MECHANISMS OF CELL-WALL GROWTH IN SECONDARY XYLEM[1]

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ABSTRACT

Current concepts of the growth of the cell wall in secondary xylem are briefly reviewed. Following a description of the organization of the mature cell wall, its development is traced from initiation in the cambial zone to maturity. Multinet growth and various concepts concerning the origin of microfibrils are discussed.

The mature cell wall of tracheids in the secondary xylem of gymnosperms and of fibers in the angiosperms is a layered structure of great complexity to which many properties of wood and its behavior in processing can be attributed. A number of theories have been advanced concerning the growth of these cells, which are derived from the vascular cambium. It is the purpose of this paper to review briefly current concepts of the mechanisms of cell-wall deposition and growth from initiation to maturity as proposed by various authors.

ORGANIZATION OF THE MATURE CELL WALL[2]

As shown in Fig. 1, intercellular substance, the true middle lamella, completely encloses the individual cell and constitutes a continuous matrix rich in lignin that cements each cell completely to its immediate neighbors. The thin outer layer of the cell wall proper, distinguishable only with difficulty from the middle lamella, is known as the primary wall. The primary wall is also highly lignified but, in addition,

it contains noncellulosic carbohydrates and some cellulose. Together these two layers are known as the compound middle lamella.

The major portion of the cell wall is the secondary wall. It is basically cellulosic in nature. Three layers of the secondary wall are recognized: a thin S1 layer lies immediately inside the primary wall. Next in sequence is a relatively thick S2 layer, the organization of which exerts a major influence on wood properties. Variations in total wall thickness reflect variation in thickness of the S2 layer. Finally, there is a thin S3 layer adjacent to the cell cavity. An elaboration of this somewhat general characterization of the layered structure of the cell wall is shown in Fig. 2.

Cellulose occurs in the various layers of the cell wall in the form of discrete microfibrils of varying orientation and compactness. Microfibrils are long slender strands about 35 Å in thickness and ranging up to 100 Å in width in wood cells, although they may be considerably larger in some seaweeds such as *Valonia* (Frey-Wyssling and Mühlethaler 1965; Preston 1962).

The microfibrillar mesh of the primary wall is loosely woven and seemingly of more or less random orientation. The microfibrillar system of the secondary wall is more compact. In the outer S1 layer, the microfibrils have a low-pitched helical arrangement and are directed at angles of 70° or more to the long axis of the fiber. This layer is only a few microfibrils thick. Most of the cellulose of wood is contained in the strongly oriented S2 layer. The microfibrils in this layer occur in steep counterclockwise (Z-type) helices at angles
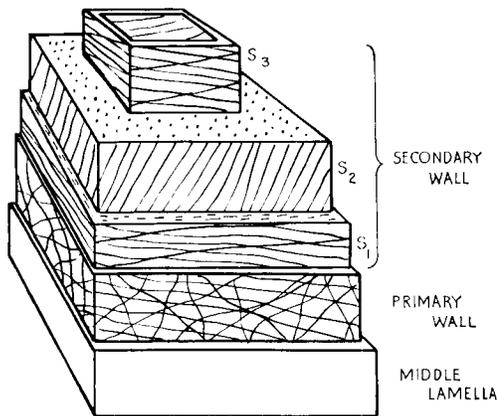
FIG. 1. Cutaway view of cell-wall layers showing general orientation of microfibrils (Preston 1960).

FIG. 3. Diagrammatic representation of microfibrillar structure according to Rånby (1958) showing a group of microfibrils in cross section and a single microfibril in longitudinal section.

commonly ranging from a few degrees to 30° from the fiber axis. Electron micrographs have disclosed lamellae of slightly different orientation within the S2 layer of the same cell wall. The innermost S3 layer

of the secondary wall consists of a less compact microfibrillar network of variable helical orientation, but the strands commonly are at a large angle to the fiber axis, similar to their orientation in the S1 layer.

