4CL-1* genes in transgenic tobacco plants identified sequences required for expression in the xylem tissue and those required for silencing in the phloem (Hatton and others 1995). These sequences include *cis*-elements to which Myb-type transcription factors can bind (Grotewold and others 1994). Thus, a Myb protein is thought to play a role in the regulation of lignin precursor synthesis (Jin and Martin 1999) during TE morphogenesis.

Recently, further search on the *trans*-factors of bean *PAL2* expression was performed (Séguin and others 1997). A screen for proteins that can bind to the *cis*-element in a tobacco stem cDNA expression library identified a clone for an AC-rich binding factor (ACBF). Unexpectedly, this clone encodes a protein distinct from Mybs or other known transcription factors. The partial sequence of ACBF is homologous to a yeast RNA-binding protein. ACBF is expressed in all tissues, and forms a gene family in tobacco genomic DNA. Because the tandemly linked heptamer of these 42-bp oligonucleotides that include this *cis*-element can direct the xylem-associated expression of a minimal promoter-reporter gene, ACBF would play a role in xylem-associated *PAL2* gene expression by binding to this *cis*-element. Repeating this type of analytical process may lead to the successful isolation of the fundamental regulator of TE formation-related gene cascades.

Basic leucine zipper (bZip) transcription factors. French bean glycine-rich protein (GRP)1.8 is a cell wall component of vascular cells (Keller and others 1988). The *grp1.8* gene is expressed only during a short period of vascular tissue differentiation, and its promoter region contains several *cis*-elements that regulate xylem tissue-specific gene expression (Keller and Heierli 1994). One of the transcription factors that can bind to such *cis*-elements is a bZIP-type protein, VSF-1 (Torres-Schumann and others 1996). Detailed gel-shift assays identified the minimal promoter region for *grp1.8,* which is adequate for conferring vascular tissue-specific expression (Ringli and Keller 1998). The GUS reporter gene fused with a *vsf-1* gene promoter appears also in vascular tissues of transgenic tobacco plants (Ringli and Keller 1998).

Another bZIP-type transcription factor involved in the regulation of vascular tissue-associated gene expression was cloned from rice (Yin and others 1997). The rice cDNA clone encodes RF2a bZIP DNA-binding protein that can bind to the promoter sequence of rice tungro bacilliform virus, which exploits a gene expression system of phloem cells for its replication. The *rf2a* gene is present in the rice genomic sequence as a single copy gene, and its mRNA is distributed to roots, leaf blade, and leaf sheath tissues. The RF2a protein, however, preferentially accumulated in leaf blade and leaf sheath tissues. The nuclei of phloem, xylem, and other parenchyma cells were all stained by an immunodetection method using anti-RF2a antibody. The ectopic expression of the *rf2a* gene in antisense orien-

tation resulted in the dwarf and twisted leaf phenotypes. The vascular tissue of these plants is severely reduced and disorganized. Instead, sclerenchyma and air spaces are enlarged at the expense of vascular tissues and mesophyll cells.

Homeobox proteins. The expression of *Arabidopsis homeobox gene 8* (*ATHB8*), a group III homeobox gene, is well associated with developing vascular tissues (Baima and others 1995). *ATHB8* mRNA localizes specifically in procambial cells between the xylem and phloem of developing vascular tissues. In fully developed vascular cells, the signal for *ATHB8* mRNA is not detected. These expression patterns strongly suggest that all vascular cells undergo *ATHB8* expression when they are procambial cells. The ectopic overexpression of this gene resulted in the formation of extra xylem tissues (Baima and others 2000). *ATHB8* is involved in xylem tissue formation in *Arabidopsis.* In light of the fact that TEs are likely to arise from procambial cells in culture (Fukuda 1996), the *ATHB8* may regulate the expression of genes that work at the early stage of TE differentiation.

Arabidopsis INTERFASCICULAR FIBERLESS (IFL1) gene mutation completely suppresses interfascicular fiber differentiation in inflorescence stems, resulting in the characteristic pendent stem phenotype of mutant plants (Zhong and others 1997). The *IFL1* gene encodes a HD-ZIP type transcription factor that is highly homologous to other group III HD-ZIP proteins, ATHB8, ATHB9, and ATHB14. *IFL1* transcript localizes not only in interfascicular fibers but also in vascular bundles (Zhong and Ye 1999). The lack of normal *IFL1* transcript in the stem vascular bundle region causes abnormal xylem development followed by a reduction of xylary fibers and vessel elements. *IFL1* gene commonly functions in such different cell types.

In rice plants, a HD-ZIP II gene *Oshox1* plays a role in the regulation of provascular cell differentiation (Scarpella and others 2000). Extensive expression analyses using a chimeric gene that consists of an *Oshox1* promoter sequence fused with the GUS reporter gene and using an *in situ* hybridization method indicated that *Oshox1* is expressed in provascular and vascular strands. In roots, *Oshox1* expression always starts in cells that do not yet show any sign of vascular cell differentiation. These cells are induced to differentiate into vascular cells, but do not seem to be restricted to this fate as they can become other cell types. The ectopic expression of this *Oshox1* gene caused vascular cell differentiation at the site where it does not yet occur in wild-type plants. Protoxylem cells and TEs also appear at more distal sites in the root of *Oshox1*-overexpressing plants than they do in that of wild-type plants. *Oshox1* can promote vascular cell differentiation by promoting provascular cell specification.

