REVIEW / SYNTHÈSE

The cell biology of wood formation: from cambial divisions to mature secondary xylem[1]

A.L. Samuels, M. Kaneda, and K.H. Rensing

Abstract: The development of secondary xylem has been studied historically from an anatomical point of view, but recent developments in cell and molecular biology have revitalized this field. An integrated view of cell structure with physiology is emerging for each stage of the developing xylem cells' lives, from birth in the cambium to programmed cell death. High-quality structural information is essential in building this type of integrated view, but conventional electron microscopy of developing wood cells has been problematic. The importance of adequately preserving cells of the secondary vascular system is illustrated with examples from dormant cambium, cell division, and secondary cell wall deposition. In many cases, contemporary gene expression studies can be viewed in the context of both new structural information and pioneering live cell studies done in the early 1900s to increase our understanding of secondary xylem development.

Key words: xylogenesis, secondary xylem, cell wall, cytokinesis, cambial zone, vascular cambium.

Résumé : Le développement du xylème secondaire a été historiquement étudié du point de vue anatomique, mais les récents développements de la biologie cellulaire et moléculaire suscitent un intérêt nouveau pour ce sujet. Il se dessine une vision holistique des structures cellulaires, intégrant la physiologie, pour chaque stade de la vie des cellules de xylème en développement, de la naissance dans le cambium à la mort cellulaire programmée. Une information structurale de qualité est essentielle pour établir ce type de vision intégrée, mais la microscopie électronique conventionnelle a posé un problème. On illustre l'importance de bien conserver les cellules du système vasculaire secondaire, avec des exemples provenant de cambium dormant, de divisons cellulaires et de déposition de la paroi secondaire. Dans plusieurs cas, des études contemporaines de l'expression génétique peuvent être analysées dans le contexte à la fois de nouvelle information structurale et d'études pionnières sur les cellules vivantes, effectuées au début des années 1900, pour étendre notre compréhension du développement du xylème secondaire.

Mots clés : xylogenèse, xylème secondaire, paroi cellulaire, cytocinèse, zone cambiale, cambium vasculaire.

[Traduit par la Rédaction]

Introduction

The production of secondary xylem (wood) requires both mitosis in the vascular cambium and xylogenesis, a developmental process whereby thin-walled cambial cells mature into functional water-conducting tubes with elaborate lignified secondary cell walls. In recent years, advances in cell biology have produced insights into how the cambial cells divide and overwinter, produce secondary cell walls, and mature following programmed cell death. Xylogenesis can be defined as the series of common developmental steps found in differentiation of both primary xylem (produced via procambium by apical meristems) and secondary xylem (produced by the vascular cambium) (Fig. 1). These common events of xylogenesis have been illustrated in the in vitro transdifferentiation of *Zinnia elegans* tracheary elements (Fukuda 1996; also outlined here in Fig. 1A). They include cell expansion, secondary cell wall synthesis, programmed cell death, and lignification of wall thickenings. In secondary xylem, variations in these basic developmental steps produce the tracheids of gymnosperms and vessels or fibres of angiosperms.

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The vascular cambium

The cambium has been called the "least understood plant meristem" (Groover 2005), because of the technical difficulties associated with working with trees. Historical accounts of the development of our thinking about the cambium were provided by Larson (1994). He traced the concept of the cambium as a vital sap back to the 14th century, but credited the idea that the growing zone of wood was located in the inner bark to Malpighi in the 1670s. Following Schwan's and Schleiden's development of cell theory in 1839, the cambium was viewed as the cell layer between the "bast" (phloem) and wood. This paved the way for Sanio who, in

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Fig. 1. Common elements of xylogenesis in primary (top) and secondary (boxed, below) xylem. (Top) Xylogenesis in the *Zinnia elegans* in vitro tracheary element model system, where mesophyll cells are cultured under conditions that induce them to differentiate into tracheary elements. The steps of differentiation are (1) expansion, (2) secondary cell wall polysaccharide deposition, followed by (3) lignification and programmed cell death (PCD). (Bottom) Similar steps in secondary xylem, shown here for angiosperm vessels and fibres, arising from the vascular cambium. For vessels of angiosperms, and to a lesser extent for gymnosperm tracheids, expansion is strongly in the radial direction, followed by secondary cell wall production, lignification, and protoplast lysis. For angiosperm fibres, expansion is axial as the cells elongate by intrusive growth, followed by secondary cell wall synthesis, lignification, and protoplast lysis.