Within the microfibril are regions of highly oriented and closely packed cellulose molecules arranged in a regular crystalline lattice structure. Interspersed among these crystallites are so-called amorphous regions

Outer surface   Inner surface

Primary wall

Inner layer of secondary wall   Outer layer of secondary wall

(S3)   (S1)

(0-6 Laminae)

(4-6 Laminae alternately of S or Z microfibrillar orientation)

Middle layer of secondary wall (S2)

(Several Laminae intermediate between those of the S2 and S3 layers)

(CA. 30-150 Laminae)

(Several Laminae intermediate between those of the SI and S2 layers)

FIG. 2. Cell-wall organization showing microfibrillar textures. Attributed to Wardrop in Mark (1967).
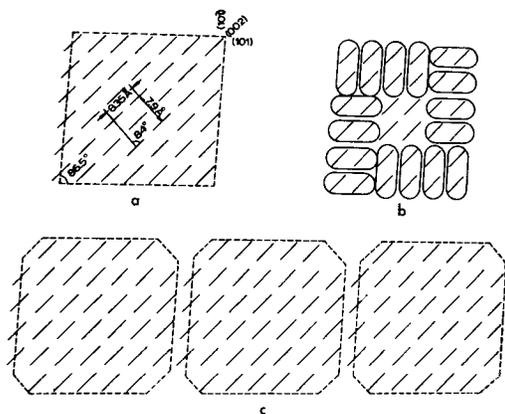
Fig. 4. Cross section of cellulose crystallite showing arrangement of cellulose chains: a) a crystalline elementary fibril 35 Å on each side; b) antiparallel chains forming molecular pairs; c) three elementary fibrils grouped to form a strap-shaped microfibril. Attributed to Frey-Wyssling and Mühlethaler (1965) in Mark (1967).

Fig. 5. The classical model of Meyer and Misch for the unit cell of native cellulose (Mark 1967).

in which cellulose molecules vary in degree of disorder and closeness of packing. Figure 3 is a diagrammatic representation of a microfibril in longitudinal section and a group of microfibrils in cross section. The longitudinal section shows long-chain cellulose molecules passing through crystalline and noncrystalline regions. The cellulose molecule is many times longer than an individual crystallite, which is on the order of 600 Å in length. The cross-sectional view shows crystallites about 100 Å wide and 30 to 40 Å thick consisting of molecules having parallel orientation. The crystallites are enclosed in a noncrystalline sheath of similar molecules having a lesser degree of lateral order. A more recent interpretation of a strap-shaped microfibril consisting of elementary fibrils 35 Å in diameter is portrayed in Fig. 4. The dimensions and crystallographic planes indicated in Fig. 4a refer to the unit cell of the crystal lattice of cellulose depicted in Fig. 5. The occurrence of such elementary fibrils in softwood tracheids has reportedly been demonstrated by Heyn (1969).

DEVELOPMENT OF THE PRIMARY WALL

The cambial zone consisting of a single layer of cambial initials and a varied number of layers of actively dividing mother cells is the source of all fibers and tracheids in the xylem. Following the formation of a tangential-longitudinal cell plate as the concluding phase of cell division, the primary wall is initiated through the deposition of highly hydrated uronides and hemicelluloses that polymerize in the wall and lose their solubility in water. The Golgi vesicles in the protoplasm presumably play an important role in this sequence of development, as synthesizers of these matrix substances (Mühlethaler 1965).

The contrasting size and appearance of the primary wall in the cambial zone and the greatly thickened secondary walls of mature latewood tracheids are shown in cross section in Fig. 6. The cambial initial also differs in length from the mature xylem cell, particularly in the angiosperms, in which the fibers typically increase two to three times in length during cell growth, whereas in the gymnosperms the tracheids commonly increase in length by only a few per cent (Bailey 1920). All of this growth in length and diameter occurs through expansion of the primary wall, the plastic gel matrix of which is reinforced by the dep-
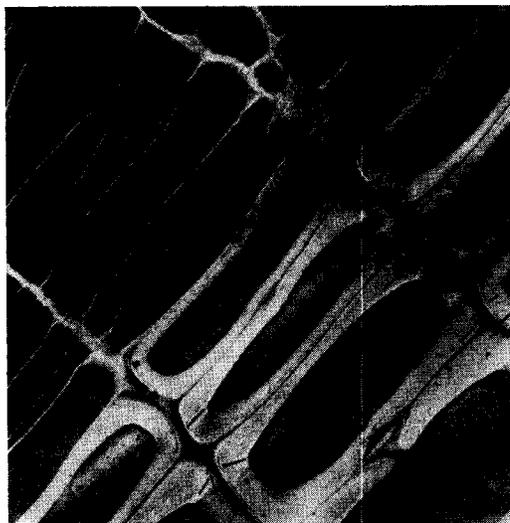
FIG. 6. Cambial cells (left) and mature late-wood tracheids (right) showing cell enlargement and wall thickening (Murmanis and Sachs 1969).

osition of cellulosic microfibrils, which may constitute as much as 12% of the hydrated wall (Frey-Wyssling 1962; Wardrop 1965).