In contrast, another group II gene *ATHB2* acts as a negative regulator for vascular system formation during the secondary growth of roots and hypocotyls (Steindler and others 1999). The *ATHB2* gene is activated in response to natural changes in the ratio of red/far-red light, and regulates cell expansion for shade avoidance. Transgenic *Arabidopsis* plants overexpressing this gene exhibit reduced vasculature, whereas plants expressing the antisense sequence of this gene show expanded vascular systems that result from increased secondary growth. ATHB2 can bind to the sites of a promoter region to which the ATHB8 can also bind. Secondary growth-related vascular development might be regulated through a competitive action of the ATHB2 and ATHB8 on the expression of downstream target genes.

Kawahara and others (1995) reported the cloning of six carrot HD-ZIP I homeobox genes (*CHB1–CHB6*). Hiwatashi and Fukuda (2000) found that four such homeobox genes (*CHB3–CHB6*) are expressed in vascular tissues of carrot cotyledons and hypocotyls. During somatic embryo development, *CHB3–CHB5* appears in the cortex, whereas *CHB6* is consistently localized in vascular tissues (especially the procambium). Functional analyses of these genes will provide important insights into the mechanisms of vascular tissue formation during the early stage of vascular plant development. A very effective transgenic system has recently been developed for the functional analysis of carrot genes during embryo development (Tokuji and Fukuda 1999).

The fact that exogenous auxin and wound stimulus can induce high levels of *ATHB8* and *Oshox1* mRNA expression in (pro) vascular tissues (Baima and others 1995; Scarpella and others 2000) indicates that auxin and wound signals might be converted intracellularly to homeobox proteins that promote vascular cell differentiation. Furthermore, sucrose can also induce the expression of *Oshox1,* thereby influencing provascular cell fate commitment (Scarpella and others 2000). Although previous reports questioned the regulatory roles of sucrose in vascular cell specification (Fukuda and Komamine 1985; Aloni 1987), these studies will help to elicit unambiguous answers.

At the same time, these homeobox genes may regulate multiple aspects of cell development or multiple cell type differentiation. It is possible that these proteins help to transcribe the same effector genes in different cell types. However, the genes directly activated by these homeobox transcription factors are currently unknown. The identification of

such downstream target genes will be essential to unravel the complete picture of gene expression cascades.

Genes regulating the continuity of vascular tissues. To address the nature of vascular patterning in relation to vascular cell development, direct screens for vascular pattern mutants have been performed. Mutations of *COTYLEDON VASCULAR PATTERN1* and *2* (*CVP1* and *2*) genes generate abnormal vascular patterns in cotyledons (Carland and others 1999). The *cvp1* mutants form thicker, but discontinuous cotyledon vascular bundles, whereas *cvp2* mutants are defective in lateral vein formation and their marginal loop structures are missing. The double mutants of *cvp1* and *2* show a *cvp2*-like vascular patterning with a slight increase in *cvp1*-characteristic disconnected vasculature. These mutations affect pattern formation in the provascular tissue, resulting in discontinuity of both xylem and phloem. The gross morphology of these mutants is almost the same as those of wild-type plants. In contrast to the expression of a *cvp1* phenotype, which cannot be seen in other leaves, the *cvp2* phenotype (vascular discontinuity) also appears in higher order veins of adult plant leaves. The *cvp1cvp2* double mutation affects the inflorescence stem and causes overproduction of vascular tissue. Interestingly, in these mutants, auxin synthesis, transport, and perception are normal, suggesting that factors independent of auxin-related events are involved in proper vascular pattern formation in cotyledons (and leaves) of these mutants.

Koizumi and others (2000) also determined loci that encode genes involved in cotyledon vascular pattern formation from the M_3 generation of mutagenized plants. In contrast to *CVP1* and *2,* all mutations of their novel genes *VASCULAR NETWORK1-6* (*VAN1-6*) result in severe sterility, possibly due to the deleterious effects of cotyledon vascular pattern disruption. In cotyledons of these *van* mutants, overall vascular architecture is preserved, but disconnected lateral veins appear in which the vessels consist of larger TEs than those of wild type plants. Aberrant vascular patterning or structure also appears in leaves, roots, and hypocotyls. In particular, the hypocotyls of *van* mutants, except *van3,* lack normal phloem strands. Discontinuous vascular strands can also be seen in the *van1* roots. Among these *van* mutants, *van3* exhibits the most drastic vascular pattern defects. Transgenic *van3* plants carrying β-glucuronidase (GUS) reporter genes fused with a *ATHB8* or *TED3* promoter sequence indicate that not only are the vessel patterns of the xylem affected but the patterns of the provascular tissue are also affected. Although the relationships between these mutations and actions of auxin have not been investigated, an auxin signal flow canalization hypothesis (Berleth and others 2000; Sachs 2000; see above sections) alone seems inadequate to explain the discontinuous lateral vein formation (Koizumi and others 2000). Rather, these results suggest the significant involvement of a mechanism postulated by the diffusion-reaction prepattern hypothesis (Nelson and Dengler 1997).

