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Wood Formation in Trees¹

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TREES ARE MAJOR COMPONENTS OF THE BIOSPHERE, AND THEIR WOOD HAS A MAJOR ROLE AS A SUSTAINABLE AND RENEWABLE ECOMATERIAL

Wood is the most important natural and endlessly renewable source of energy and therefore has a major future role as an environmentally cost-effective alternative to burning fossil fuels. The major role of wood is not only the provision of energy but also the provision of energy-sufficient material for our buildings and many other products. In addition, developing wood cells represent one of the most important sinks for excess atmospheric CO₂, thereby reducing one of the major contributors to global warming.

WOOD IS ALSO A RAW MATERIAL FOR A MAJOR GLOBAL INDUSTRY, AND ITS DEMAND IS INCREASING

Wood is the fifth most important product of the world trade. Vast quantities of wood are logged by foresters to provide fuel, fibers (for pulp, paper products, and boards), and sawn timber (for house building and furniture) as commodities. The complex chemical makeup of wood (cellulose, hemicelluloses, lignin, and pectins) also makes it an ideal raw material for what could be a future "ligno-chemical" industry that could replace the petrochemical industry, in providing not only plastic and all kinds of chemical products, but also food and textile products.

ENVIRONMENTAL BENEFITS OF TREES CONFLICT INCREASINGLY WITH INDUSTRIAL FORESTRY PRACTICES

Resource analyses have led to the conclusion that wood and fiber needs over the next 40 years can only

be met by logging 20% to 40% of the total present standing timber inventory in the natural forest (Food and Agriculture Organization). However, most agree that some of these forests should be either left completely alone or managed with only a minimum of wood extraction to preserve their environmental value.

WHERE COULD THE WORLD FIND MORE WOOD IN THE NEW MILLENNIUM?

The solution is to try to accomplish what agriculture has been doing for the last few centuries: grow wood as a crop in the same way that we grow wheat and maize. The future of the world's forests as well as the forest products industry will therefore depend to a huge extent on our ability to domesticate wild tree species and tailor them for maximum economic yield in the highly controlled environments typical of agriculture. The domestication of forest trees must be accomplished rationally, using the best available modern scientific methods, to develop high-yielding, intensively managed plantation forests, occupying a small percentage of existing forested land. In particular, the safe and careful application of biotechnology (marker-assisted breeding, genetic engineering, and *in vitro* propagation) to forestry practices, should help develop genetically superior trees in a time span of only a few decades.

OUR UNDERSTANDING OF HOW WOOD DEVELOPS IS NOT COMPLETE

Considering the important role that wood is foreseen to play in the near future, it is surprising to see that our understanding of how wood develops is far from complete. With a few exceptions, very little is known about the cellular, molecular, and developmental processes that underlie wood formation. Xylogenesis represents an example of cell differentiation in an exceptionally complex form. This process is controlled by a wide variety of factors both exogenous (photoperiod and temperature) and endogenous (phytohormones) and by interaction between them. It is driven by the coordinated expression of numerous structural genes (some of known function) involved in cell origination, differentiation, programmed cell death, and heartwood (HW) formation

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and by virtually unknown regulatory genes orchestrating this ordered developmental sequence. The presence of gene families and the extreme plasticity of the metabolism involved (as exemplified by the unusual behavior of plants with transformed cell walls; for review, see Fagard et al., 2000) add a further complexity to our understanding of the process of wood formation.

The making of wood spans a broad range of topics. Rather than to attempt a detailed review of all recent works in the different fields of wood formation, it is an objective of this contribution to give a brief overview and generalized concepts of this developmental process. We first open this *Update* by reviewing the well-known stages leading to the formation of wood, starting from the activities in a secondary meristem (the vascular cambium) to programmed cell death via the maturation of cambial derivatives. Emphasis is made on the biosynthesis and deposition of the main cell wall compounds, as well as the mediation of this ordered developmental process by phytohormones and transcription factors. The considerable plasticity in anatomical, chemical and physical wood properties is then described in view of providing a unique opportunity to dissect the molecular and biochemical mechanisms involved in such differences. Finally, the current progress in the molecular biology of wood development is presented.

WOOD BIOSYNTHESIS

Wood (secondary xylem) is manufactured by a succession of five major steps, including cell division, cell expansion (elongation and radial enlargement), cell wall thickening (involving cellulose, hemicellulose, cell wall proteins, and lignin biosynthesis and deposition), programmed cell death, and HW formation. The developmental biology of wood has been recently extensively reviewed and the following reading will provide the reader with essential references in research on vascular differentiation: Chaffey (1999), Lachaud et al. (1999), Savidge et al. (2000), Chaffey (2001), and Mellerowicz et al. (2001).

Wood Cells Originate from Vascular Cambium Activity

The vascular cambium is a secondary meristem derived from the procambium, which in turn develops from the apical meristem (Larson, 1994). The cambium plays a major role in the diametral growth of gymnosperm and angiosperm shoots and roots and is of great significance, particularly in respect to the wood that is produced. Cambial activity ensures the perennial life of trees through the regular renewing of functional xylem and phloem. The cambial zone includes the cambium *sensu stricto* made up of only one layer of juvenile cells, called initials, and the phloem and xylem mother cells, which are both produced by the dividing cambial initials.