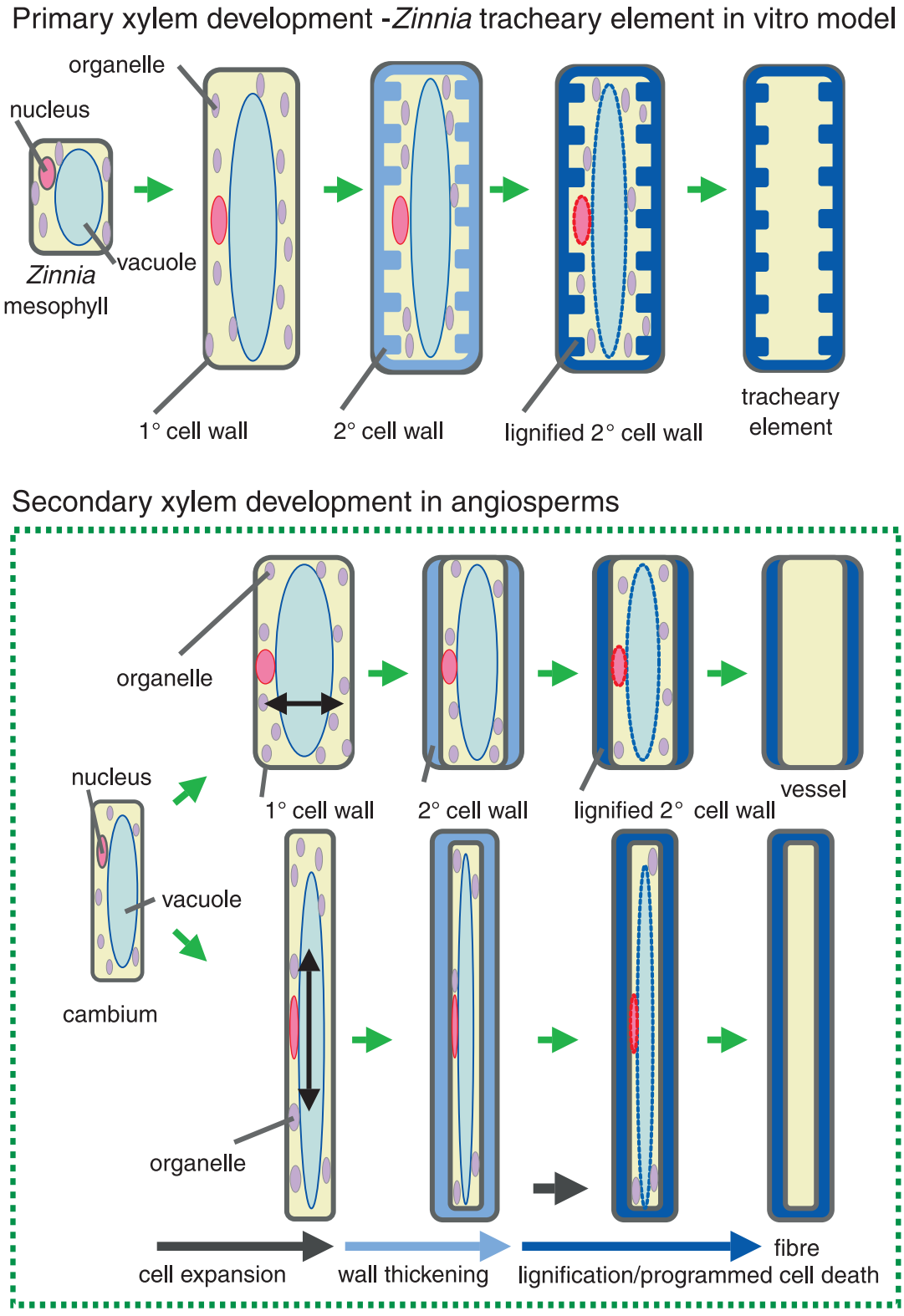
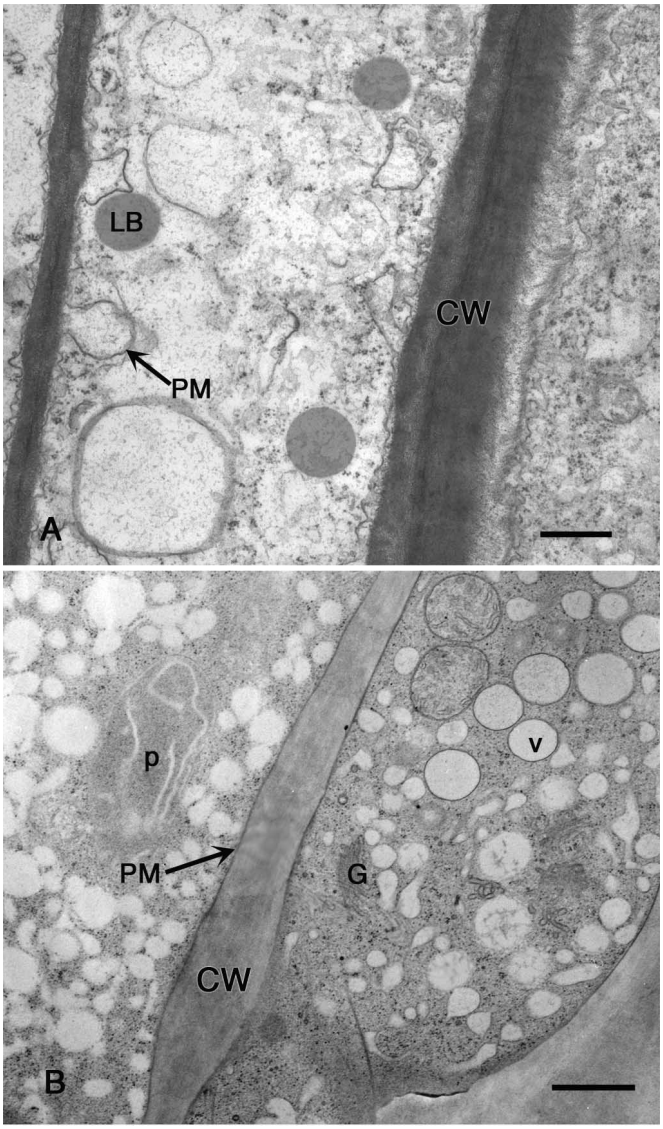


Fig. 2. Chemical versus cryo-fixation. (A) Chemically fixed dormant cambial cells with vacuoles and lipid bodies (LB). Typical infoldings of the plasma membrane (PM) are artifacts of this type of preparation. CW, cell wall. (B) High-pressure freezing (HPF) or freeze substitution preserves the plasma membrane association with the cell wall and retains the cytoplasm and organelles such as plastids (p), Golgi (G), and vacuoles (v). A large number of small vacuoles that are not seen following chemical fixation are present in HPF samples. Scale bars = 1 μm.



the 1850s, developed key concepts such as the cambial initial theory, whereby the cambial initial cell gives rise to two daughter cells, one of which retains the cambial initial identity, while the other undergoes a committed fate as a xylem or phloem mother cell, dividing several more times and undergoing terminal differentiation. This strict view of a single "cambium" layer led to some controversy over whether the cambium is uniseriate or multiseriate (Larson 1994). The concept of a single cambial "initial" was derived from retrospective analysis of cambial anatomy during times of little or no activity. Because a complete primary cell wall is laid down following each division, the tangential wall opposite the new cell wall becomes thicker than the newly produced wall thereby outlining the history of each cell of a radial file. These grouped, recently divided, cells enclosed in a thicker surrounding wall, called "Sanio's four" can often be identified. The most recently divided cell with the thinnest wall would be defined as the fusiform initial with the presumption that only this cell would continue to produce new xylem and phloem mother cells. Murmanis (1977) found that cambium in *Quercus rubra* branches fit this pattern, but the derived cells did not, because they became distorted outside this cell group. It must be noted that there are no subcellular differences in the protoplast that would identify an initial from the other actively dividing cells of the cambial zone (Evert and Deshpande 1970). The controversy of whether single or multiple cells retain meristematic competence can be avoided by using the terminology "cambial zone" to include the full region of active mitosis. The cambial zone persists because there is a dynamic balance between cells capable of acting as initials and daughter cells that commit to differentiation into secondary phloem and xylem.

A functional definition of the cambial zone as the region where anticlinal divisions add new cell files to both the xylem and phloem has been used in a recent study in aspen (*Populus tremula*), where gene expression data using microarrays was gathered from 20 μm thick sections, representing about three cell layers of cambial zone (Schrader et al. 2004). In the region adjacent to the phloem where anticlinal divisions were most often observed, the set of genes with peak expression were distinctly different than the adjacent xylem mother cell region that was rich in cell cycle genes. This study supports the view that the cambial zone can be subdivided into a region of meristematic identity and two regions of xylem and phloem mother cells committed to differentiation.

