Identifying and characterizing genes that regulate vascular tissue-specific functions

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ABSTRACT

Vascular tissues provide both the mechanical support to the plant body and the conducting cells for the transport of water, mineral solutes, hormones and other signaling molecules, amino acids, and sugars. To identify genes that may regulate vascular tissue-specific functions, we isolated xylem, phloem-cambium, and nonvascular tissues from the Arabidopsis root-hypocotyl, performed a genome-wide comparative analysis of tissue-specific transcripts using the 24K Affymetrix Arabidopsis ATH1 Genome Array (24K GeneChip), and identified potential genes that are required for xylem and phloem differentiation or tissue-specific functions.

Based on this comparative analysis, two phloem-specific G2-like transcription factors, *MYR1* and *MYR2*, and a xylem-specific NAC domain family member, *XND1*, were selected for further characterization. Under continuous light, *myr2* plants flowered early, while *myr1* plants did not differ significantly from wild type controls. However, double mutant *myr1myr2* plants exhibited a novel phenotype characterized by elongated petioles, semi-erect leaf orientation, and suppression of lateral shoot outgrowth. These characteristics are reminiscent of *yucca*, a dominant Arabidopsis mutant with elevated levels of free auxin. Preliminary results indicated that like *yucca*, *myr1myr2* plants were more resistant than wt plants to 5-mT, a toxic tryptophan analog, suggesting that *MYR1* and *MYR2* may be involved in regulating tryptophan-dependent auxin biosynthesis. Overexpression of any one of *MYR1* isoforms resulted in a phenotype that in some cases resembled that observed in the double mutant, indicating that the regulation mediated by MYR1 and MYR2 may depend on formation of specific heterodimers consisting of isoforms of MYR1 and/or MYR2, and that the dimerization was susceptible to disruption both by overexpression and loss-of-function of *MYR1/MYR2*.

Overexpression of *XND1* resulted in the absence of TEs as determined from the absence of both secondary cell wall deposition and TE death. Using 3 tissue-specific promoter-GUS lines as genetic backgrounds, we demonstrated that overexpression of *XND1* suppressed only TE-specific GUS expression but not phloem-specific GUS expression. Three T-DNA/transposon insertion lines, *xnd1-1*, *-2*, and *-3*, were identified. Under normal conditions, *xnd1* did not exhibit significantly different growth and development compared to wild type plants. However, preliminary data indicated that *xnd1* plants were ABA and cold hypersensitive. Yeast-two hybrid screening using the N-terminal portion of XND1 as bait identified a novel RING finger protein, At3g62970 that may function as the ubiquitin ligase (E3). These results suggested that XND1 functions as a negative regulator of xylem cell differentiation, and that the regulation mediated by XND1 may be integrated with the ubiquitin/26S proteasome pathway.

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Dedication

This dissertation is dedicated to my parents, Zhao, Shougui and Ma, Zhongxiu, my brother, Zhao, Chengan, my sister, Zhao, Fengying, my wife, Jin, Ying and my daughter, Zhao, Biyuan for their love and support.

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Chapter 1

Literature Review

In higher plants, the vascular system is composed of three tissue types: xylem, cambium, and phloem. The cambial zone lies between xylem and phloem and gives rise to xylem, the central region, and phloem, the peripheral region of the stem and root. Vascular tissues form from procambium during primary growth or vascular cambium during secondary growth. The vascular cambium can originate from the procambium, which is derived from the apical meristem, or parenchyma or pericycle cells during secondary growth. Xylem, responsible for transporting water and mineral solutes, and for providing the mechanical support to the plant body, is composed of water-conducting tracheary elements (TEs) and nonconducting parenchyma and fiber cells. Phloem, responsible for distribution of nutrients, such as the photosynthetic product sucrose, and for the long distance transport of signaling molecules important to normal growth and development and responses to biotic or abiotic stresses, is composed of conducting sieve elements (SEs) and their associated companion cells (CCs), and nonconducting phloem parenchyma and fiber cells (Mauseth, 1988).