Xylem mother cells always divide more compared with phloem mother cells, which explains the considerable disproportion existing between phloem and xylem tissues (Fig. 1A). The cambial zone consists of a few layers of narrow, elongated, thin-walled cells and comprises two types of highly vacuolated cells (Fig. 1A). Short radial initials give rise to rays that are essential to the translocation of nutrients between phloem and xylem (Fig. 1B). Elongated fusiform initials divide length-wise and produce secondary vascular tissues through periclinal divisions (tangential plane) in a position-dependent manner: on the inner side, wood elements (mostly tracheids in gymnosperms, but also vessel elements, vessel-associated cells, axial parenchyma and fibers in dicotyledons) and, on the outer side, phloem cells (sieve tubes, and, in dicotyledons, companion cells, axial parenchyma, and fibers). Anticlinal (radial) divisions of the fusiform initials also produce daughter cells similar to mother cells and ensure the harmonious increase in circumference of the cambium (Fig. 1A). New ray initials are formed by subsequent anticlinal divisions of fusiform initials.

The Differentiation of Xylem Cells Involves Four Major Steps

The daughter cells, produced by the cambial initials, give rise to a wide variety of wood cells, whose unique characteristics and three-dimensional associations define the intrinsic structure of wood (Fig. 1B). In the following section we will focus on the maturation process engaged by the xylem cells: tracheids, vessels, and fibers involved in water transport and mechanical support of the entire tree. The differentiation of these cells involves four major steps: cell expansion, followed by the ordered deposition of a thick multilayered secondary cell wall, lignification, and cell death.

Derivative cells expand longitudinally and radially to reach their final size during the formation of the primary wall. Xyloglucan endotransglycosylases, endoglucanases, expansins, pectin methyl esterases, and pectinases are among the primary determinants of this process. Once expansion is completed, the formation of the secondary cell wall begins, driven by the coordinated expression of numerous genes specifically involved in the biosynthesis and assembly of four major compounds: polysaccharides (cellulose, hemicelluloses), lignins, cell wall proteins and other minor soluble (stilbenes, flavonoids, tannins, and terpenoids), and insoluble (pectins and cell wall proteins) compounds in a neutral solvent (Higuchi, 1997).

Between 40% and 50% of wood consists of cellulose. The fundamental structure units are the microfibrils (MFs), which are the result of a strong association of inter- and intrachain hydrogen bonds between the different chains of β -linked Glc residues

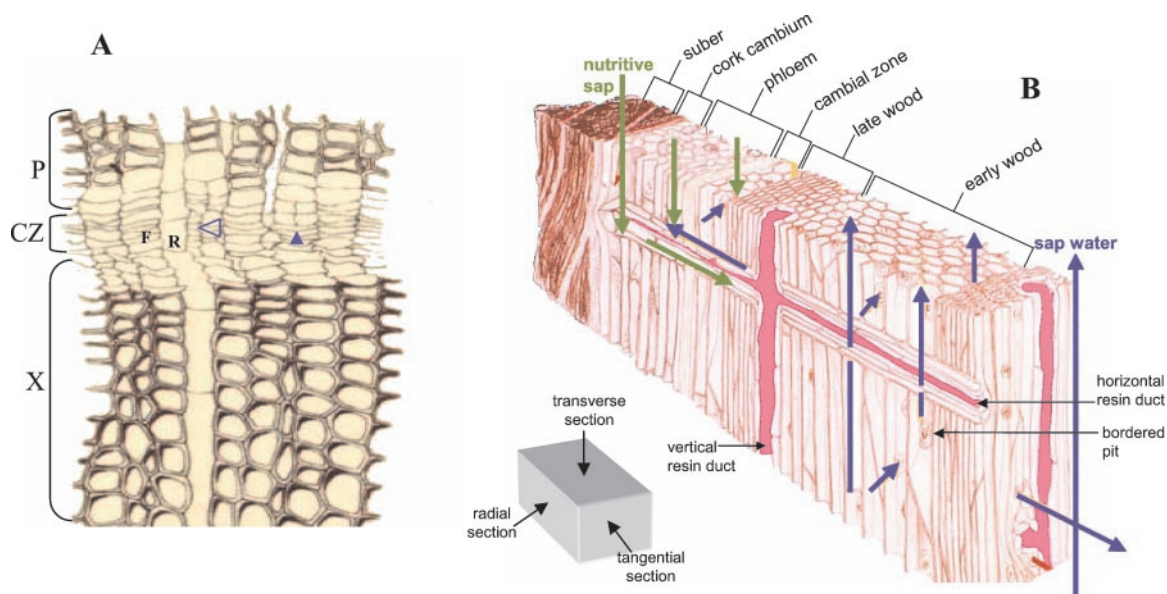


Figure 1. A, Drawing of a transverse section of the cambial zone of maritime pine (*Pinus pinaster*) showing the fusiform (F) and ray (R) initial cells in the cambial zone (CZ). X, Centripetal xylem differentiation with radially enlarging, maturing, and mature xylem. P, Centrifugal phloem differentiation with radially enlarging, maturing, and mature phloem. Empty arrowhead indicates a newly deposited radial wall (anticlinal division). Full arrowhead indicates a newly deposited tangential wall (periclinal division). B, Three-dimensional scheme of maritime pine wood showing the relatively homogeneous structure of conifer xylem. Ninety percent of the wood is made of tracheids, and the remainder is composed of ray parenchyma and longitudinal parenchyma cells, as well as resin ducts in certain species. Sap water ascends via the xylem and nutritive sap descends via the phloem. Sap can also be transported radially via the ray cells and tangentially by bordered pits. It is important to note the different direction and faces which are needed to describe wood structure: transverse, radial, and tangential sections.