A mutant carrying a dysfunctional *SCARFACE* (*SFC*) gene, the chromosomal position of which is very similar to that of *VAN3,* has also been reported (Deyholos and others 2000). The *sfc* mutants exhibit abnormal cotyledon and leaf vascular patterning due to the abnormal development of provascular bundles and increased auxin sensitivity. Double mutants of *sfc* and putative *mp* null allele aggravate the defect in the formation of cotyledon vasculature. Remarkable shortening of the midvein can be observed, implying partially overlapped function of the *SFC* and *MP* genes. However, double mutants of *sfc* and auxin-resistant mutants or an auxin-overproducing mutant did not reverse the defect of *sfc.* The *SFC* gene would encode a negative regulator in a distinct auxin signaling pathway.

In the case of *amphivasal vascular bundle 1* (*avb1*) mutants (Zhong and others 1999), a gene closely associated with vascular tissue architecture is likely to be disrupted. The *avb1* carries amphivasal vascular bundles in which the phloem, cambium, and xylem are arranged concentrically. These bundles are occasionally branched and penetrate the pith in stems in contrast to the case of wild type plants, but venation patterns in cotyledons or primary roots are normal. In these mutants, machinery for polar auxin transport is unaffected. The *AVB1* gene plays a pivotal role in the determination of tissue arrangement within the vasculature.

The roles of *CVP*s, *VAN*s, *SFC,* and *AVB1* are relatively confined to vascular development and/or patterning. Thus it is possible that these genes are closely associated with vascular or TE cell differentiation. These mutants may also be very useful in unraveling the nature of the elaborate and flexible mechanisms underlying preservation of a strict vascular bundle or TE cell file continuity.

There are many other *Arabidopsis* genes, the mutations of which cause disruption of vascular tissue structures. Several mutants with abnormal plant body organization patterns (Mayer and others 1991) include *fackel* (Jang and others 2000; Schrick and others 2000) and *fass* (Torres-Ruiz and Jürgens 1994), in which the formation of normal continuous lateral veins is prevented in cotyledons. Defects in specific cell division in the stele can also affect stem

vascular bundle development, as suggested with *wooden leg* (*wol*) mutants (Scheres and others 1995). In *lion's tail* (*lit*) mutants, stem vascular tissues are disrupted by disabled cell expansion-related machinery (Hauser and others 1995). Furthermore, *ectopic lignification1* (*eli1*) mutants show that disorganized programming of cell lignification in tissues results in discontinuous TE cell file formation in the stem (Caño-Delgado and others 2000). These aberrant vascular-associated phenotypes might be secondary effects of defective cell division or expansion. It is unknown, however, whether these cell growth and developmental aspects are fully independent of vascular or TE cell differentiation. These mutants will provide opportunities to examine the involvement of cell division or expansion-related machinery in vascular or TE cell development.

To understand regulatory mechanisms for vascular and TE cell differentiation exactly, it is preferable that gene expression cascades involving the above genes are studied in the same experimental systems. For this reason, the expression analysis of *Zinnia* genes homologous to the ACBF (Séguin and others 1997), bZips (Ringli and Keller 1998; Yin and others 1997), or *ATHB*s (Baima and others 1995; Hiwatashi and Fukuda 2000; Scarpella and others 2000; Söderman and others 1999; Steindler and others 1999; Zhong and Ye 1999) or to those derived from *Arabidopsis* vascular patterning mutants (Berleth and others 2000) using the *Zinnia* culture system may be effective. Some of them could be regarded as temporal and spatial marker genes for (pro)vascular tissue formation. The determination of their expression patterns will provide new insight into the mechanism of vascular cell fate specification.

Recent Topics of Morphogenesis and Programmed Cell Death during TE Differentiation

The most striking features of TE formation are the development of patterned secondary walls and the autolysis of cell contents and walls that occurs in stage III (Figure 1). The secondary cell wall is composed of cellulose microfibrils arranged in parallel with one another and of cementing substances that contain lignin, hemicellulose, pectin, and proteins, which add strength and rigidity to the wall (Fukuda 1996). These substances are synthesized and deposited cooperatively during secondary wall formation. The autolysis is a typical example of programmed cell death. Hydrolytic enzymes, such as DNases, RNases, and proteases, accumulate in the vacuole of differentiating TEs. In association with the inhibition of organic anion transport into the vacuole, the vacuole swells (Kuriyama 1999) then bursts, shrinks, and fragments (Groover and others 1997; Groover and Jones, 1999; Kuriyama 1999). Vacuole collapse causes hydrolytic enzymes to invade the cytoplasm and attack various organelles, resulting in the degradation of cell contents including the plasma membrane (Obara and others 2001) and a part of primary cell walls. Finally, the opening of a pore leads TEs to lose all cell contents and form mature hollow tubes fortified by secondary walls. Details of secondary wall formation and cell death programs are beyond the scope of this article, but readers may obtain relevant information from recent review articles (Beers and others 2000; Fukuda 1996, 1997a, b, 2000; Jones 2000, 2001). Herein, two new aspects, one related to cell death and the other with cell polarity, are described. These new aspects provide valuable opportunities to specify genetic regulation for TE development and to consider the way in which local cell–cell interactions are important for vascular tissue development.