Apical meristems (shoot and root apical meristems) are generated during embryogenesis. Since they are not activated until the seedling geminates, apical meristems do not contribute to the formation of the embryo. Recent genetic and molecular analyses have shown that complex networks, including the CLV-WUS pathway, a feedback signaling loop between stem cells and the organizing center, control the balance between stem cell renewal and differentiation, which allows the plant to maintain the organization of the shoot and root meristems (for review, see Doerner, 2001; 2003; Byrne et al., 2003; Gross-Hardt and Laux, 2003; Birnbaum and Benfey, 2004). Relative to the apical meristems, little is known about the regulatory networks in the vascular cambium (Schrader et al., 2004).

The HD-ZIP/KANADI genetic system for regulating central versus peripheral cell fate

Recent investigations have demonstrated that two families of genes, class III HD-ZIP and KANADI, control central/adaxial (xylem) and peripheral/abaxial (phloem) identity in stem, root, and leaf (Kerstetter et al., 2001; McConnell et al., 2001; Emery et al., 2003; Hawker and Bowman, 2004). The class III HD-ZIP and KANADI genes, encoding homeodomain leucine zipper-containing proteins and GARP transcriptional regulators, respectively, have antagonistic roles in vascular patterning. Gain-of-function mutations of the class III HD-ZIP family members resulted in adaxialization of lateral organs and a *rev phb phv* triple loss-of-function mutant exhibited abaxialized cotyledons (Emery et al., 2003). Misexpression of KANADI genes in developing lateral organs leads to complete abaxialization of those organs (Kerstetter et al., 2001; Emery et al., 2003).

Xylem and phloem differentiation

Once polarity of the cambium derivatives is established, the newly formed xylem mother cells undergo a highly ordered developmental process involving cell division, expansion, secondary cell wall synthesis/deposition, lignification, and programmed cell death, leading to progressive degradation of the organelles and the removal of the protoplast and parts of unlignified primary walls, thus producing mature TEs (for review, see Fukuda, 1996; Roberts and McCann, 2000; Kuriyama and Fukuda, 2002; Ye, 2002; Dahiya, 2003; Kozela and Regan, 2003; Nieminen et al., 2004). Compared with TEs, xylem fiber differentiation is slower but still involves expansion, extensive synthesis of the secondary walls, and in some cases cell death (Moreau et al., 2005). The division of a phloem mother cell produces a SE and a nucleated sister cell, the companion cell (CC). SEs undergo a developmental program leading to selective degradation of the nuclei and vacuoles, and the formation of sieve pores. Without a nucleus, SEs lack the capacity for autonomous gene expression and need CCs to synthesize most of the required RNA and proteins for primary metabolism (Sjolund, 1997). Unlike TEs, SEs and associated CCs lack characteristic patterned secondary cell walls to aid with cell-type identification.

Due to the easily visualized patterned cell walls in xylem, most of the research on vascular differentiation has focused on xylem, especially on secondary cell wall biosynthesis, with a relative few studies being reported for phloem differentiation (Bonke

et al., 2003). To date, only one mutant, *apl*, has been reported to exhibit widespread absence of a subset of phloem cell types. Roots of *apl* seedlings lack both sieve SEs and CCs (Bonke et al., 2003).

Xylem secondary cell wall biosynthesis

During xylem differentiation, secondary cell walls are deposited after plant cells stop expanding. Even though the composition of secondary cell walls varies widely among different species and different cell types, in general, they are composed of a complex mixture of lignin, cellulose/hemicellulose, proteins and other minor components (for review, see Showalter, 1993; Gibeaut and Carpita, 1994; Reiter, 2002). Early biochemical studies have aided our understanding of secondary cell wall composition, but our understanding of the enzymology and genetic mechanisms involved in plant secondary cell wall biosynthesis is highly fragmented. Indeed, prior to 1995 no enzyme involved in plant cell wall biosynthesis had been identified and characterized (for review, see Doblin et al., 2002).

By screening for a radial swelling phenotype (*rsw*), which mimics responses of wild type roots to cellulose synthesis inhibitors such as dichlorobenzonitrile (Baskin et al., 1992), and the subsequent map-based cloning, Arioli et al. (1998) reported that the Arabidopsis *RSW1* locus encodes a subunit of a putative cellulose synthase (Pear et al., 1996), providing the first functional link between cellulose biosynthesis and a genetic locus.