Especially in tubular cells that undergo considerable increase in length, the pattern of changing helical pitch of the microfibrils from a relatively flat orientation at the inner face to a steeper helical pattern at the outer surface of the primary wall has been observed. The cottonseed hair illustrates this effect. Numerous theories have been advanced to account for the orientation of the microfibrils in the expanding primary wall. These include streaming of the cytoplasm, stress and/or strain in the cell wall, and genetic predestination (Frey-Wyssling 1962).

The explanation most widely accepted today for the observed changes throughout the successively deposited layers of the primary wall is that attributed to Houwink and Roelofsen (1954) and described in detail by Roelofsen in "The Plant Cell-Wall" (1959) as multinet growth. As noted by him, the primary wall texture of elongating cells varies as shown diagrammatically in Fig. 7. The outer surface of the primary wall exhibits a loose network of more or
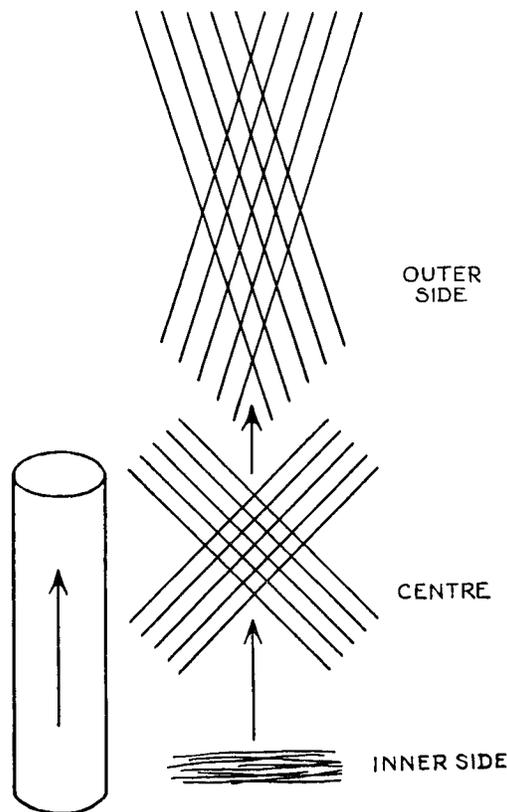
FIG. 7. Orientation of microfibrils in successive layers of the primary wall according to multinet growth theory (Roelofsen 1959).

less axially or irregularly oriented microfibrils, whereas the inner surface is denser in texture and the microfibrils are transversely oriented. Roelofsen theorizes that all of the microfibrillar layers of the primary wall are laid down with more or less transverse orientation and that the turgor pressure within the cell stretches the first layer deposited particularly in the direction of cell elongation, thus opening up the fibrillar mesh. Successive layers are deposited transversely on this expanded surface but with decreasing extension, until the last formed layer of the primary wall remains as it is laid down without subsequent extension. The elongation of the cell, or at least that part of it to which the foregoing narrative applies, has come to an end.

Roelofsen describes this type of passive growth as analogous to a set of superimposed fishing nets, successively stretched in the same direction. Wardrop has confirmed this pattern in the primary wall of *Pinus radiata* in an electron micrograph presumably taken of the elongating tip of a tracheid. Most electron micrographs of the primary walls of tracheids show a more or less transverse orientation throughout their thickness as would be expected in view of the slight elongation that is known to occur throughout the major portion of the length of tracheids.