An apoplastic protection from injury derived from hydrolytic activities. As outlined above, soon after TEs die, their plasma membrane disintegrates (Obara and others 2001) and leaks cell contents including autolysis-related enzymes (Fukuda 1996, 1997b). Such hydrolytic enzymes must be deleterious to other living cells. Actually, in *Zinnia* culture, proteasome activity appears in the medium concurrently with TE cell death, and affects the viability of other non-TE cells (Endo and others 2001). To avoid this toxic effect of the extracellular proteasome, cultured cells synthesize and excrete the TED4 protein before TE cell death (Demura and Fukuda 1993, 1994; Endo and others 2001). The TED4 protein is homologous to nonspecific lipid transfer proteins (Demura and Fukuda 1993, 1994), but binds to the proteasome in the medium and reduces its proteolytic activity. Thus, the TED4 protein probably works in xylem tissues to protect living TEs and other xylem non-TE cells surrounding dead TEs that are releasing the proteasome. These findings imply the existence of a novel mechanism that provides a safety apparatus based on local cell–cell interactions. This mechanism is potentially very important as it ensures the intactness of TE precursors or other xylem cells, and thereby indirectly contributes to the progression of normal TE or xylem cell differentiation processes.

Cell polarity. Polar auxin transport may be involved in the formation and maintenance of the apical-basal axis of plant bodies. The polarity through the plant body is closely associated with cell polarity, as typically shown by the intracellular localization of PIN1 auxin efflux carrier protein (Berleth and

Mattsson 2000; Steinmann and others 1999). Nakashima and others (2000) found that single TEs formed from isolated and cultured *Zinnia* mesophyll cells always have pores only at the one-sided tip of their cell wall. This result is consistent with the behavior of TEs in plants: when TEs mature, they lyse their wall tip proximal to the vessel or tracheid cell files and connect themselves with conductive tissues, thereby helping the extension of these conduits without interrupting the continuity. This observation suggests that even cultured single cells can retain or express a kind of cell polarity by themselves in the course of TE differentiation. The polarity of particular cells is integrated in tissues of TE cell files via cell–cell interactions (Nakashima and others 2000).

There is another line of findings that support the presence of cell polarity in developing TEs. Shinohara and others (2000) raised antibodies against cell wall components of *Zinnia* cultured cells using a phage display method. To obtain many kinds of antibodies that selectively recognize TE cell wall antigens, they enriched their phage clones via a subtractive selection process. The antibody showing the highest specificity to TE cell wall samples was named CN 8 and further characterized. The CN 8 antibody reacts with a carbohydrate compound in a hemicellulose fraction. By indirect immunofluorescence microscopy, the compound was shown to locate at one side of cultured TE cells, although its presence is transient. The CN 8 antigen is also associated with young TEs of stem cross-sections in a similar manner. This polar localization pattern may reflect the TE cell polarity that underlies the pore formation at the one-sided tip of the cell wall (Nakashima and others 2000). Moreover, it is possible to think that this component itself is involved in the expression, reinforcement, or maintenance of TE cell polarity.

Interestingly, a spherical, non-TE cell type that appears at the late stage of culture can also be stained with this antibody. In contrast to the case of TEs, the signal is uniformly distributed on their surface. Shinohara and others (2000) speculate that this cell type shares features with xylem parenchyma cells, which are also labeled with CN 8 in stem cross-sections. The CN 8 epitope-related expression of cell polarity might necessitate some cytological background (such as polar transport systems for some cell wall components) of TE cells.

Conclusion and Perspectives

Recent studies have revealed several important features in the process of TE differentiation. (1) All known plant hormones seem to be involved in processes of vascular or TE cell development (see for example, Fukuda 1992; Kalev and Aloni 1998, 1999; Ryan 2000; Söderman and others 1999; Yamamoto and others 1997, 2001). The role of auxin is especially distinctive, as it can act as a non-autonomous regulator for vascular and TE cell differentiation. (2) Based on marker gene expression patterns, procambial-like cells are likely to appear in *Zinnia* culture (Fukuda 1996, 1997a). Even in a single cell-based suspension culture, TEs arise from procambial-like cells undergoing sequential changes in cell differentiation states as they do during xylem tissue establishment in plants (Baima and others 2000; Fukuda 1996; Northcote 1995; Scarpella and others 2000). (3) The presence of many different genes related to vascular cell development in the *Arabidopsis* genome (Berleth and others 2000; Carland and others 1999; Deyholos and others 2000; Koizumi and others 2000) indicates that the establishment of vascular patterns is subject to complicated genetic regulation. (4) Local cell–cell interactions important for TE formation in vascular tissues may involve the production of brassinosteroids (Yamamoto and others 2001), the production of a local cell density-dependent conditioning factor(s) (Motose and others 2001), the construction of a safety apparatus for protection from hydrolytic activities (Endo and others 2001), and the integration of cell polarity (Nakashima and others 2000; Shinohara and others 2000).

Identification and isolation of genes that have a key role in vascular or TE development are urgent themes for future work. However, it may be difficult to achieve this by popular strategies such as standard mutant analyses, as the mutation of such genes often culminates in lethality before the establishment of embryos. For this reason, the massive cloning of genes whose expression changes in association with TE differentiation using DNA microarrays will become a powerful tool for the isolation of key genes. Detailed analyses with the *in vitro Zinnia* culture system have suggested that intercellular communication is an important factor initiating specific processes of TE differentiation (Motose and others 2001; Yamamoto and others 2001). Therefore, the identification of such intercellular factors and the characterization of their signaling cascades are essential for further analysis of the regulation of TE differentiation.