Turner et al. (1997; 2000) identified a series of *irregular xylem* (*irx*) mutants by examining hand-cut Arabidopsis stem sections stained with toluidine blue under the light microscope to screen for altered xylem cell morphology. From these efforts, the *IRX1*, *IRX3*, and *IRX5* genes were cloned and shown to encode distinct classes of catalytic subunits of cellulose synthase, the complex required for cellulose synthesis in secondary cell walls of developing xylem vessels (Taylor et al., 1999; 2000; 2003). The cellulose synthesis complex can be visualized in freeze-fracture studies as a “rosette” structure (Herth et al., 1985; Haigler and Brown, 1986). Not all *irx* mutants are affected in cellulose synthase subunit genes. The *irx2* mutant is caused by a point mutation in the

KORRIGAN (KOR) beta, 1-4 endoglucanase gene. KOR is not an integral part of the cellulose complex and may play a role in processing of the growing microfibrils or release of the cellulose synthase complex (Szyjanowicz et al., 2004).

Using a strategy similar to that of Turner et al. (1997; 2000), Zhong et al. (2002; 2004; 2005) and Burk et al. (2001; 2002) identified a series of *fra* mutants with reduction in fiber mechanical strength, and fiber length and secondary wall thickness. *FRA1* and *FRA2* encode a kinesin-like protein and a katanin-like protein, respectively. Katanin has a microtubulus-severing activity in animal cells and kinesin can function as a microtubule-binding motor protein, suggesting that cortical microtubules play important roles in regulating the orientation of cellulose microfibrils. *FRA3* and *FRA7* encode Type II inositol polyphosphate 5-phosphatases (5PTases) and a SAC domain phosphoinositide phosphatase, respectively, indicating that phosphoinositide metabolism may be involved in secondary wall synthesis.

Lignin, the second most abundant biopolymer in secondary cell walls, is mainly composed of three hydroxycinnamyl alcohol monomers: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin plays important roles in supporting plants and enabling transport of water and mineral solutes through the vascular tissue by waterproofing the cell wall. Lignin deposition takes place in all three layers of the secondary cell wall with the bulk of lignin in the S3 layer. Using in vitro assays and radiolabeling experiments, significant progress has been made in characterizing the monolignol biosynthetic pathway (for review, see Boerjan et al., 2003; Peter and Neale, 2004) and a few mutants with modified expression of monolignol biosynthesis genes, such as *fah-1* (Humphreys et al., 1999), *irx4* (Jones et al., 2001), and *ref8* (Franke et al., 2002), have been reported (for review, Boerjan et al., 2003).

Xylem and phloem function

Vascular tissues function as conduits for water, mineral nutrients, and a diverse range of compounds, including products of photosynthesis, proteins, RNAs, and pathogens. Research on xylem function has focused on xylem loading and unloading of ions and

characterization of xylem-specific transporters (for review, see Zimmermann and Sentenac, 1999; Very and Sentenac, 2002). Research on phloem function has focused on determining the site of phloem loading and unloading, long-distance transport, and the roles of plasmodesmata. Both apoplastic and symplastic pathways of loading and unloading of molecules have been described. For example, sugar enters the SE/CC complex through transporters located in the plasma membrane for apoplastic loading or via plasmodesmata for symplastic loading (for review, see Sjolund, 1997, Oparka and Turgeon, 1999). In addition to the roles as conduits, the vascular tissue system is an important site for biosynthesis of various compounds, such as L-ascorbic acids (Hancock et al., 2003) and glucosinolates (Wittstock and Halkier, 2002).

Genomics of vascular tissue development

Using forward genetics, significant progress has been made in understanding xylem and phloem differentiation and function. However, there is still much to be discovered about the genetic mechanisms required for vascularization. To provide a complement to forward genetic screens, several models, such as Zinnia mesophyll cell suspension cultures, and pine, poplar, and Arabidopsis wood-forming tissues have been used for reverse genetic studies.