The multinet theory is consistent with other concepts of growth in wall thickness and area by such mechanisms as apposition and intussusception. It clearly provides no explanation for the initial deposition of microfibrils in the transverse direction and, for this, we are confronted by mystery. Having discarded most of the concepts based on cytoplasmic flow and stress or strain, we can do little more than cite the opinion expressed by Frey-Wyssling (1962) that "It is more sensible to invoke morphogenesis as an organizer which imposes on the cell wall an adequate texture for its future function." This statement might well be remembered as we confront other aspects of cell-wall development for which ready explanations are still beyond our grasp.

DEVELOPMENT OF THE SECONDARY WALL

The surface growth of the cell through development of the primary wall is followed by the phase of wall thickening or secondary wall formation. Actually both processes may proceed at the same time in different parts of the same cell (Wardrop 1964, 1965). Optical and autoradiographic observations of cells from plants grown in an atmosphere of labelled carbon dioxide have shown that secondary wall formation begins near midlength of the fiber and proceeds towards the ends where elongation of the fiber (and hence primary wall expansion) may still be occurring (Wardrop 1964). The progres-
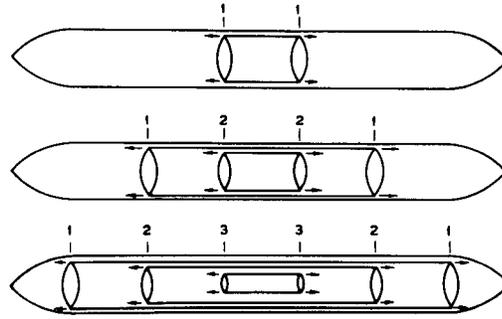
FIG. 8. Diagrammatic representation of the formation of three successive lamellae of the outer layer of the secondary wall of a differentiating fiber. Tip growth of the primary wall is not shown (Wardrop 1965).

sive development of successive lamellae of the S1 layer of the secondary wall is represented diagrammatically in Fig. 8. Because of opposing directions of helical orientation in successive lamellae of the S1, it follows that Z- and S-type microfibrillar helices are being laid down simultaneously in different parts of the same cell. From this argument it is difficult to understand how there can be an overall template in the cell cytoplasm that controls the orientation of microfibrils.

Microfibrillar orientation in the numerous lamellae of the S2 layer of the secondary wall has been the subject of study by many investigators applying many techniques (Bailey and Kerr 1935; Côté and Day 1969; Frey-Wyssling and Mühlethaler 1965; Harada 1958; Preston 1962, 1965). The electron micrograph in Fig. 9 illustrates the typical steep helical orientation in the S2 layer and the more nearly transverse orientation of the overlying S3 layer as seen from the inner face of the cell wall. The lamellar nature of microfibrillar deposition is evident. In interpreting the compactness of cell walls from an electron micrograph such as that shown in Fig. 9, it should be borne in mind that the replica is that of a desiccated wall. Recent work has shown that swollen cell walls contain water-filled voids on the order of 0.30 to 0.45 cc/g of dry solids (Kellogg and Wangaard 1969). Most of these voids are in the secondary cell wall.

Fig. 9. Microfibrillar orientation in the S2 layer and the overlying S3 layer as viewed from the inner surface of an earlywood tracheid of spruce (Harada 1958).

An interesting relationship between helical pitch (fibril angle) in the S2 layer and length of tracheid was first recognized as long ago as 1934 (Preston 1965; Wardrop 1964). The steeper helix is associated with the longer cell. This was at one time thought to reflect the extent to which the helical structure had been stretched. In view of the present concept of secondary wall formation following the completion of cell expansion, however, this hypothesis must be rejected and the underlying reason for this correlation remains unknown. We are reminded again of Frey-Wyssling's reference (1962) to morphogenesis as an organizer for future function.

Lignification is the final phase in the development of fibers and tracheids. Lignin is deposited in the matrix of the middle lamella and in the intermicrofibrillar spaces

of the primary and secondary walls, but this occurs only after the cessation of surface growth. Wardrop (1964) has suggested that it may be part of a mechanism limiting further surface enlargement of the cell wall.

THE ORIGIN OF MICROFIBRILS

It is paradoxical that despite the predominant occurrence of cellulose in wood, almost all that is known concerning its biosynthesis and its deposition in the form of microfibrils has come from the study of bacteria, yeasts, and seaweeds (Colvin 1964). The overall similarity, except for differences in size, of cellulose microfibrils from different sources argues in favor of a common mechanism of synthesis and suggests the applicability to woody plants of information gained from study of other organisms. The structure of the elementary fibril shown in Fig. 4 involves a crystalline lattice of 1, 4 β-polyglucosan chains.