The cambium also supports the production of transverse elements such as parenchyma and radial tracheary elements. These cells are produced in files by ray initials within the cambial zone and produce generally shorter, more isodiametric radial elements. In addition to radial conduction of water and nutrients, the rays play a supportive role in secondary xylem development.

With the sequencing of the poplar genome (http://www.ornl.gov/sci/ipgc/) and the recognition that all the genes required for environmental induction of secondary growth are present in herbaceous plants like *Arabidopsis thaliana* (Zhao et al. 2005), molecular genetic investigations into how the meristematic identity is maintained and the correct number and geometry of derivatives produced have become possible (Groover 2005). These studies will rely on loss- or gain-of-function experiments to test the roles that candidate genes play in cambium structure and function. To make valid observations of the cambial zone structure at the subcellular level, for example, localization of gene products at the transmission electron microscopic (TEM) level, advanced microscopy tools, such as cryo-fixation, will be required because of the sensitive nature of these long, thin, highly vacuolated active cells (Rensing 2002).

An example of how sensitive the secondary tissues are to conventional electron microscopy techniques can be seen in cambial cells during seasonal dormancy (Fig. 2). Pro-

Fig. 3. (A–F) From Bailey (1919) drawings of live *Pinus strobus* dividing cambial cells. (A) start of cytokinesis in radial view; (B) later stage of cytokinesis with extending cell plate and opposing phragmoplasts in radial view; (C) similar stage of cytokinesis in tangential view; (D) enlarged diagram of telophase and initiation of the phragmoplast; (E) beginning of expansion of the cell plate as the nuclei reform; (F) enlarged view of the expanding cell plate and phragmoplasts. (G–J) High-pressure frozen dividing cambial cells of *Pinus contorta*; Figs. 3G, 3H, and 3I are equivalent to Figs. 3D, 3E, and 3F, respectivel. (G) transmission electron micrographs (TEM) showing the initiation of the phragmoplast between the recently separated chromosomes; (H) light micrograph (LM) showing the formation of the cell plate between the reforming nuclei; (I) light micrograph showing the expansion of the cell plate by advancement of the phragmoplast through the central vacuole; (J) transmission electron micrograph showing the greatly extended cell plate and, within the phragmoplast, the delicate newly formed cell plate. Scale bars = 1 μm for Figs. 3G and 3J; Scale bars = 10 μm for Figs. 3H and 3I.

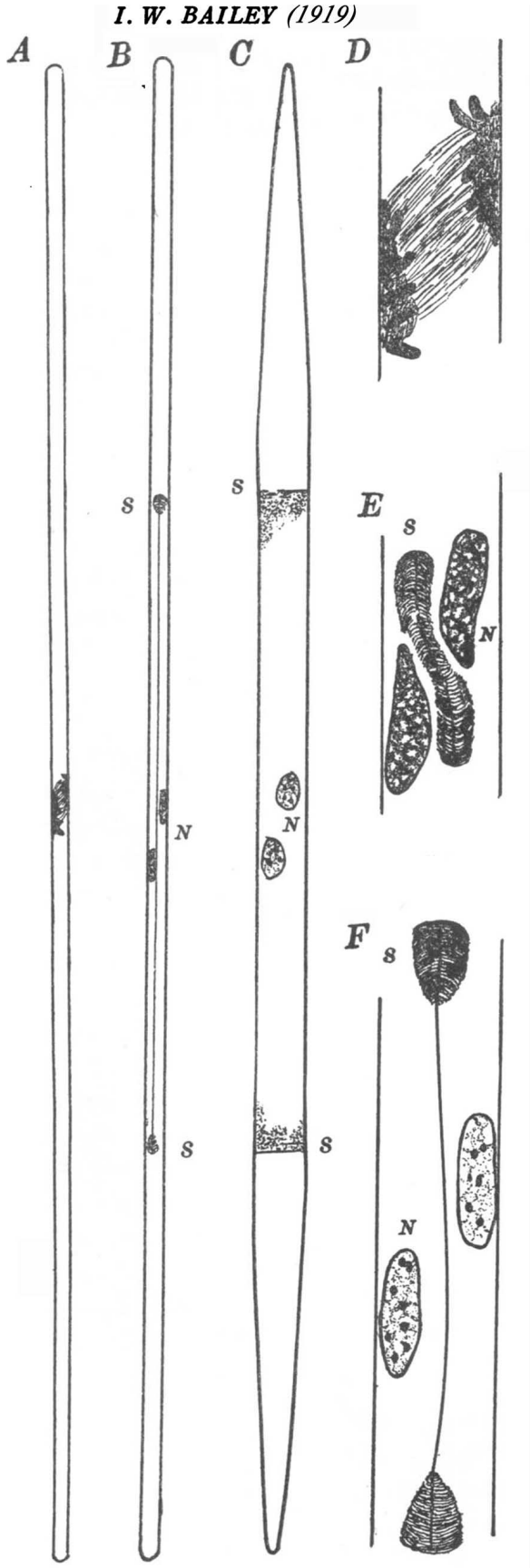
nounced seasonal changes in cytoplasmic organization occur in cambial cells; dormant cambial cells are filled with many small vacuoles surrounded by organelles within a dense cytoplasm. These abundant, small vacuoles were observed in live cells, and at least two heterogenous populations of vacuoles were identified on the basis of staining with neutral red (Bailey and Zirkle 1931). Following chemical fixation for electron microscopy, vacuole morphology was different than in live cells as fewer, larger vacuoles were observed (Rao and Catesson 1987). In addition, the plasma membranes of these cells were highly convoluted and "plasmalemma invaginations (PLI)" were frequently observed in cambial cells during autumn rest in angiosperms (*Aesculus hippocastanum*) (Rao and Catesson 1987), gymnosperms (*Pseudotsuga menziesii*) (Rensing and Owens 1994), and *Pinus contorta* (Fig. 2A). Such membrane in-foldings were interpreted to be the result of active membrane trafficking of vesicles from the cell membrane (endocytosis) following cessation of growth, or areas of active sucrose uptake by endocytosis, presumably for carbon sequestration (Rao and Catesson 1987). Such studies relied upon conventional sample preparation methods for TEM using chemical fixatives (typical results of chemical fixation are illustrated in Fig. 2A). However, when dormant cambium of *Pinus contorta* was rapidly frozen using high-pressure freezing and the cells were preserved with freeze substitution, the dormant cells showed dramatically different cell structure (Fig. 2B; Rensing and Samuels 2004). The plasma membrane was smooth and tightly pressed to the cell wall, unlike the chemically fixed material. The lack of membrane infoldings in all cryofixed samples suggests that endocytotic uptake of sucrose is unlikely to be a mechanism of sucrose uptake in these cells. In addition, the cryofixed cells had cytoplasm filled with many, smaller vacuoles, as reported in live cell microscopy studies by Bailey et al. in the 1930s and confirmed using confocal microscopy on live cells. Differences between vacuoles of cells in the quiescent and rest stages of dormancy were also found (Rensing and Samuels 2004).