in a manner so precise that microfibrillar cellulose is largely crystalline. Until recently, not even one single enzyme involved in cellulose biosynthesis and deposition was purified or cloned (Arioli et al., 2000). A first breakthrough had been the identification of genes encoding the catalytic subunit of the cellulose synthase (Ces) complex. In *Arabidopsis*, at least six genes encode putative catalytic subunits of Ces. In addition, a large gene family of over 20 more distantly related genes, so-called Ces-like (Csl) genes, exists, whose gene products most likely are involved in the synthesis of other polysaccharides. In higher plants, the substrate for Ces (UDP-D-Glc) is provided by Suc synthase. The complex Ces/Suc synthase is thought to have a cytoplasmic localization and the growing cellulose chain may be secreted through the membrane via a pore. Cortical microtubules (mainly composed of α - and β -tubulin) may determine the wall pattern by defining the position and orientation of cellulose MFs during the differentiation of conducting elements (Chaffey, 2000), probably by guiding the movement of the cellulose-synthesizing complex in the plasma membrane. However, although in many cases co-orientation of microtubules and MFs were observed, mathematical models (that remain to be tested in wood-forming tissue) relying on the geometry of the cell, have been proposed to challenge this dogma (Mulder and Emons, 2001).

The water-insoluble cellulose MFs are associated with mixtures of soluble noncellulosic polysaccharides, the hemicelluloses, which account for about 25% of the dry weight of wood. They generally occur as heteropolymer such as glucomannan, galactoglucomannan, arabinogalactan, and glucuronoxylan, or as a homopolymer like galactan, arabinan, and β -1,3-glucan. The biosynthesis of these polysaccharides occurs in the Golgi apparatus by a process that can be divided into two main steps: the synthesis of the backbone by polysaccharide synthases, and the addition of side chain residues in reactions catalyzed by a variety of glycosyltransferases (Keegstra and Raikhel, 2001).

The third major component of wood (25%–35%) is lignin, a phenolic polymer derived from three hydroxycinnamyl alcohols (monolignols): *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, giving rise to H, G, and S units, which differ from each other only by their degree of methoxylation. Lignin embeds the polysaccharide matrix giving rigidity and cohesiveness to the wood tissue as a whole, and providing the hydrophobic surface needed for the transport of water. Lignin content and monomeric composition vary widely among different taxa, individuals, tissues, cell types, and cell wall layers. As an example, no S units are detected in gymnosperms compared with angiosperms. Lignin biosynthesis has

been the most studied pathway, resulting in the cloning of several structural genes (Whetten et al., 1998; Christensen et al., 2000). However, it is somewhat surprising that recent attempts at engineering lignin biosynthesis have demonstrated that our current models of the pathway are incomplete (Grima-Pettenati and Goffner, 1999).

Cell wall proteins and pectins are among the other minor compounds of the cell wall. Although different proteins are present in the cell wall at different times during development, the amount of protein remaining in the wood is small. Nevertheless, such proteins could play important roles determining the composition and morphology of xylem cell walls (Cassab, 1998). Abundant cell wall associated proteins have been found in many plants and have traditionally been classified into four main groups: Gly-rich proteins, Pro-rich proteins, arabinogalactan proteins, and Hyp-rich glycoproteins (or extensins). These proteins are cross-linked into the cell wall and probably have structural functions. Pectins are thought to play a fundamental role in the regulation of cell wall extensibility. They are also thought to be exported from the Golgi apparatus as highly esterified galacturonan and then de-esterified in muro by cell wall-bound pectin methylesterases, thus allowing the formation of intermolecular bonds through calcium ions (Guglielmino et al., 1997; Higuchi, 1997).

When lignification is completed, conducting xylem elements undergo programmed cell death, involving cell-autonomous, active, and ordered suicide, in which specific hydrolases (Cys and Ser proteases, nucleases, and RNase) are recruited (Roberts and McCann, 2000). Several factors (auxins, cytokinins, and Suc) prepare the cell to die by determining the profile of hydrolases synthesized by the cell. These hydrolases are inactive in the vacuole. By a signal that remains unknown, a calcium flux provokes the vacuoles to collapse with the release of hydrolases (Jones, 2001) that degrade all of the cellular content but not the secondary cell wall.

Regulation of Wood Biosynthesis

The role of phytohormones in procambium initiation, cambial cell division, primary cell wall expansion, and secondary wall formation has been reviewed by Sundberg et al. (2000) and Mellerowicz et al. (2001). Recent findings have demonstrated the existence of an auxin (indole-3-acetic acid [IAA]) gradient across the developing vascular tissues of pine and poplar. This IAA concentration gradient seems to have a function in positional signaling, i.e. cambial derivatives develop according to their position along the gradient, and neighboring cell files receive the same dose to develop in a synchronized manner. A Suc gradient has been observed as well and may provide additional positional information for xylem and phloem differentiation. Other hormones than

auxins have been shown to be involved in xylogenesis by interacting with IAA in a synergetic (gibberellins, cytokinins, and ethylene) or inhibitory (abscisic acid) manner.