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REFERENCES

Aloni R. 1987. Differentiation of vascular tissues. Annu Rev Plant Physiol 38:179–204.

Aoyama T, Chua N-H. 1997. A glucocorticoid-mediated transcriptional induction system in transgenic plants. Plant J 11:605–612.

Asami T, Min YK, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S. 2000. Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. Plant Physiol 123:93–99.

Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I, Morelli G. 1995. The expression of the Athb-8 homeobox gene is restricted to provascular cells in *Arabidopsis thaliana.* Development 121:4171–4182.

Baima S, Tomassi M, Mateucci A, Altamura MM, Ruberti I, Morelli G. 2000. Role of the *ATHB-8* gene in xylem formation. In: Savidge RA, Barnett JR, Napier R, editors. Cell and molecular biology of wood formation. Oxford: BIOS Scientific Publishers. p 445–455.

Barnett JR. 1981. Xylem cell development. London: Castle House Publications.

Beers EP, Woffenden BJ, Zhao C. 2000. Plant proteolytic enzymes: possible roles during programmed cell death. Plant Mol Biol 44:399–415.

Berleth T, Mattsson J. 2000. Vascular development: tracing signals along veins. Curr Opin Plant Biol 3:406–411. DOI: 10.1016/S1369-5266(00)00104-7

Berleth T, Mattsson J, Hardtke CS. 2000. Vascular continuity and auxin signals. Trends Plant Sci 5:387–393. DOI: 10.1016/S1360-1385(00)01725-8

Berleth T, Jürgens G. 1993. The role of the *MONOPTEROS* gene in organizing the basal body region of the *Arabidopsis* embryo. Development 118:575–587.

Caño-Delgado AI, Metzlaff K, Bevan MW. 2000. The *eli1* mutation reveals a link between cell expansion and secondary cell wall formation in *Arabidopsis thaliana.* Development 127:3395–3405.

Carland FM, Berg BL, FitzGerald JN, Jinamornphongs S, Nelson T, Keith B. 1999. Genetic regulation of vascular tissue patterning in Arabidopsis. Plant Cell 11:2123–2137.

Carland FM, McHale NA. 1996. *LOP1:* a gene involved in auxin transport and vascular patterning in *Arabidopsis.* Development 122:1811–1819.

Chaudhury AM, Letham S, Craig S, Dennis ES. 1993. *amp1*—a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering. Plant J 4:907–916.

Choe S, Noguchi T, Fujioka S, Takatsuto S, Tissier CP, Gregory BD, Ross AS, Tanaka A, Yoshida S, Tax FE, Feldmann KA. 1999. The Arabidopsis *dwf/ste1* mutant is defective in the Δ^7 sterol C-5 desaturation step leading to brassinosteroid biosynthesis. Plant Cell 11:207–221.

Christensen SK, Dagenais N, Chory J, Weigel D. 2000. Regulation of auxin response by the protein kinase PINOID. Cell 100:469–478.

Clouse SD, Sasse JM. 1998. Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451.

Conway LJ, Poethig RS. 1997. Mutations of *Arabidopsis thaliana* that transform leaves into cotyledons. Proc Natl Acad Sci USA 94:10209–10214. DOI: 10.1073/pnas.94.19.10209

Creelman RA, Mullet JE. 1997. Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381.

Demura T, Fukuda H. 1993. Molecular cloning and characterization of cDNAs associated with tracheary element differentiation in cultured *Zinnia* cells. Plant Physiol 103:815–821.

Demura T, Fukuda H. 1994. Novel vascular cell-specific genes whose expression is regulated temporally and spatially during vascular system development. Plant Cell 6:967–981.

Deyholos MK, Cordner G, Beebe D, Sieburth LE. 2000. The *SCARFACE* gene is required for cotyledon and leaf vein patterning. Development 127:3205–3213.

Eklund L. 1991. Relations between indoleacetic acid, calcium ions and ethylene in the regulation of growth and cell wall composition in *Picea abies.* J Exp Bot 42:785–789.

Eklund L, Tiltu A. 1999. Cambial activity in 'normal' spruce *Picea abies* Karst (L.) and snake spruce *Picea abies* (L.) Karst f. *virgata* (Jacq.) Rhed in response to ethylene. J Exp Bot 50:1489–1493.

Endo S, Demura T, Fukuda H. 2001. Inhibition of proteasome activity by the TED4 protein in extracellular space: a novel mechanism for protection of living cells from injury caused by dying cells. Plant Cell Physiol 42:9–19.

Eriksson ME, Israelsson M, Olsson O, Moritz T. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. Nature Biotechnol 18:784–788.

Fukuda H. 1992. Tracheary element formation as a model system of cell differentiation. Int Rev Cytol 136:289–332.

Fukuda H. 1996. Xylogenesis: initiation, progression, and cell death. Annu Rev Plant Physiol Plant Mol Biol 47:299–325.

Fukuda H. 1997a. Tracheary element differentiation. Plant Cell 9:1147–1156.

Fukuda H. 1997b. Programmed cell death during vascular system formation. Cell Death Differ 4:684–688.