With resumption of cell-division activity, the many small vacuoles filling the cambial cells coalesce, so in active cambial cells, the cytoplasm occupies a narrow peripheral layer surrounding a single large vacuole (Bannan 1955; Rensing et al. 2002). Following the break from dormancy, the cambial cells have very active cytoplasmic streaming (Thimann and Kaufman 1958). Immunofluorescence and transmission electron microscopy observations of prominent microfilament bundles with associated organelles are consistent with an actin–myosin system supporting this streaming (Chaffey et al. 1997; Samuels et al. 2002; see Figs. 4B, 4C).

Mitotic activity in the cambium was beautifully described in the observations of live cells and drawings of Bailey (1919; reprinted in Fig. 3), including the oblique position of the mitotic spindle and the two opposing phragmoplasts, which stretch the newly formed cell wall down the central axis of the long, thin cambial cell (Figs. 3A–3F). The nuclei of cambial cells generally maintain a peripheral position until just prior to mitosis when a "phragmosome", a central bridge of cytoplasm, suspends the nucleus across the narrow radial width of the cell (typical width is about 6 μm). Immediately following separation of chromosomes, Golgi-derived vesicles begin to accumulate at the midzone of the spindle equator, and vesicle fusion begins to form the new cell plate (Evert and Deshpande 1970; Rensing et al. 2002). When dividing cambial cells of *Pinus contorta* were cryo-fixed and examined with transmission electron microscopy, the early fusion events of Golgi vesicles forming the nascent cell plate could be accurately observed, and the cell plate was revealed as a delicate network of membranes encased in phragmoplast microtubules (Rensing et al. 2002; Figs. 3E, 3H, 3J). The basic stages of cell plate formation were equivalent to those observed in divisions in primary meristems (Samuels et al. 1995), but the cell plate was greatly elongated along the axial length of the fusiform cambial cells (Figs. 3A, 3B, 3I, 3J). The delicate tubulo-vesicular networks at the phragmoplast periphery were encased by the two migrating balls of cytoplasm that moved along the length of the cell (Figs. 3B, 3F, 3I, 3J), followed by the callose-filled tubular network and completed daughter cell wall in the central zone of the cell where the daughter nuclei were located (Figs. 3F, 3I). Immunofluorescence results from cytokinesis studies in pine, hybrid poplar, and *Aesculus hippocastanum* also showed callose in the cell plate behind the advancing segment of cell plate encased in the phragmoplast (Chaffey and Barlow 2002).

Xylogenesis

Following cell division, cambial cell derivatives undergo a period of cellular expansion. Variation in differentiated xylem cells begins immediately at this early stage of development. Angiosperm vessels and gymnosperm tracheids, the cells that will conduct water, undergo radial expansion, while supportive fibres of angiosperms undergo intrusive elongation (reviewed by Mellerowicz et al. 2001). Expansins, proteins which are reported to aid in wall loosening (Im et al. 2000), are co-expressed with cell-cycle genes in xylem mother cells, indicating that completion of mitosis and cell expansion are closely linked (Schrader et al. 2004). While several studies have reported that expansin genes are