Knowledge of the molecular events that determine the differentiation pathway of a cambial derivative is embryonic. Even if the intervening signal transduction steps remain mysterious, we can assume that these signaling inputs result in altered patterns of gene transcription, which in turn requires the activity of specific transcription factors. In particular, considerable progress has been made in understanding the roles of transcription factors in controlling lignification. The analysis of lignification genes have also shown the presence in the promoter of conserved motifs that have been demonstrated to be important in xylem localized gene expression (Lacombe et al., 2000). Proteins that can bind this motif and activate the transcription, belong to the MYB family. Two MYB genes preferentially expressed in *Pinus taeda* xylem were proposed to be involved in regulating transcription during xylogenesis (Newman and Campbell, 2000).

Wood Cell Walls Are Highly Structured

The cell wall is composed of several layers that are fabricated at different periods during cell differentiation (Fig. 2). The first layer to be developed after cell division is called the middle lamella, which is found between the wood cells, and ensures the adhesion of a cell with its neighbors. The middle lamella is only 0.5 to 1.5 μm thick and is made up of pectic substances to which lignin may be added during the differentiation period. At the beginning of cell differentiation, the primary cell wall forms. This new, highly elastic layer is attached to the middle lamella and is approximately 0.1 μm thick. The primary cell wall is made up of several layers of MFs, which are

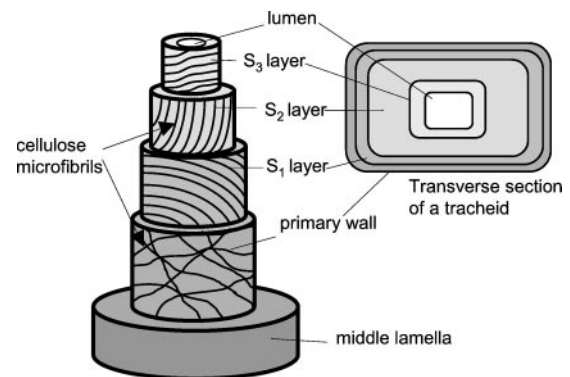


Figure 2. Three-dimensional structure of the secondary cell wall of a tracheid (xylem cell). The cell wall is divided into different layers, each layer having its own particular arrangement of cellulose MFs, which determine the mechanical and physical properties of the wood in that cell. These MFs may be aligned irregularly (as in the primary cell wall), or at a particular angle to the cell axis (as in layer S1, S2, and S3). The middle lamella ensures the adhesion between cells.

arranged randomly within this wall. Pectic substances, lignin, and hemicelluloses can be found between these MFs. As the developing cell reaches its definitive size, a new layer is formed inside the primary cell wall, which is the most important layer for the cell, in terms of mechanical strength. This new secondary cell wall is divided into three different layers, S_1 , S_2 , and S_3 (Timell, 1986). Each of these layers is composed of cellulose MFs, aligned in an ordered, parallel arrangement, which differs from S layer to S layer. Hemicelluloses and lignin are also present in each of these layers. These three S layers can be modified during cell maturation, which lasts for several days after the birth of the wood cell, e.g. the amount of lignin and cellulose laid down in the secondary cell wall may be influenced by abiotic factors such as mechanical stress, i.e. wind and stem lean.

The S_1 layer is the thinnest of the S layers, being only 0.1 to 0.35 μm thick, and representing just 5% to 10% of the total thickness of the cell wall. This layer is considered as an intermediate between the primary cell wall and the S_2 and S_3 layers. The MF angle with regard to the cell axis is 60° to 80° . The S_2 layer is the thickest layer in the secondary cell wall, and is the most important, with regard to mechanical support. The thickness of the S_2 layer varies between 1 and 10 μm , and accounts for 75% to 85% of the total thickness of the cell wall. The MF angle in this layer is 5° to 30° to the cell axis, and can be even higher, depending on external mechanical stress (see later section on reaction wood). The angle of the cellulose MFs in the S_2 layer can influence greatly the physical and mechanical properties of the cell and even stem wood as a whole. As the MF angle increases, with regard to the cell axis, wood becomes less rigid, and the longitudinal modulus of elasticity decreases, as in the case of juvenile and compression wood (CW). The innermost layer of the secondary cell wall, the S_3 layer, is relatively thin, being only 0.5 to 1.10 μm thick. The MFs are ordered in a parallel arrangement, but less strictly than in the S_2 layer, and the MF angle is 60° to 90° with regard to the cell axis.