Fukuda H. 2000. Programmed cell death of tracheary elements as a paradigm in plants. Plant Mol Biol 44:245–253.

Fukuda H, Komamine A. 1980. Establishment of an experimental system for the tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans.* Plant Physiol 65:57–60.

Fukuda H, Komamine A. 1985. Cytodifferentiation. In: Vasil IK, editor. Cell culture and somatic cell genetics of plants 2 Cell growth, nutrition, cytodifferentiation, and cryopreservation 1985. London: Academic Press. p 149–212.

Fukuda H, Watanabe Y, Kuriyama H, Aoyagi S, Sugiyama M, Yamamoto R, Demura T, Minami A. 1998. Programming of cell death during xylogenesis. J Plant Res 111:253–256.

Groover A, DeWitt N, Heidel A, Jones A. 1997. Programmed cell death of plant tracheary elements differentiating in vitro. Protoplasma 196:197–211.

Groover A, Jones AM. 1999. Tracheary element differentiation uses a novel mechanism coordinating programmed cell death and secondary cell wall synthesis. Plant Physiol 119:375–384.

Grotewold E, Drummond B, Bowen B, Peterson T. 1994. The *Myb*-homologous *P* gene controls phlobaphene pigmentation in maize floral organs by directly activating a subset of flavonoid biosynthetic genes. Cell 76:543–553.

Hamman T, Mayer U, Jürgens G. 1999. The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. Development 126:1387–1395.

Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J 17:1405–1411. DOI: 10.1093/emboj/17.5.1405

Hatton D, Sablowski R, Yung M-H, Smith C, Schuch W, Bevan M. 1995. Two classes of *cis* sequences contribute to tissue-specific expression of a *PAL2* promoter in transgenic tobacco. Plant J 7:859–876.

Hause B, Stenzel I, Miersch O, Maucher H, Kramell R, Ziegler J, Wasternack C. 2000. Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. Plant J 24:113–126. DOI: 10.1046/j.1365-313X.2000.00861.x

Hauser M-T, Morikami A, Benfey PN. 1995. Conditional root expansion mutants of *Arabidopsis.* Development 121:1237–1252.

Hedden P, Kamiya Y. 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. Annu Rev Plant Physiol Plant Mol Biol 48:431–460.

Hennion S, Little CHA, Hartmann C. 1992. Activities of enzymes involved in lignification during the post-harvest storage of etiolated asparagus spears. Physiol Plant 86:474–478.

Hiwatashi Y, Fukuda H. 2000. Tissue-specific localization of mRNA for carrot homeobox genes, *CHB*s, in carrot somatic embryos. Plant Cell Physiol 41:639–643.

Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY, Bandyopadhyay A, Estelle M. 1999. The *axr6* mutants of *Arabidopsis* define a gene involved in auxin response and early development. Development 127:23–32.

Igarashi M, Demura T, Fukuda H. 1998. Expression of the *Zinnia TED3* promoter in developing tracheary elements of transgenic *Arabidopsis.* Plant Mol Biol 36:917–927.

Ingemarsson BSM, Eklund L, Eliasson L. 1991. Ethylene effects on cambial activity and cell wall formation in hypocotyls of *Picea abies* seedlings. Physiol Plant 82:219–224.

Iwasaki T, Shibaoka H. 1991. Brassinosteroids act as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. Plant Cell Physiol 32:1007–1014.

Jang J-C, Fujioka S, Tasaka M, Seto H, Takatsuto S, Ishii A, Aida M, Yoshida S, Sheen J. 2000. A critical role of sterols in embryonic patterning and meristem programming revealed by the *fackel* mutants of *Arabidopsis thaliana.* Genes Dev 14:1485–1497.

Jefferson RA, Kavanagh TA, Bevan MW. 1987. GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907.

Jin H, Martin C. 1999. Multifunctionality and diversity within the plant *MYB*-gene family. Plant Mol Biol 41:577–585.

Jones A. 2000. Does the plant mitochondrion integrate cellular stress and regulate programmed cell death? Trends Plant Sci 5:225–230. DOI: 10.1016/S1360-1385(00)01605-8

Jones AM. 2001. Programmed cell death in development and defense. Plant Physiol 125:94–97.

Kalev N, Aloni R. 1998. Role of auxin and gibberellin in regenerative differentiation of tracheids in *Pinus pinea* seedlings. New Phytol 138:461–468.

Kalev N, Aloni R. 1999. Role of ethylene and auxin in regenerative differentiation and orientation of tracheids in *Pinus pinea* seedlings. New Phytol 142:307–313.

Kawahara R, Komamine A, Fukuda H. 1995. Isolation and characterization of homeobox-containing genes of carrot. Plant Mol Biol 27:155–164.

Keller B, Heierli D. 1994. Vascular expression of the *grp1.8* promoter is controlled by three specific regulatory elements and one unspecific activating sequence. Plant Mol Biol 26:747–756.

Keller B, Sauer N, Lamb CJ. 1988. Glycine-rich cell wall proteins in bean: gene structure and association of the protein with the vascular system. EMBO J 7:3625–3633.

Kieber JJ. 1997. The ethylene response pathway in Arabidopsis. Annu Rev Plant Physiol Plant Mol Biol 48:277–296.