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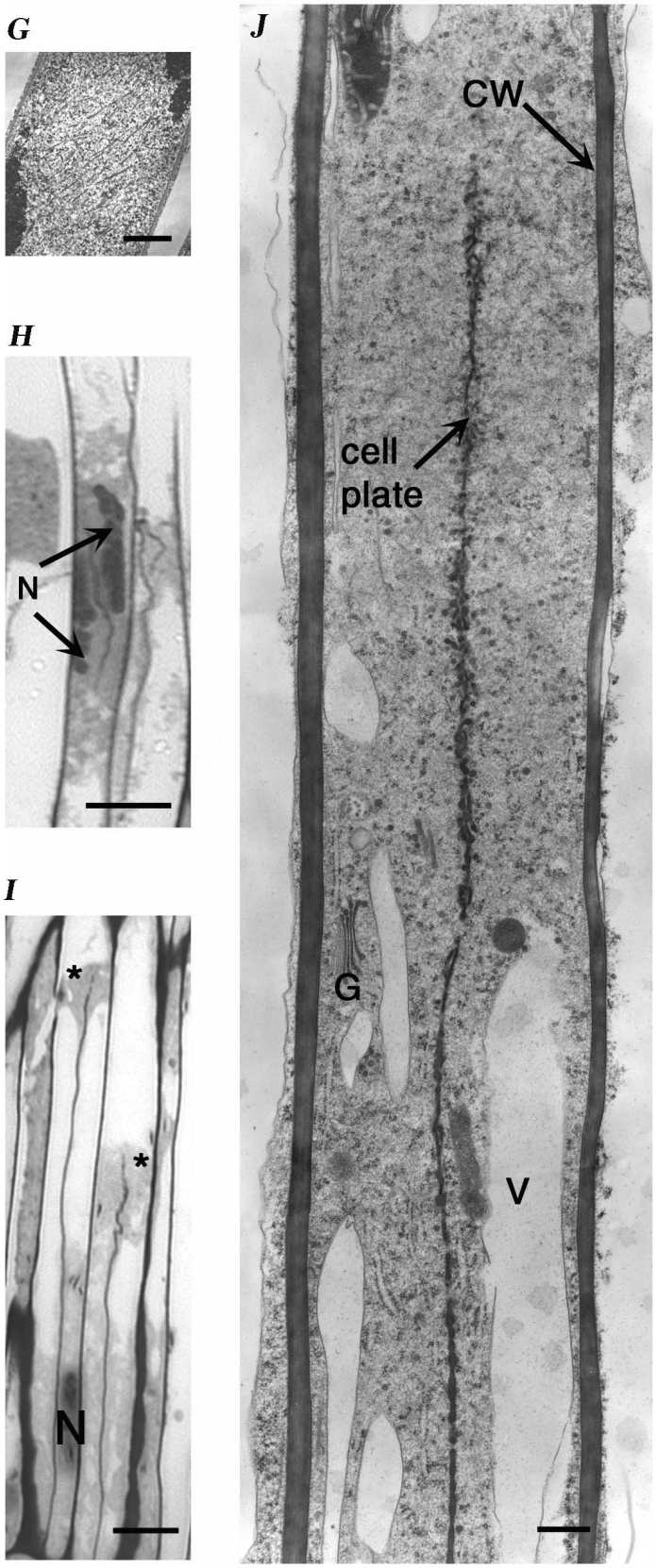
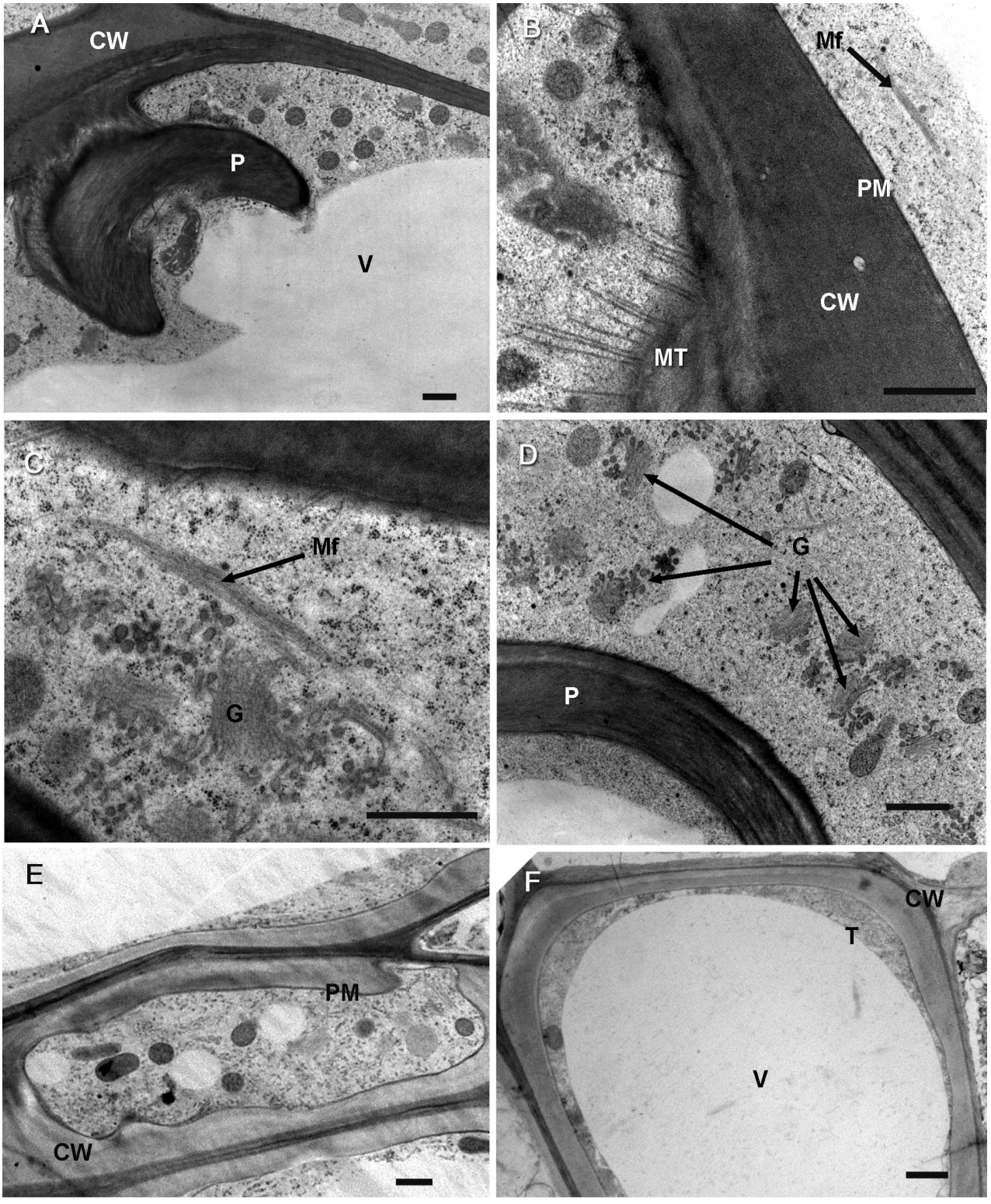


PINUS CONTORTA

Fig. 4. Cryo-fixed (high-pressure frozen or freeze substituted) transmission electron micrographs showing developing secondary xylem cells from *Pinus* (A–D) and *Populus* (E, F) showing intact, smooth membranes and internal cell structure during deposition of secondary cell wall (CW). (A) Tracheid with developing secondary cell wall including pit (P), typical peripheral cytoplasm and large central vacuole (V) during active growth season. (B, C) The cytoskeleton of developing tracheids is dominated by cortical microtubules lining the plasma membrane (PM) and actin microfilament (Mf)bundles. (D) Abundant Golgi (G) in developing xylem adjacent to developing pit. (E) Poplar fibre during secondary cell wall deposition with smooth plasma membrane. (F) Poplar vessel during secondary cell wall development showing peripheral cytoplasm, large central vacuole with tonoplast (T) and smooth turgid plasma membrane. Scale bars = 1 μm.



present during xylogenesis (Zhao et al. 2005), a recent study not only identified which hybrid poplar expansin genes family members are present in the expanding cambial derivatives (α-expansin subfamily A), but also demonstrated by elegant in situ reverse transcription polymerase chain reaction (RT-PCR) that the *PttEXP1* mRNA is found in the tips of fusiform initials as well as in intrusively growing fibres (Gray-Mitsumune et al. 2004).

The first physical manifestation of a cambial cell's commitment to differentiation is remodeling of the cortical microtubules, which have been observed with indirect immunofluorescence on dissected, sectioned cambium and developing xylem. Microtubules are initially in random orientations in the cambium (Chaffey 2000; Chaffey et al. 2002; Abe and Funada 2005). When the cortical microtubules are still randomly arranged during expansion, multiple microtubule-free circular regions form in the cortical cytoplasm in the locations where either bordered pits of tracheids in conifers (Funada et al. 2000) or intervessel bordered pits in hybrid aspen will be located (Chaffey et al. 2002). As the radial expansion phase nears completion, the cortical microtubules assume a helical ordered array, wrapping transversely around the late radially expanding cells in a pattern that strongly correlates with the microfibril deposition of first secondary cell wall layer (Funada et al. 2000; Chaffey et al. 2002). It is interesting to note that in conifer tracheids, the radial direction of cell expansion is parallel, rather than perpendicular, to the transverse cortical microtubule array. So in late radial expansion the presence of transverse microtubules and corresponding transverse cellulose microfibrils per se are not enough to constrain growth in the radial dimension. In contrast, in developing fibres of *Aesculus hippocastanum*, the cortical microtubules are transverse to the long axis of the cells during their intrusive axial growth phase (Chaffey et al. 1997).