Zinnia mesophyll cell suspension culture model system

The Zinnia *in vitro* xylem cell differentiation system is the most remarkable *in vitro* system developed since tissue culture has been used to study the effects of hormones on xylem and phloem differentiation (Fukuda and Komamine, 1980; Aloni et al., 1987; Fukuda, 1997). In this model, single photosynthetic mesophyll cells isolated from Zinnia leaves can be induced to transdifferentiate into TEs in the presence of auxin and cytokinin. The advantage of this system is the semi-synchronous transdifferentiation of a high percentage (up to 70%) to TEs, thus allowing establishment of the chronology of molecular and biochemical events during the commitment and differentiation to a specific cell fate (Roberts and McCann, 2000; Milioni et al., 2001). By using this system, Milioni et al. (2002) performed an extensive cDNA-amplified fragment length polymorphism (cDNA-AFLP) study to determine the range of genes involved in this

complex process. Demura et al. (2002) performed microarray analysis of gene expression, using > 8,000 Zinnia cDNA clones isolated from the equalized cDNA library of differentiating xylem cells. These projects provide fundamental information on the gene expression underlying TE formation and give new insight into the regulation mechanisms in primary and secondary xylem formation.

Pine and poplar systems

The advantage of pine and poplar systems is that the developing secondary xylem (wood) is highly organized with easily recognized and distinct boundaries between the different developmental stages (i.e., meristematic cells, early expansion, late expansion, secondary wall formation, and late cell maturation), and the physical size of the vascular meristem (cambium) is large enough to obtain samples from different positions to cover the developmental sequence by tangential cryo-sectioning (Uggla et al., 1996). Using these systems, the large-scale sequencing of the expressed sequences from cambium and staged secondary xylem of pine (Allona et al., 1998) and poplar (Sterky et al., 1998) has been performed. The transcriptional profiling using microarrays of nearly 3,000 poplar genes from different developmental stages of wood-forming tissues has identified genes encoding lignin and cellulose biosynthetic enzymes and other potential regulators of xylem differentiation (Hertzberg et al., 2001).

Compared with Arabidopsis, Zinnia, poplar, and pine all have significant deficiencies in research infrastructure. Currently, only with Arabidopsis is it possible to study the complex process of xylem and phloem differentiation and function from a genome-wide perspective and to combine the power of microarray data with a vast network of functional genomics resources.

Arabidopsis system

Because of its small size, short generation time, prodigious seed production, and small genome with little repeated DNA (Redei, 1975; Pruitt and Meyerowitz, 1986), Arabidopsis has become one of the most important genetics models. Examples of tools and databases developed in support of this model system are high-resolution physical and

genetic maps and related YAC, BAC, and lambda libraries, transposon and T-DNA tagging (Hehl, 1994; Azpiroz-Leehan and Feldmann, 1997), enhancer trap and gene trap constructs (Sundaresan et al., 1995), a rapid transformation protocol (Chang et al., 1994), the complete sequence of the genome (AGI, 2000), and genome-wide chip technology, e.g., from Affymetrix (http://www.affymetrix.com). Arabidopsis, like many annual plants, is capable of developing a true vascular cambium and small amounts of secondary xylem under normal conditions, and producing relatively larger quantities of secondary vascular tissue when grown at low-population density and under conditions that prevent senescence, i.e., removal of reproductive structures (Lev-Yadun, 1994; Dolan and Roberts, 1995; Busse and Evert, 1999). By exploiting the potential for secondary growth combined with manual dissection of vascular tissues, we succeeded in isolating biochemical quantities of xylem and phloem for analysis of tissue-specific protease activities, and produced the first Arabidopsis xylem and bark cDNA libraries (Zhao et al., 2000; Beers and Zhao, 2001). Our approach has not been fully exploited, however, and the secondary tissue dissection method offers further possibilities for analysis of xylem and phloem differentiation and function via genome-wide expression profiling for gene discovery linked to reverse genetics.

In this study, we isolated xylem, phloem-cambium, and nonvascular tissues from the Arabidopsis root-hypocotyl, performed a genome-wide comparative analysis of tissue-specific transcripts using the 24K Affymetrix Arabidopsis ATH1 Genome Array (24K GeneChip), and identified potential genes that are required for xylem and phloem differentiation or tissue-specific functions.

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Overexpression of *XND1* resulted in the absence of TEs as determined from the absence of both secondary cell wall deposition and TE death. Using 3 tissue-specific promoter-GUS lines as genetic backgrounds, we demonstrated that overexpression of *XND1* suppressed only TE-specific GUS expression but not phloem-specific GUS expression. Three T-DNA/transposon insertion lines, *xnd1-1*, *-2*, and *-3*, were identified. Under normal conditions, *xnd1* did not exhibit significantly different growth and development compared to wild type plants. However, preliminary data indicated that *xnd1* plants were ABA and cold hypersensitive. Yeast-two hybrid screening using the N-terminal portion of XND1 as bait identified a novel RING finger protein, At3g62970 that may function as the ubiquitin ligase (E3). These results suggested that XND1 functions as a negative regulator of xylem cell differentiation, and that the regulation mediated by XND1 may be integrated with the ubiquitin/26S proteasome pathway.