Newly Developed Wood Is Stretched Longitudinally and Compressed Tangentially

Immediately after cell birth, the newly developed cells undergo a several day long “maturation” period, in which two mechanisms take place in the cell wall: (a) as lignin is deposited, the amorphous cellulose matrix swells transversely, and (b) when cellulose crystallization occurs, MFs shrink longitudinally. The combined effect of these two mechanisms results in a longitudinal shrinking of the cell (Fig. 3). However, as the maturing wood cells are attached to older, already lignified cells, they cannot shrink completely. Hence, these maturing cells are held in a state of tensile stress, and it is only on cutting the wood, that

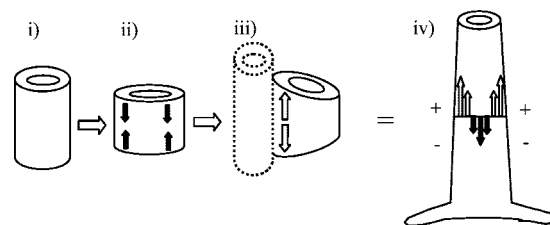


Figure 3. As the newly developed wood cell (i) begins to differentiate (ii), the deposition of lignin and cellulose in the secondary cell wall tends to stretch the cell laterally and cause it to shrink longitudinally (black arrows). However, as the differentiating wood cell is attached to older wood (iii), it cannot deform completely, thereby setting up a mechanical stress in the cell wall (empty arrows). In normal wood (NW), this translates into a tensile stress, therefore, the wood in the outer surface of a tree (iv) is usually held in tension (+ and empty arrows). However, as the new wood accumulates year after year, each new layer exerts a tangential force on the wood already present in the tree, thereby gradually placing the wood inside the tree under compression (– and full arrows).

these “maturation stresses” can be released in the form of residual strains along the longitudinal axis (Archer, 1986). The wood cells at the surface of a tree are therefore stretched longitudinally and compressed tangentially and can be said to be held in tension. However, as more and more wood is added to the tree surface, the wood cells inside the trunk are slowly compressed, until they are completely held in compression, toward the center of the trunk. This gradient of mechanical stress in a trunk, whereby the outside is held in tension, and the center in compression, is called growth stress, and can be highly detrimental to wood quality, resulting in warping and twisting of boards and planks.

HW: The Final Transformation of Xylem Cells

On cutting a mature tree, several different colored zones may be observed, a lighter colored external zone: the sapwood (SW), and often a darker colored wood: the HW situated at the center of the tree (Fig. 4). A third zone, often intermediate in color—the transition zone, TZ—may exist between the two. SW is known as the functional part of the tree and is sometimes termed the “living” part of the tree. Although SW contains living cells, most of its mass is comprised of terminally differentiated non-living tracheids or libriform fibers. Soon after their birth in the cambium, wood cells die, except for the longitudinal and radial parenchyma cells, which remain alive and functional until several years later, when they too die. It is when these parenchyma cells become dysfunctional, that HW forms. A specific role for HW has not yet been determined, although it has been suggested that it forms to provide long-term resistance to pathogens or even provides a mechanical role in tree support. Recent research suggests that HW forms as a response to a hydraulic stimulus and that HW may even develop irregularly, both radially

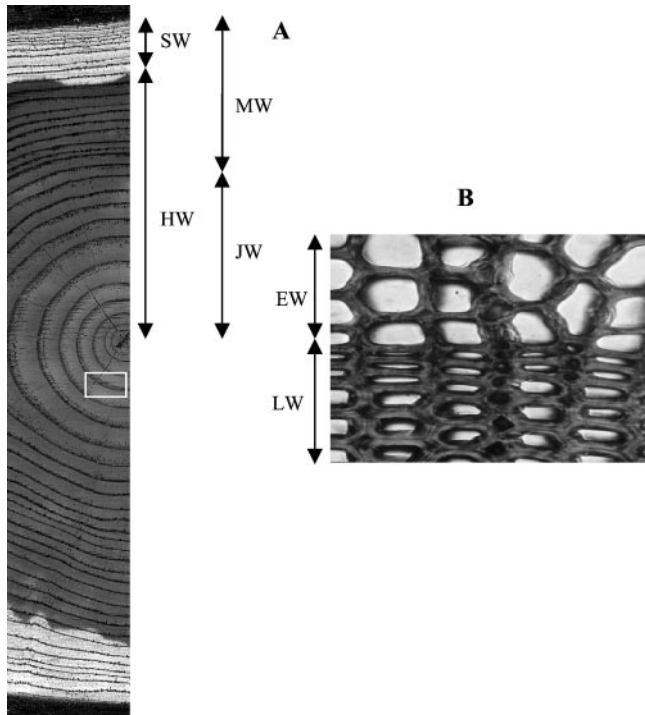


Figure 4. A, Photograph of a wood disc (*Pinus nigra* var. Laricio) showing the different types of wood which can be present within a tree (photograph courtesy of P. Rozenberg). B, A higher power view of the wood cells shows the transition between early wood (EW) and late wood (LW). LW cells have smaller lumens and thicker cell walls.

and longitudinally in the trunk, to maintain a constant and optimal proportion of SW in the tree stem (Berthier et al., 2001). As yet, there is no clear explanation as to why or how HW forms, although a theory has been put forward by Higuchi (1997), which is supported by recent research. Higuchi (1997) proposed that endogenous factors trigger expression of genes involved in phenolics biosynthesis in parenchyma cells bordering the TZ. As the parenchyma cells die, these phenolics are released and diffused into the neighboring cell walls and lumens.