At the transition between primary cell wall and secondary cell wall production, the cells' biosynthetic machinery must undergo a dramatic shift in secretion from supporting active expansion of the pectin-rich primary cell wall to supporting assembly of a secondary cell wall consisting predominantly of the ordered cellulose and hemicellulose layers that will eventually be lignified. During this transition in hybrid poplar, xyloglucan endotransglycosylase (XET) activity has been demonstrated and candidate XETs have been characterized (Bourquin et al. 2002). XETs, now called xyloglucan endotransglycosylase hydrolases (XTH), can cut and rejoin xyloglucan chains, a process believed to be important in control of cell wall expansion (Vissenberg et al. 2005). In the hybrid poplar, the XTH was suggested to act in restructuring the border between the primary and secondary cell walls (Bourquin et al. 2002).

At the end of the cell-expansion phase, the protoplast begins to produce the thickened secondary cell wall, a three-layered structure (S_1, S_2, S_3) made of cellulose microfibrils (Wardrop and Harada 1965; Donaldson 2001). The microfibril orientations of secondary cell wall layers during development have been examined using field emission scanning electron microscopy (FESEM), which confirmed earlier studies using light microscopy with birefringence and transmission electron microscopy (reviewed by Abe and Funada 2005). During secondary wall synthesis of bordered pits, each pit is encircled by a ring-shaped pattern of cortical microtubules that can be observed with immunofluorescence (Chaffey 2000; Abe and Funada 2005). With transmission electron microscopy, the growing bordered pits of pine and poplar can be observed to be filled with cytoplasm and rich in organelles and cytoskeletal elements (Figs. 4A, 4D, 4E).

The hemicelluloses of the secondary cell wall are produced and secreted by the Golgi, as illustrated by developing *Pinus contorta*, where Golgi structure changed dramatically during the transition from primary to secondary cell wall when production of the hemicellulose, galactoglucomannan was high (Samuels et al. 2002). In developing xylem cells with high secretory activity, the cellular membranes seem particularly susceptible to chemical fixation artifacts such as invaginations of membranes into the vacuole. As with dormant pine cambium, developing xylem cells from pine and poplar prepared for transmission electron microscopy by rapid freezing show smooth turgid plasma membranes, entirely without infoldings or vesiculation (Inomata et al. 1992; Samuels et al. 2002; Figs. 4A–4D). In developing secondary xylem of poplar, both vessels and fibres prepared by cryo-fixation have intact organelles and smooth tonoplasts without infoldings of plasma membrane (Figs. 4E, 4F). In contrast, when poplar developing secondary xylem, particularly fibres, was prepared by chemical fixation and TEM, extensive infoldings of plasma membrane intruding into the vacuoles were seen (Arend and Fromm 2003).

The cellulose synthases responsible for secondary cell wall deposition in hybrid poplar (Joshi et al. 2004) and pine (Nairn and Haselkorn 2005) have been identified. As in Arabidopsis, a generally accepted model of cellulose synthesis is that the functional cellulose synthase complex (called a rosette or terminal complex) consists of β-glucosyltransferase (cellulose synthase) subunits encoded by *CESA* genes (Doblin et al. 2002). In a variety of taxa, recent studies support the view that cellulose synthesis enzymes for secondary cell wall formation are different from the cellulose synthases used in primary cell wall formation. In Arabidopsis, three *CesA* genes (*AtCesA1*, *AtCesA3*, and *AtCesA6*) are required for primary cell wall deposition, while other CesA genes (*AtCesA4*, *AtCesA7*, and *AtCesA8*) must work together to produce controlled secondary cell wall. Similarly, in situ mRNA hybridization results indicated that three poplar *CesA* genes (*PtrCes1*, *PtrCes2*, and *PtrCes3*) were expressed in developing xylem and phloem fibres undergoing secondary cell wall formation (Joshi et al. 2004). In loblolly pine, semiquantitative RT-PCR was used to examine developing wood, and these data suggest three *CesA* genes (*PtCesA1*, *PtCesA2*, and *PtCesA3*) are highly expressed in wood but not in the needle and lateral shoot (Nairn and Haselkorn 2005). A multiple alignment of full-length CesA protein sequences showed that secondary CesAs in pine are homologues of Arabidopsis and poplar secondary wall CesAs (Nairn and Haselkorn 2005). Primary cell wall CesAs and secondary cell wall CesAs might be specialized to form cellulose microfibrils in different environments, e.g., the pectin-rich primary cell wall during expansive growth or the relatively low-pectin, ordered wall layers of secondary cell wall.

As the later stages of polysaccharide biosynthesis pro-

ceed, lignification of cell corners and the middle lamella of tracheary elements and fibres begins (reviewed by Donaldson 2001). Our knowledge of phenylpropanoid and monolignol biosynthetic enzymes (and their encoding genes) has been increased by studies of Arabidopsis mutants with defects in primary xylem and interfascicular fibre development (Raes et al. 2003). With the sequencing of the poplar genome and large-scale EST projects in poplar and pine, genes involved in phenylpropanoid metabolism and monolignol biosynthesis in poplar and pine genomes are beginning to be identified (Sterky et al. 2004).

There are several lines of evidence that indicate that lignification of xylem cells is the result of metabolic activity of developing xylem cells themselves, as well as the surrounding axial and radial parenchyma cells (Hosokawa et al. 2001; Li et al. 2001; van Raemdonck et al. 2005). Phenylpropanoid biosynthetic enzymes, such as PAL, CCR, and CAD, have been localized in the cytosol of lignifying cells such as vessels, fibres, in addition to adjacent rays (Takabe et al. 2001). While the majority of phenylpropanoid biosynthetic enzymes have been localized to the cytoplasm, cytochrome P450 enzymes such as C4H, C3H, and F5H associate with the endoplasmic reticulum (Raes et al. 2003). The location of these enzymes suggests that monolignols are synthesized in the cytosolic compartment and, to be transported to the apoplast, must cross the plasma membrane. How the monolignols are exported from their sites of biosynthesis remains unknown. Early studies using autoradiography must be reconsidered carefully, since they used conventional chemical fixatives that have been shown to be inadequate for preservation of delicate secondary vascular tissues (Fig. 2; Rensing 2002; Rensing et al. 2002; Rensing and Samuels 2004).