References

Allona I, Quinn M, Shoop E, Swope K, St Cyr S, Carlis J, Riedl J, Retzel E, Campbell MM, Sederoff R, Whetten RW. 1998. Analysis of xylem formation in pine by cDNA sequencing. Proc Natl Acad Sci U S A. 95: 9693-8.

Aloni R. 1987. Differentiation of vascular tissues. Annu Rev Plant Physiol. 38: 178-204.

Arioli T, Peng L, Betzner AS, Burn J, Wittke W, Herth W, Camilleri C, Hofte H, Plazinski J, Birch R, Cork A, Glover J, Redmond J, Williamson RE. 1998. Molecular analysis of cellulose biosynthesis in Arabidopsis. Science. 279:717-20.

Azpiroz-Leehan R, Feldmann KA. 1997. T-DNA insertion mutagenesis in Arabidopsis: going back and forth. Trends Genet. 13: 152-6.

Baskin TI, Betzner AS, Hoggart R, Cork A, Williamson RE. 1992. Root morphology mutant in Arabidopsis thaliana. Aust J Plant Physiol. 19, 427-37.

Beers EP and Zhao C. 2001. Arabidopsis as a model for investigating gene activity and function in vascular tissues. In Molecular breeding of woody plants (Morohoshi, N. and Komamine, A., eds). Elsevier Science B.V. 43-52.

Birnbaum K, Benfey PN. 2004. Network building: transcriptional circuits in the root. Curr Opin Plant Biol. 7:582-8.

Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. Annu Rev Plant Biol. 54: 519-46.

Bonke M, Thitamadee S, Mahonen AP, Hauser MT, Helariutta Y. 2003. APL regulates vascular tissue identity in Arabidopsis. Nature. 426: 181-6.

Burk DH, Liu B, Zhong R, Morrison WH, Ye ZH. 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. Plant Cell. 13: 807-27.

Burk DH, Ye ZH. 2002. Alteration of oriented deposition of cellulose microfibrils by mutation of a katanin-like microtubule-severing protein. Plant Cell. 14: 2145-60.

Busse JS, Evert RF: 1999. Vascular differentiation and transition in the seedling of *Arabidopsis thaliana* (Brassicaceae). Int J Plant Sci. 160:241-251.

Byrne ME, Kidner CA, Martienssen RA. 2003. Plant stem cells: divergent pathways and common themes in shoots and roots. Curr Opin Genet Dev. 13:551-7.

Chang SS, Park SK, Kim BC, Kang BJ, Kim DU, Nam HG. 1994. Stable genetic transformation of *Arabidopsis thaliana* by *Agrobacterium* inoculation *in planta*. Plant J. 5: 551-558

Dahiya P. 2003. Role of death in providing lifeline to plants. Trends Plant Sci. 8: 462-5.

Demura T, Tashiro G, Horiguchi G, Kishimoto N, Kubo M, Matsuoka N, Minami A, Nagata-Hiwatashi M, Nakamura K, Okamura Y, Sassa N, Suzuki S, Yazaki J, Kikuchi S, Fukuda H. Visualization by comprehensive microarray analysis of gene expression programs during transdifferentiation of mesophyll cells into xylem cells. Proc Natl Acad Sci U S A. 99: 15794-9.

Doblin MS, Kurek I, Jacob-Wilk D, Delmer DP. 2002. Cellulose biosynthesis in plants: from genes to rosettes. Plant Cell Physiol. 43:1407-20.

Doerner P. 2001. Plant meristems: a menage a trois to end it all. Curr Biol. 11:R785-7.

Doerner P. 2003. Plant meristems: a merry-go-round of signals. Curr Biol. 13:R368-74.

Dolan L, Roberts K.1995. Secondary thickening in roots of *Arabidopsis thaliana*: anatomy and cell surface changes. New Phytol. 131**:** 121–128.

Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL. 2003. Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. Curr Biol. 13: 1768-74.

Franke R, Hemm MR, Denault JW, Ruegger MO, Humphreys JM, Chapple C. 2002. Changes in secondary metabolism and deposition of an unusual lignin in the *ref8* mutant of *Arabidopsis*. *Plant J.* 30:47–59

Fukuda H. 1996. XYLOGENESIS: INITIATION, PROGRESSION, AND CELL DEATH. Annu Rev Plant Physiol Plant Mol Biol. 47: 299-325.

Fukuda H. 1997. Tracheary Element Differentiation. Plant Cell. 9: 1147-1156.

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

Gibeaut DM, Carpita NC. 1994. Biosynthesis of plant cell wall polysaccharides. FASEB J. 8:904-15.

Gross-Hardt R, Laux T. 2003. Stem cell regulation in the shoot meristem. J Cell Sci.116(Pt 9):1659-66.

Hancock RD, McRae D, Haupt S, Viola R. 2003. Synthesis of L-ascorbic acid in the phloem. BMC Plant Biol. 3: 7.
Hawker NP, Bowman JL. 2004. Roles for Class III HD-Zip and KANADI genes in Arabidopsis root development. Plant Physiol. 135: 2261-70.
Hehl R. 1994. Transposon tagging in heterologous host plants. Trends Genet. 10: 385-6.
Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G. 2001. A transcriptional roadmap to wood formation. Proc Natl Acad Sci U S A. 98: 14732-7.
Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS. 2001. KANADI regulates organ polarity in Arabidopsis. Nature. 411: 706-9.
Kozela C, Regan S. 2003. How plants make tubes. Trends Plant Sci. 8: 159-64.
Kuriyama H, Fukuda H. 2002. Developmental programmed cell death in plants. Curr Opin Plant Biol. 5: 568-73.
Humphreys JM, Hemm MR, Chapple C. 1999. New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5-hydroxylase, a multifunctional cytochrome P450-dependent monooxygenase. Proc. Natl. Acad. Sci. USA 96:10045–50.
Jones L, Ennos AR, Turner SR. 2001. Cloning and characterization of *irregular xylem4* (*irx4*): a severely lignin-deficient mutant of *Arabidopsis*. Plant J. 26:205–16.
Lev-Yadun S. 1994. Induction of sclereid differentiation in the pith of *Arabidopsis thaliana* (L.) Heynh. J Exp Bot. 45: 1845-1849.
Mauseth JD. 1988. Plant Anatomy. Menlo Park, CA: The Benjamin/Cummings Publish Co.
McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. 2001. Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. Nature. 411: 709-13.
Milioni D, Sado PE, Stacey NJ, Domingo C, Roberts K, McCann MC. 2001. Differential expression of cell-wall-related genes during the formation of tracheary elements in the Zinnia mesophyll cell system. Plant Mol Biol. 2001 Sep;47: 221-38.
Milioni D, Sado PE, Stacey NJ, Roberts K, McCann MC. 2002. Early gene expression associated with the commitment and differentiation of a plant tracheary element is

revealed by cDNA-amplified fragment length polymorphism analysis. Plant Cell. 14: 2813-24.
Moreau C, Aksenov N, Lorenzo MG, Segerman B, Funk C, Nilsson P, Jansson S, Tuominen H. 2005. A genomic approach to investigate developmental cell death in woody tissues of Populus trees. Genome Biol. 2005;6: R34.
Nieminen KM, Kauppinen L, Helariutta Y. 2004. A weed for wood? Arabidopsis as a genetic model for xylem development. Plant Physiol. 135: 653-9.
Oparka KJ, Turgeon R. 1999. Sieve elements and companion cells-traffic control centers of the phloem. Plant Cell. 11: 739-50.
Pear JR, Kawagoe Y, Schreckengost WE, Delmer DP, Stalker DM. 1996. Higher plants contain homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. Proc Natl Acad Sci U S A. 93:12637-42.
Peter G, Neale D. 2004. Molecular basis for the evolution of xylem lignification. Curr Opin Plant Biol. 7: 737-42.
Pruitt RE, Meyerowitz EM. 1986. Characterization of the genome of Arabidopsis thaliana. J Mol Biol. 187: 169-83.
Redei GP. 1975. Arabidopsis as a genetic tool. Annu Rev Genet. 9: 111-27.
Reiter WD. 2002. Biosynthesis and properties of the plant cell wall. Curr Opin Plant Biol. 5:536-42.
Roberts K, McCann MC. 2000. Xylogenesis: the birth of a corpse. Curr Opin Plant Biol. 3: 517-22.
Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G. 2004. A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. Plant Cell.16:2278-92.
Showalter AM. 1993. Structure and function of plant cell wall proteins. Plant Cell. 5:9-23.
Sjolund RD. 1997. The Phloem Sieve Element: A River Runs through It. Plant Cell. 9: 1137-1146.
Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, Holmberg A, Amini B, Bhalerao R, Larsson M, Villarroel R, Van Montagu M, Sandberg G, Olsson O, Teeri TT, Boerjan W, Gustafsson P, Uhlen M, Sundberg B, Lundeberg J. 1998. Gene discovery in the