Klee HJ, Horsch RB, Hinchee MA, Hein MB, Hoffmann NL. 1987. The effects of overproduction of two Agrobacterium tumefaciens T-DNA auxin biosynthetic gene products in transgenic petunia plants. Genes Dev 1:86–96.

Kobayashi H, Fukuda H. 1994. Involvement of calmodulin and calmodulin-binding proteins in the differentiation of tracheary elements in *Zinnia cells.* Planta 194:388–394.

Koizumi K, Sugiyama M, Fukuda H. 2000. A series of novel mutants of *Arabidopsis thaliana* that are defective in the formation of continuous vascular network: calling the auxin signal flow canalization hypothesis into question. Development 217:3197–3204.

Kubigsteltig I, Laudert D, Weiler EW. 1999. Structure and regulation of the *Arabidopsis thaliana* allene oxide synthase gene. Planta 208:463–471. DOI: 10.1007/s004250050583

Kuriyama H. 1999. Loss of tonoplast integrity programmed in tracheary element differentiation. Plant Physiol 121:763–774.

Leung J, Giraudat J. 1998. Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol 49:199–222.

Matsubayashi Y, Sakagami Y. 1996. Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. Proc Natl Acad Sci USA 93:7623–7627. DOI: 10.1073/pnas.93.15.7623

Matsubayashi Y, Takagi L, Omura N, Morita A, Sakagami Y. 1999. The endogenous sulfated pentapeptide phytosulfokine-α stimulates tracheary element differentiation of isolated mesophyll cells of zinnia. Plant Physiol 120:1043–1048.

Mattsson J, Sung ZR, Berleth T. 1999. Responses of plant vascular systems to auxin transport inhibition. Development 126:2979–2991.

Maucher H, Hause B, Feussner I, Ziegler J, Wasternack C. 2000. Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome): tissue specific regulation in seedling development. Plant J 21:199–213. DOI: 10.1046/j.1365-313X.2000.00669.x

Mayer U, Torres Ruiz RA, Berleth T, Miséra S, Jürgens G. 1991. Mutations affecting body organization in the *Arabidopsis* embryo. Nature 353:402–407.

McCann M. 1997. Tracheary element formation: building up to a dead end. Trends Plant Sci 2:333–338. DOI: 10.1016/S1360-1385(97)84620-1

McCourt P. 1999. Genetic analysis of hormone signaling. Annu Rev Plant Physiol Plant Mol Biol 50:219–243.

Motose H, Fukuda H, Sugiyama M. 2001. Involvement of local intercellular communication in the differentiation of zinnia mesophyll cells into tracheary elements. Planta (in press).

Nakashima J, Takabe K, Fujita M, Fukuda H. 2000. Autolysis during in vitro tracheary element differentiation: formation and location of the perforation. Plant Cell Physiol 41:1267–1271.

Nelson T, Dengler N. 1997. Leaf vascular pattern formation. Plant Cell 9:1121–1135.

Northcote DH. 1995. Aspects of vascular tissue differentiation in plants: parameters that may be used to monitor the process. Int J Plant Sci 156:245–256.

Obara K, Kuriyama H, Fukuda H. 2001. Direct evidence of active and rapid nuclear degradation triggered by vacuole rupture during programmed cell death in zinnia. Plant Physiol 125:615–626.

O'Donnell PJ, Calvert C, Atzorn R, Wasternack C, Leyser HMO, Bowels DJ. 1996. Ethylene as a signal mediating the wound response of tomato plants. Science 274:1914–1917. DOI: 10.1126/science.274.5294.1914

Oh MH, Romanow WG, Smith RC, Zamski E, Sasse J, Clouse SD. 1998. Soybean *BRU1* encodes a functional xyloglucan endotransglycosylase that is highly expressed in inner epicotyl tissues during brassinosteroid-promoted elongation. Plant Cell Physiol 39:124–130.

Ringli C, Keller B. 1998. Specific interaction of the tomato bZIP transcription factor VSF-1 with a non-palindromic DNA sequence that controls vascular gene expression. Plant Mol Biol 37:977–988.

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JAH. 1999. Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Scienece 283:1541–1544.

Roberts AW, Haigler CH. 1990. Tracheary element differentiation in suspension-cultured cells of *Zinnia* requires uptake of extracellular Ca^{2+}. Planta 180:502–509.

Roberts AW, Haigler CH. 1994. Cell expansion and tracheary element differentiation are regulated by extracellular pH in mesophyll cultures of *Zinnia elegans* L. Plant Physiol 105:699–706.

Roberts K, McCann MC. 2000. Xylogenesis: the birth of a corpse. Curr Opin Plant Biol 3:517–522. DOI: 10.1016/S1369-5266(00)00122-9

Romano CP, Hein MB, Klee HJ. 1991. Inactivation of auxin in tobacco transformed with the indoleacetic acid-lysine synthetase gene of *Pseudomonas savastanoi*. Genes Dev 5:438–446.

Rupp H-M, Frank M, Werner T, Strnad M, Schmülling T. 1999. Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. Plant J 18:557–563. DOI: 10.1046/j.1365-313X.1999.00472.x

Ryan CA. 2000. The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta 1477:112–121.

Sachs T. 2000. Integrating cellular and organismic aspects of vascular differentiation. Plant Cell Physiol 41:649–656.