Possible candidates that may trigger the molecular signal include an increase in ethylene, which is needed for polyphenol production, or a rapid change in water content near the TZ resulting in bordered pit aspiration, and even external mechanical stress, i.e. wind loading or stem lean, as trees which have been subjected to such a stress, show greater HW formation near the zone of mechanical loading (Berthier et al., 2001). However, HW formation is also under species-specific genetic control as some species exist which do not even produce HW, even at a very advanced age (Hillis, 1987). Once genes in the TZ have been activated by a molecular signal, a cascade of events occurs resulting in eventual cell death. Very little work has been carried out on the identification of the genes involved in this process. However, recent research has shown that in black walnut (*Juglans nigra*), flavanol biosynthesis was up-regulated in the

TZ. Flavonoids are highly soluble polyphenols and in black walnut, the flavanol production was found to be highly correlated with the transcript levels of genes encoding the enzymes chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*), and dihydroflavonol 4-reductase (*DFR*), all of which are implicated in the flavonoid pathway (Beritognolo et al., 2001). At the same time that these genes are being expressed in the TZ, carbohydrate content decreases drastically in this zone. Magel et al. (1994) found that in black locust (*Robinia pseudoacacia*) non-structural storage carbohydrates (Glc, Fru, Suc, and starch) decrease from the outer to the inner SW, with only trace amounts present in the TZ, thereby suggesting that these sugars and starch are necessary for polyphenol synthesis.

To elucidate the mechanism by which HW forms, future research should concentrate on the genes expressed in the TZ of different species, as well as the factors, endogenous or exogenous, inducing molecular activity in this zone.

Wood Is a Highly Variable Raw Material

Wood differs among trees. Use of the terms “softwood” for gymnosperms and “hardwood” for dicotyledon angiosperms is a crude acknowledgment of this difference. This variability is due to the heterogeneity of the cell types that make up the different woods and the structure of the individual cells. Anatomical, chemical, and physical differences in wood characteristics within a single tree are also a common feature. These include: (a) variation within the annual ring in temperate zones, i.e. early versus late wood, (b) variation due to juvenile wood (JW) with extremely variable properties, ranging from the pith to the bark, particularly in the early years of cambium activity, and (c) variation between normal and reaction wood. Wood can also be modified by damage from pathogens and by wounding.

Wood Formation Varies during the Growing Season

In temperate regions, the annual course of cambial activity (dormancy and activation) is induced by temperature and/or photoperiod (Uggla et al., 2001 and refs. therein). In temperate zones, EW is formed early in the growing season when temperature and photoperiod are favorable for active growth. EW has shorter cells and a lower density resulting from thin-walled tracheids or fibers of large radial diameter (Fig. 4B). LW is formed in the late summer or autumn when cambial cell division and expansion declines. LW has high density resulting from the small tracheid/fiber radial diameter and large tangential wall thickness. Having narrower lumens in the wood cells, the LW is much less vulnerable to water-stress-induced xylem embolism, and so increases the safety of water conductance. The passage of one type of

wood cell to another is always abrupt between two growing seasons, i.e. between autumn and the following spring. However, within one growing season, this passage may be relatively progressive or abrupt depending on the species.

Wood Formation Varies during the Aging Process

The transition from juvenile wood (formed during the first 10–20 years of a tree's life, i.e. in young trees and in the crown of older trees; Zobel and Sprague, 1998) to mature wood (MW) is another major transition typically found in wood during a tree's life (Fig. 4A). As demand for wood continues to increase and as new trees are developed that grow faster than their predecessors and are often logged at an early stage, the proportion of JW in the harvested timber increases. One of the characteristics of JW compared with MW is a lower density and a higher MF angle, which generally leads to such wood being considered "inferior" (lower tensile and tear strength) to MW.

Reaction Wood: A Mechanism by Which Trees Respond to Stem Displacement

Reaction wood is generally formed in response to a non-vertical orientation of the stem caused by prevailing winds, snow, slope, or asymmetric crown shape. This abnormal type of wood forms as part of a developmental process, which aims to re-orient a leaning stem or branch, so as to enable the tree find a more favorable position (for review, see Timell, 1986; Zobel and van Buijtenen, 1989). In both softwood and hardwood species, such tissue is often associated with eccentric radial growth of the leaning stem and in conifers is called CW as it often appears in localized zones of the tree held in compression, e.g. on the underside of a leaning stem. CW is highly lignified with more *p*-hydroxyphenyl subunits, and contains less cellulose than NW. The density of CW is up to 50% higher than that of NW. In CW, the MF angle of the cellulose fibers in the S₂ layer of the cell wall is high (<45°), tracheid length is reduced, cell cross-sectional profile is rounder, and the intercellular spaces larger.

In hardwood species, reaction wood is called tension wood (TW) as it tends to form in zones of the tree held in tension e.g. the upper side of a leaning stem. The overall lignin content of TW is lower, the cellulose content is higher and MF angle is lower than that of corresponding NW. The wood produced on the side of a stem or branch opposite to the reaction wood (opposite wood) may also be of a special nature and can be viewed as having many properties intermediate between NW and reaction wood (Timell, 1986). The signaling pathway which controls reaction wood formation is still poorly understood but may be a gravitropic response of the tree, related to intrinsic growth direction and phytohormone dis-

tribution and interaction, particularly that of ethylene and auxin (Timell, 1986; Sundberg et al., 1994; Little and Eklund, 1999).