The final stage of development for secondary xylem cells, such as vessel elements, fibres, and tracheids, is autodigestion of their living protoplast due to programmed cell death (PCD), resulting in the cell becoming an empty tube. Cell death in xylogenesis has been extensively studied in the in vitro tracheary element differentiation of *Zinnia*, where major events of PCD are vacuole swelling followed by tonoplast rupture and rapid nuclear degradation (Fukuda 1996). While earlier studies had suggested that Ca^{2+} influx is the trigger to initiate vacuolar collapse, more complex upstream signaling mechanisms including nitric oxide and cGMP might also be involved (Lam 2004). The result of tonoplast rupture is the release of accumulated hydrolytic enzymes such as DNase, Rnase, and protease from the vacuole to digest cytoplasm and organelles. Multiple enzymes involved in degradation of tracheary elements have been identified in both the *Zinnia* in vitro xylogenesis experimental system and more recently in hybrid aspen (Lam 2004; Moreau et al. 2005). Major protease proteins for animal apoptosis, cysteine-dependent aspartate-directed proteases called caspases, have not been found in plants, but caspase-like protease has been identified and demonstrated to be activated during PCD (Lam 2004; see also review by Trobacher et al. (2006) on the role of cysteine proteinases in PCD in this issue).

Conclusions

Despite recent advances in techniques and knowledge, secondary xylem development remains a fascinating and enigmatic process. Developing xylem cells are exquisitely sensitive to experimental manipulation including dissection and fixation for electron microscopy. New rapid-freezing techniques such as high-pressure freezing or freeze substitution have allowed us to preserve even the late stages of programmed cell death during xylem development. Recent thoughtful studies, which combine anatomical, physiological, and molecular data, are models of how respect for and awareness of the old anatomy literature can be joined with modern techniques. This approach allows us to see how the gene products work in the context of the cell structures required for secondary xylem cell development.

References

Abe, H., and Funada, R. 2005. Review–The orientation of cellulose microfibrils in the cell walls of tracheids in conifers. A model based on observations by field emission-scanning electron microscopy. IAWA J. **26**: 161–174.

Arend, M., and Fromm, J. 2003. Ultrastructural changes in cambial cell derivatives during xylem differentiation in poplar. Plant Biol. **5**: 255–264.

Bailey, I.W. 1919. Phenomena of cell division in the cambium of arborescent gymnosperms and their cytological significance. Proc. Natl. Acad. Sci. U.S.A. **5**: 283–285. PMID: 16576393.

Bailey, W., and Zirkle, C. 1931. The cambium and its derivative tissues: VI. The effects of hydrogen ion concentration in vital staining. J. Gen. Physiol. **14**: 363–383. doi:10.1085/jgp.14.3.363.

Bannan, M.W. 1955. The vascular cambium and radial growth in *Thuja occidentalis* L. Can. J. Bot. **33**: 113–138.

Bourquin, V., Nishikubo, N., Abe, H., Brumer, H., Denman, S., Eklund, M., Christiernin, M., Teeri, T.T., Sundberg, B., and Mellerowicz, E.J. 2002. Xyloglucan endotransglycosylases have a function during the formation of secondary cell walls of vascular tissues. Plant Cell, **14**: 3073–3088. doi:10.1105/tpc.007773. PMID: 12468728.

Chaffey, N.J. 2000. *In* Cell and molecular biology of wood formation. *Edited by* R. Savidge, J. Barnett, and R. Napier. Bios Scientific Publishers, Oxford, UK. pp. 30–42.

Chaffey, N., and Barlow, P. 2002. Myosin, microtubules, and microfilaments: co-operation between cytoskeletal components during cambial cell division and secondary vascular differentiation in trees. Planta, **214**: 526–536. doi:10.1007/s004250100652. PMID: 11925036.

Chaffey, N.J., Barlow, P.W., and Barnett, J.R. 1997. Cortical microtubules rearrange during differentiation of vascular cambial derivatives, microfilaments do not. Trees, **11**: 333–341.

Chaffey, N., Barlow, P., and Sundberg, B. 2002. Understanding the role of the cytoskeleton in wood formaton in angiosperm trees: hybrid aspen (*Populus tremula* × *P. tremuloides*) as the model species. Tree Physiol. **22**: 239–249. PMID: 11874720.

Doblin, M.S., Kurek, I., Jacob-Wilk, D., and Delmer, D.P. 2002. Cellulose biosynthesis in plants: from genes to rosettes. Plant Cell Physiol. **43**: 1407–1420. doi:10.1093/pcp/pcf164. PMID: 12514238.

Donaldson, L.A. 2001. Review: Lignification and lignin topochemistry — an ultrastructural view. Phytochemistry, **57**: 859–873.

Evert, R.F., and Deshpande, B.P. 1970. An ultrastructural study of cell division in the cambium. Am. J. Bot. **57**: 942–961.

Fukuda, H. 1996. Xylogenesis: initiation, progression and cell death. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47**: 299–325. doi:10.1146/annurev.arplant.47.1.299. PMID: 15012291.

Funada, R., Shibagaki, M., Miura, H., Miura, T., Abe, H., and Oh-

tani, J. 2000. The role of cytoskeleton in secondary xylem differentiation in conifers. *In* Cell and molecular biology of wood formation. *Edited by* R. Savidge, J. Barnett, and R. Napier. Bios Scientific Publishers, Oxford, UK. pp. 255–264.

Gray-Mitsumune, M., Mellerowicz, E.J., Abe, H., Schrader, J., Winzell, A., Sterky, F., Blomqvist, K., McQueen-Mason, S., Teeri, T.T., and Sundberg, B. 2004. Expansins: Abundant in secondary xylem belong to subgroup A of the a-expansin gene family. Plant Physiol. **135**: 1552–1564. doi:10.1104/pp.104.039321. PMID: 15247397.

Groover, A.T. 2005. What genes make a tree a tree? Trends Plant Sci. **10**: 210–214. doi:10.1016/j.tplants.2005.03.001. PMID: 15882652.

Hosokawa, M., Suzuki, S., Umezawa, T., and Sato, Y. 2001. Progress of lignification mediated by intercellular transportation of monolignols during tracheary element differentiation of isolated Zinnia mesophyll cells. Plant Cell Physiol. **42**: 959–968. doi:10.1093/pcp/pce124. PMID: 11577190.

Im, K.-H., Cosgrove, D.J., and Jones, A.M. 2000. Subcellular localization of expansin mRNA in xylem cells. Plant Physiol. **123**: 463–470. doi:10.1104/pp.123.2.463. PMID: 10859177.

Inomata, F., Takabe, K., and Saiki, H. 1992. Cell wall formation of conifer tracheid as revealed by rapid-freeze and substitution method. J. Electron Microsc. (Tokyo), **41**: 369–374.