wood-forming tissues of poplar: analysis of 5, 692 expressed sequence tags. Proc Natl Acad Sci U S A. 95: 13330-5.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JD, Dean C, Ma H, Martienssen R. 1995. Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. 9: 1797-810.

Szyjanowicz PM, McKinnon I, Taylor NG, Gardiner J, Jarvis MC, Turner SR. 2004. The irregular xylem 2 mutant is an allele of korrigan that affects the secondary cell wall of Arabidopsis thaliana. Plant J. 37:730-40.

Taylor NG, Howells RM, Huttly AK, Vickers K, Turner SR. 2003. Interactions among three distinct CesA proteins essential for cellulose synthesis. Proc Natl Acad Sci U S A. 100:1450-5.

Taylor NG, Laurie S, Turner SR. 2000. Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in Arabidopsis. Plant Cell. 12:2529-2540.

Taylor NG, Scheible WR, Cutler S, Somerville CR, Turner SR. 1999. The irregular xylem3 locus of Arabidopsis encodes a cellulose synthase required for secondary cell wall synthesis. Plant Cell. 11:769-80.

Turner SR, Hall M. 2000. The gapped xylem mutant identifies a common regulatory step in secondary cell wall deposition. Plant J. 24:477-88.

Turner SR, Somerville CR. 1997. Collapsed xylem phenotype of Arabidopsis identifies mutants deficient in cellulose deposition in the secondary cell wall. Plant Cell. 9:689-701.

Uggla C, Moritz T, Sandberg G, Sundberg B. 1996. Auxin as a positional signal in pattern formation in plants. Proc Natl Acad Sci U S A. 93: 9282-6.

Very AA, Sentenac H. 2002. Cation channels in the Arabidopsis plasma membrane. Trends Plant Sci. 7: 168-75.

Wittstock U, Halkier BA. 2002. Glucosinolate research in the Arabidopsis era. Trends Plant Sci. 7: 263-70.

Ye ZH. 2002. Vascular tissue differentiation and pattern formation in plants. Annu Rev Plant Biol. 53: 183-202.

Zhao C, Johnson BJ, Kositsup B, Beers EP. 2000. Exploiting secondary growth in Arabidopsis. Construction of xylem and bark cDNA libraries and cloning of three xylem endopeptidases. Plant Physiol. 123: 1185-96.

Zhong R, Burk DH, Morrison WH 3rd, Ye ZH. 2002. A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. Plant Cell. 14: 3101-17.

Zhong R, Burk DH, Morrison WH 3rd, Ye ZH. 2004. FRAGILE FIBER3, an Arabidopsis gene encoding a type II inositol polyphosphate 5-phosphatase, is required for secondary wall synthesis and actin organization in fiber cells. Plant Cell. 16: 3242-59.

Zhong R, Burk DH, Nairn CJ, Wood-Jones A, Morrison WH 3rd, Ye ZH. 2005. Mutation of SAC1, an Arabidopsis SAC Domain Phosphoinositide Phosphatase, Causes Alterations in Cell Morphogenesis, Cell Wall Synthesis, and Actin Organization. Plant Cell. 17: 1449-66.

Zimmermann S, Sentenac H. 1999. Plant ion channels: from molecular structures to physiological functions. Curr Opin Plant Biol. 2: 477-82.

Chapter 2

The xylem and phloem transcriptomes from secondary tissues of the Arabidopsis root-hypocotyl

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