A high proportion of reaction wood in a trunk is considered to be a major problem for several industrial applications. CW constitutes major defects in wood quality (the increase in S₂ MF angle in CW, causes an increase in longitudinal shrinkage, and consequently, lumber with CW is more likely to warp during drying) and fiber products (decrease of pulp yield). TW is also a problem for the solid wood industry as it increases longitudinal, radial, and tangential shrinkage during the drying process.

The most important mechanism by which reaction wood allows a tree to return to the vertical, occurs during the cell maturation process. In CW (gymnosperms), the MF angle in the S₂ layer is much higher (30°–45° to the cell axis) than in NW (5°–30°), due to a greater deposit of lignin laid down between the MF chains. As a result of this change in cell wall chemical composition, the newly developed wood cells tend to expand longitudinally. However, as explained in a previous section for NW, the new cells are attached to older cells, and cannot deform fully, therefore, they too exist in a state of mechanical stress. On cutting CW in a tree trunk, the wood therefore expands longitudinally, as these stresses are released. In TW (angiosperms), an extra layer exists between the lumen and the S₃ layer, called the gelatinous, or G layer. The G layer is almost entirely made up of cellulose, with an almost vertical MF angle. During maturation, this layer shrinks strongly in the longitudinal direction, thereby creating a very strong state of tensile stress in the cell. The combined result of reaction wood cells is to "push" the tree upright in the case of conifers, and to "pull" the tree to a vertical position in hardwoods (Archer, 1986; Timell, 1986).

This considerable plasticity in wood properties, as reflected by the coexistence of these several different types of wood within a single tree, is certainly due to natural variations in the expression patterns of genes/proteins. This variation provides a unique opportunity to dissect the molecular and biochemical mechanisms underlying such differences.

MOLECULAR STUDIES OF SECONDARY CELL WALL FORMATION

Wood properties are known to vary among species and among genotypes within species. This variability is heritable and thus presents an opportunity to select for improved wood properties, i.e. superior product quality (Zobel and Jett, 1995). Such selection is currently hampered by costly traditional chemical and technological assays and the necessity to wait until the trees are nearly mature to evaluate wood properties. Application of modern genomic sciences to identify the genes controlling these properties, should increase selection efficiency and/or reduce

the time and costs associated with measuring such properties, by providing early selection criteria and tools to alter cell wall properties in transgenic trees. As a highly specialized developmental process that is likely to involve a unique set of genes, it is obvious that the first place to study the molecular mechanisms of wood formation in woody perennials is trees.

As the secondary xylem of softwoods and hardwoods is different in many aspects, both gymnosperm and angiosperm species should be studied to fully understand the process of wood formation. *Populus* spp. and *Eucalyptus* spp. are evident candidates among the hardwood species, because of their ease of vegetative propagation, their suitability for genetic transformation, their small genome size (5×10^8 and 6×10^8 bp respectively, i.e. only a little larger than that of *Arabidopsis*) and the availability of genetic maps. With regard to the conifer model, and considering the high conservation of chromosome number and gene sequences in conifer species as well as the homogeneous cellular components of the wood, it is likely that the results gathered in the few studied species will be of benefit to research on other conifers.

The formation of the secondary cell wall is driven by the coordinated expression of numerous genes specifically involved in the biosynthesis, assembly, and deposition of polysaccharides, lignins, pectins, and cell wall protein. The use of molecular techniques in wood research has allowed the identification and characterization of a rather small proportion of the key molecular players involved in this process (Higuchi, 1997; Savidge et al., 2000), such as those involved in lignification (Whetten et al., 1998) and cellulose synthesis (Arioli et al., 2000), but also hemicelluloses (Reid, 2000), cell wall-associated proteins (Zhang et al., 2000), and pectins (Reid, 2000). This lack of information is illustrated by the fact that from the several hundred genes presumably required for polysaccharide biosynthesis and deposition, only a handful have been characterized. Even if the main structural pathways are known to a certain extent, virtually nothing is known about their specific regulation.

It is likely that some unknown fraction of wood quality variability will derive from structural or regulatory genes that have not yet been identified and sequenced. Therefore, much more information at the gene and protein expression level is needed in order to understand the profound basic pathways characteristic for wood physiology and to create tools which could be targets for biotechnological approaches leading to the introduction of desired wood properties. Large scale cDNA sequencing efforts to identify genes that are related to wood formation are being developed for tree species (see the list of public-domain projects at <http://www.pierroton.inra.fr/Lignome/links.html>). Based on the determination of putative

gene function by sequence homology, a wealth of genes of known function and that are active during the formation of woody tissues were isolated, providing a pool of candidate genes (CGs) for genetic manipulation of the wood forming processes. A significant proportion of the genes were also classified as orphan (Allona et al., 1998; Sterky et al., 1998). We can speculate that some of these unknown genes could be specific regulators involved in wood formation. Transcript profiling using cDNA microarrays is being used in concert with proteomics for tracking the genes/proteins of interest and to achieve a comprehensive understanding of the molecular process of wood formation. In poplar, the expression patterns of 2,995 genes were analyzed during the successive stages in the differentiation process leading to mature xylem cells (Hertzberg, 2001). This author also showed that many structural and potential regulatory genes of xylogenesis were under a strict developmental stage-specific regulation.