Joshi, C.P., Bhandari, S., Ranjan, P., Kalluri, U.C., Liang, X., Fujino, T., and Samuga, A. 2004. Genomics of cellulose biosynthesis in poplars. New Phytol. **164**: 53–61. doi:10.1111/j.1469-8137.2004.01155.x.

Lam, E. 2004. Controlled cell death, plant survival and development. Nature Mol. Cell Biol. **5**: 305–315.

Larson, P.R. 1994. The vascular cambium: development and structure. Springer-Verlag, Berlin.

Li, L., Cheng, X.F., Leshkevich, J., Umezawa, T., Harding, S.A., and Chiang, V.L. 2001. The last step of syringyl monolignol biosynthesis in angiosperms in regulated by a novel gene encoding sinapyl alcohol dehydrogenase. Plant Cell, **13**: 1567–1585. doi:10.1105/tpc.13.7.1567. PMID: 11449052.

Mellerowicz, E.J., Baucher, M., Sundberg, B., and Bojeran, W. 2001. Unraveling cell wall formation in the woody dicot stem. Plant Mol. Biol. **47**: 239–274. doi:10.1023/A:1010699919325. PMID: 11554475.

Moreau, C., Aksenov, N., Lorenzo, M., Segerman, B., Funk, C., Nilsson, P., Jansson, S., and Tuominen, H. 2005. A genomic approach to investigate developmental cell death in woody tissues of Populus trees. Genome Biol. **6**: R34. doi:10.1186/gb-2005-6-4-r34. PMID: 15833121.

Murmanis, L. 1977. Development of vascular cambium into secondary tissue of *Quercus rubra* L. Ann. Bot. (London), **41**: 617–620.

Nairn, C.J., and Haselkorn, T. 2005. Three loblolly pine CesA genes expressed in developing xylem are orthologous to secondary cell wall CesA genes of angiosperms. New Phytol. **166**: 907–915. doi:10.1111/j.1469-8137.2005.01372.x. PMID: 15869651.

Raes, J., Rohde, A., Christensen, H.J., Van de Peer, Y., and Boerjan, W. 2003. Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiol. **133**: 1051–1071. doi:10.1104/pp.103.026484. PMID: 14612585.

Rao, K.S., and Catesson, A.-M. 1987. Changes in the membrane components of nondividing cambial cells. Can. J. Bot. **65**: 246–254.

Rensing, K.H. 2002. Chemical and cryo-fixation for transmission electron microscopy of gymnosperm cambial cells. *In* Wood formation in trees: cell and molecular biology techniques. *Edited by* N.J. Chaffey. Taylor and Francis, New York. pp. 65–81.

Rensing, K.H., and Owens, J.N. 1994. Bud and cambial phenology of lateral branches from Douglas fir (*Pseudotsuga menziesii*) seedlings. Can. J. For. Res. **24**: 286–296.

Rensing, K.H., and Samuels, A.L. 2004. Cellular changes associated with rest and quiescence in winter-dormant vascular cambium of *Pinus contorta.* Trees (Berl.), **18**: 373–380.

Rensing, K.H., Samuels, A.L., and Savidge, R.A. 2002. Ultrastructure of vascular cambial cell cytokinesis in pine seedlings preserved by cryofixation and substitution. Protoplasma, **220**: 39–49. doi:10.1007/s00709-002-0033-8. PMID: 12417935.

Samuels, A.L., Giddings, T.H., and Staehelin, L.A. 1995. Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants. J. Cell Biol. **130**: 1345–1357. doi:10.1083/jcb.130.6.1345. PMID: 7559757.

Samuels, A.L., Rensing, K.H., Douglas, C.J., Mansfield, S.D., Dharmawardhana, D.P., and Ellis, B.E. 2002. Cellular machinery of wood production: differentiation of secondary xylem in *Pinus contorta* var. latifolia. Planta, **216**: 72–82. PMID: 12430016.

Schrader, J., Nilsson, J., Mellerowicz, E., Berglund, A., Nilsson, P., Hertzberg, M., and 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**: 2278–2292. doi:10.1105/tpc.104.024190. PMID: 15316113.

Sterky, F., Bhalerao, R.R., Unneberg, P., Segerman, B., Nilsson, P., Brunner, A.M., Charbonnel-Campaa, L., Lindvall, J.J., Tandre, K., Strauss, S.H., Sundberg, B., Gustafsson, P., Uhlen, M., Bhalerao, R.P., Nilsson, O., Sandberg, G., Karlsson, J., Lundeberg, J., and Jansson, S. 2004. A Populus EST resource for plant functional genomics. Proc. Natl. Acad. Sci. U.S.A. **101**: 13951–13956. doi:10.1073/pnas.0401641101. PMID: 15353603.

Takabe, K., Takeuchi, M., Sato, T., Ito, M., and Fujita, M. 2001. Immunocytochemical localization of enzymes involved in lignification of the cell wall. J. Plant Res., **114**: 509–515.

Thimann, K.V., and Kaufman, D. 1958. Cytoplasmic streaming in the cambium of white pine. *In* The physiology of forest trees. *Edited by* K.V. Thimann, W.B. Critchfield, and M.H. Zimmermann. The Ronald Press Company, New York. pp. 479–492.

Trobacher, C.P., Senatore, A., and Greenwood, J.S. 2006. Masterminds or minions? Cysteine proteinases in plant programmed cell death. Can. J. Bot. **84.** This issue.

van Raemdonck, D., Pesquet, E., Cloquet, S., Beeckman, H., Boerjan, W., Goffner, D., El Jaziri, M., and Baucher, M. 2005. Molecular changes associated with the setting up of secondary growth in aspen. J. Exp. Bot. **56**: 2211–2227. doi:10.1093/jxb/eri221. PMID: 15996985.

Vissenberg, K., Fry, S.C., Pauly, M., Hofte, H., and Verbelen, J.P. 2005. XTH acts at the microfibril-matrix interface during cell elongation. J. Exp. Bot. **56**: 673–683. doi:10.1093/jxb/eri048. PMID: 15642717.

Wardrop, A.B., and Harada, H. 1965. The formation and structure of the cell wall in fibres and tracheids. J. Exp. Bot. **16**: 350–356.

Zhao, C., Craig, J.C., Petzold, H.E., Dickerman, A.W., and Beers, E.P. 2005. The xylem and phloem transcriptomes from secondary tissues of the Arabidopsis root-hypocotyl. Plant Physiol. **138**: 803–818. doi:10.1104/pp.105.060202. PMID: 15923329.