Scarpella E, Rueb S, Boot KJM, Hoge JHC, Meijer AH. 2000. A role for the rice homeobox gene *Oshoxl* in provascular cell fate commitment. Development 127:3655–3669.

Schmülling T, Beinsberger S, De Greef J, Schell J, Van Onckelen H, Spena A. 1989. Construction of a heat-inducible chimeric gene to increase the cytokinin content in transgenic plant tissue. FEBS Lett 289:401–406.

Schrick K, Mayer U, Horrichs A, Kuhnt C, Bellini C, Dangl J, Schmidt J, Jürgens G. 2000. *FACKEL* is a sterol C-14 reductase required for organized cell division and expansion in *Arabidopsis* embryogenesis. Genes Dev 14:1471–1484.

Séguin A, Laible G, Leyva A, Dixon RA, Lamb CJ. 1997. Characterization of a gene encoding a DNA-binding protein that interacts in vitro with vascular specific *cis* elements of the phenylalanine ammonia-lyase promoter. Plant Mol Biol 35:281–291.

Sessions A, Nemhauser JL, McColl A, Roe JL, Feldmann KA, Zambryski PC. 1997. ETTIN patterns the *Arabidopsis* floral meristem and reproductive organs. Development 124:4481–4491.

Sheres B, Di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, Weisbeek P, Benfey PN. 1995. Mutants affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. Development 121:53–62.

Shinohara N, Demura T, Fukuda H. 2000. Isolation of a vascular cell wall-specific monoclonal antibody recognizing a cell polarity by using a phage display subtraction method. Proc Natl Acad Sci USA 97:2585–2590. DOI: 10.1073/pnas.050582197

Sieburth LE. 1999. Auxin is required for leaf vein pattern in *Arabidopsis*. Plant Physiol 121:1179–1190.

Söderman E, Hjellström M, Fahleson J, Engström P. 1999. The HD-Zip gene *ATBH6* in *Arabidopsis* is expressed in developing leaves, roots and carpels and up-regulated by water-deficit conditions. Plant Mol Biol 40:1073–1083.

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I. 1999. Shade avoidance responses are mediated by the ATHB-2 HD-Zip protein, a negative regulator of gene expression. Development 126:4235–4245.

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Gälweiler L, Palme K, Jürgens G. 1999. Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286:316–318. DOI: 10.1126/science.286.5438.316

Sugiyama M, Ito J, Aoyagi S, Fukuda H. 2000. Endonucleases. Plant Mol Biol 44:387–397.

Sun T-p. 2000. Gibberellin signal transduction. Curr Opin Plant Biol 3:374–380. DOI: 10.1016/S1369-5266(00)00099-6

Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei GP, Nagy F, Schell J, Koncz C. 1996. Brassinosteroids rescue the deficiency of CPY90, cytochrome P450, controlling cell elongation and deetiolation in Arabidopsis. Cell 85:171–182.

Tokuji Y, Fukuda H. 1999. A rapid method for transformation of carrot (*Daucus carrota* L.) by using direct somatic embryogenesis. Biosci Biotechnol Biochem 63:519–523.

Torres-Ruiz RA, Jürgens G. 1994. Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. Development 120:2967–2978.

Torres-Schumann S, Ringli C, Heierli D, Amrhein N, Keller B. 1996. *In vitro* binding of the tomato bZIP transcriptional activator VSF-1 to a regulatory element that controls xylem-specific gene expression. Plant J 9:283–296.

Vicient CM, Hull G, Guilleminot J, Devic M, Delseny M. 2000. Differential expression of the *Arabidopsis* genes coding for Em-like proteins. J Exp Bot 51:1211–1220.

Wurtele ES, Wang H, Durgerian S, Nicolau BJ, Ulrich TH. 1993. Characterization of a gene that is expressed early in somatic embryogenesis of *Daucus carota*. Plant Physiol 102:303–312.

Yamamoto R, Demura T, Fukuda H. 1997. Brassinosteroids induce entry into the final stage of tracheary element differentiation in cultured *Zinnia* cells. Plant Cell Physiol 38:980–983.

Yamamoto R, Fujioka S, Demura T, Takatsuto S, Yoshida S, Fukuda H. 2001. Brassinosteroid levels increase drastically prior to morphogenesis of tracheary elements. Plant Physiol 125:556–563.

Yang H, Matsubayashi Y, Hanai H, Sakagami Y. 2000. Phytosulfokine-α, a peptide growth factor found in higher plants: its structure, functions, precursor and receptors. Plant Cell Physiol 41:825–830.

Yin Y, Zhu Q, Dai S, Lamb C, Beachy RN. 1997. RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. EMBO J 17:5247–5259.

Zhong R, Taylor JJ, Ye Z-H. 1997. Disruption of interfascicular fiber differentiation in an Arabidopsis mutant. Plant Cell 9:2159–2170.

Zhong R, Taylor JJ, Ye Z-H. 1999. Transformation of the collateral vascular bundles into amphivasal vascular bundles in an Arabidopsis mutant. Plant Physiol 120:53–64.

Zhong R, Ye Z-H. 1999. IFL1, a gene regulating interfascicular fiber differentiation in Arabidopsis, encodes a homeodomain-leucine zipper protein. Plant Cell 11:2139–2152.

Zielinski RE. 1998. Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 49:697–725.