Of particular interest are those genes/proteins that underlie the anatomical, structural, and chemical differences observed between TW or CW versus NW (Zhang and Chiang, 1997; McDougall, 2000; Plomion et al., 2000; Wu et al., 2000; Zhang et al., 2000), EW versus LW, and JW versus MW. The identification and characterization of gene products differentially expressed in developing xylem, associated with a range of wood characteristics, pinpoint important genes controlling chemical composition and architecture of cell walls and cell shape, therefore determining wood and end-use properties.

Proteins accumulating abundantly in young differentiating xylem of pine and poplar (Costa et al., 1999; Mijnsbrugge et al., 2000) also provide us with essential knowledge of secondary cell wall formation. It is remarkable that *S*-adenosyl-l-Met synthases, one of the most highly expressed genes in poplar (Sterky et al., 1998) and pine (Allona et al., 1998) developing-xylem expressed sequence tag libraries, were also strongly expressed at the proteome level in both species (Costa et al., 1999; Mijnsbrugge et al., 2000). The presence of several *S*-adenosyl-l-Met synthases points out the importance of methyl transfer reactions and the complexity of gene regulation for methyl-group donation during xylogenesis.

The identification of genes specifically expressed in the highly specialized vascular cambium or differentiating xylem tissues may also give us substantial information with respect to the molecular processes involved in xylogenesis. Such genes are thought to play critical roles in the genetic engineering of wood properties, because genetic manipulation will require precise spatial regulation of introduced sequences.

All of these studies are powerful strategies to the identification of CGs potentially involved in wood formation and in controlling the chemical and mechanical properties of wood. Whether these CGs are involved in the genetic control of wood quality traits

still needs to be tested by using (a) reverse genetics experiments in trees or model organisms or (b) forward genetics experiments: colocation study between CGs and wood quality quantitative trait loci or linkage disequilibrium mapping in natural populations. Mapping such CG is already under way in eucalyptus, pine and poplar, for which quantitative trait loci for wood and end-uses properties have been localized on genetic linkage maps.

PROSPECTS

Non-Woody Systems Shed New Light on the Cell Biology of Xylogenesis. . . .

It is obvious that for the study of wood formation, woody models are desirable. However, a deeper understanding of the molecular mechanisms involved in cell wall biosynthesis and assembly can also arise from model plant systems such as *Arabidopsis* and zinnia (*Zinnia elegans*). Crucial to the use of such wood-forming models is the degree of similarity of xylem formation in these plants to that in the tree. More and more evidence supports the view that the vast majority of the biochemical, metabolic, cellular, and developmental processes is conserved throughout the plant kingdom. This theory also applies to secondary xylem formation. Indeed, even if wood properties can be highly specific for a given species, it is reasonable to assume that the differences in wood properties are the result of natural variation in expression patterns or biochemical properties of proteins, and are also part of the biochemical, metabolic, cellular, and developmental pathways conserved by the evolution processes. Some of these conserved pathways have already been identified. They include for example, the synthesis, deposition, and orientation of cellulose MFs. The formation of reaction wood, although highly specific to trees, also involves conserved signaling pathways including thigmomorphogenesis, ethylene, and auxin signaling pathways.

Arabidopsis undergoes secondary substantial thickening in the hypocotyl under appropriate growth conditions, and can therefore be viewed as a miniature tree (Chaffey et al., 2001). This species is the most advanced model system used in plant biology, with the complete genome sequence already available. With respect to wood formation, screening for *Arabidopsis* mutants (for review, see Fagard et al., 2000; Roberts and McCann, 2000) has already yielded interesting results concerning structural and regulatory genes (e.g. homeobox genes). A gene playing a crucial role in the control of the orientation of microtubules in *Arabidopsis* has been identified (Bichet et al., 2001; Burk et al., 2001) and is very likely to play a similar role in other plant species including tree species. An alternative use of *Arabidopsis* in research in xylogenesis would be to reveal the function of CGs identified in tree genomics projects by

reverse genetics or identification of knock-out mutants.

Certain aspects of wood development are also being studied in the ornamental garden plant, zinnia. In zinnia, mesophyll cells can be induced to redifferentiate synchronously, into tracheary elements, hence, proteins and genes can be identified at different stages of xylem formation (Roberts and McCann, 2000; Milioni et al., 2001).

. . . But Trees Will Remain the Main Model for Field Testing

The limited amount of cambium and secondary xylem and the small size of these non-woody models make it difficult to isolate large amounts of tissue for investigations into the molecular biology and biochemistry of wood formation. In contrast, during the active growth of trees, kilograms of maturing xylem may be readily collected by simply peeling away the bark, thus providing highly enriched material for molecular studies. Given also the heterogeneous structure and complex functioning (i.e. seasonal cycle of cambial dormancy-activity, wood maturation, and production of HW) of wood, trees will remain the main model to investigate the whole dimension of the "making" of wood.

For a long time, the understanding of the process by which the vascular cambium produces wood has progressed very slowly. There has been an extensive descriptive literature addressing wood anatomy, chemistry and physical properties. The understanding of the molecular and physiological mechanisms of cambial activity and wood formation is now considered as the main research area which must draw upon diverse disciplines, including genomics as well as the more traditional biochemical and wood sciences.

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