Microfibrils may either be synthesized through the aggregation of individually formed cellulose chain molecules, or the microfibril may grow by end synthesis involving the transfer of glucose or small water-soluble polymers of glucose successively to the ends of chains already incorporated in the microfibril. The latter process would increase the length of the microfibril as a whole. In such a mechanism, polymerization and crystallization are essentially simultaneous events. Biosynthesis of cellulose microfibrils by end growth has been demonstrated to operate in a bacterium (Colvin 1964), and the concept has many attractive points in its favor (Preston 1962).

A problem not yet resolved in this connection is the question of the appropriateness of the Meyer and Misch model of cellulose crystalline structure (Fig. 5) in
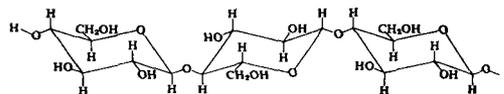
Fig. 10. Cellotriose as a model of cellulose chain end units showing a nonreducing and a reducing end.
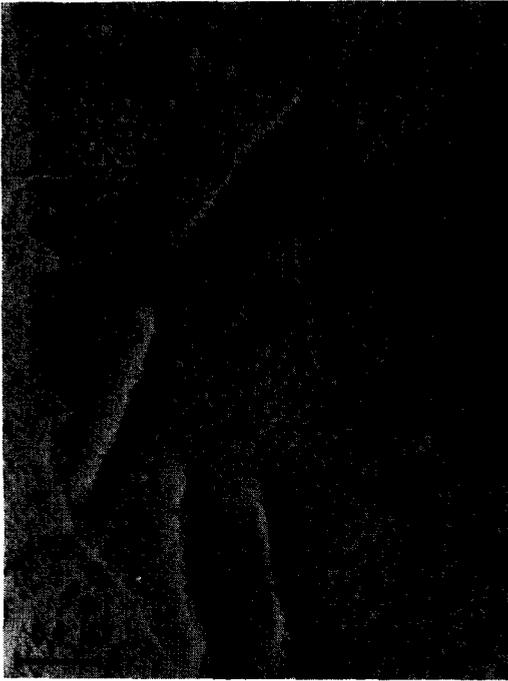
Fig. 11. Surface view of the cytoplasmic membrane (plasmalemma) of yeast (Mühlethaler 1965).

Fig. 12. Electron micrograph of innermost wall lamella of a cell of *Chaetomorpha* with adhering cytoplasmic granules (Preston 1962).

which alternate cellulose chain molecules are reversed in direction. If such be the case, the tip of a microfibril undergoing synthesis would include both nonreducing and reducing ends, as must be obvious from Fig. 10. Roelofsen (1959) notes that this would make cellulose the only polysaccharide molecule that is synthesized by the addition of monomers at both nonreducing and reducing ends. He suggests that an earlier unit cell model with parallel chains proposed in 1929 by Meyer and Mark is more plausible, and that antiparallel microfibrils in the same crystalline zone are as consistent with X-ray data as is the model of Meyer and Misch.

Current opinion points to the cytoplasmic membrane (plasmalemma) as the region containing the organizational apparatus for microfibril synthesis. A surface view of such a membrane in yeast is shown in Fig. 11. The organizational pattern of particles is distinct. Mühlethaler (1965) suggests that these particles originate in the Golgi

vesicles, and reports that they are always present when microfibrils are being laid down.

Figure 12 is an electron micrograph of the innermost surface of a wall presumably undergoing microfibrillar end growth originating in granular bodies on the wall surface, and it is revealing in support of the end-growth hypothesis (Preston 1962, 1964). In preparing this material for micrography, the cytoplasm was removed by plasmolysis; hence the granules only hint at the nature of the active cytoplasmic surface. Based on this observation, Preston (1964) has proposed a formal model (Fig. 13) to account for the simultaneous formation of fully formed microfibrils with different orientations. Neither the microfibril nor the synthesizing enzyme moves during synthesis.

By attributing microfibrillar direction to the orientation of particles on the cyto-
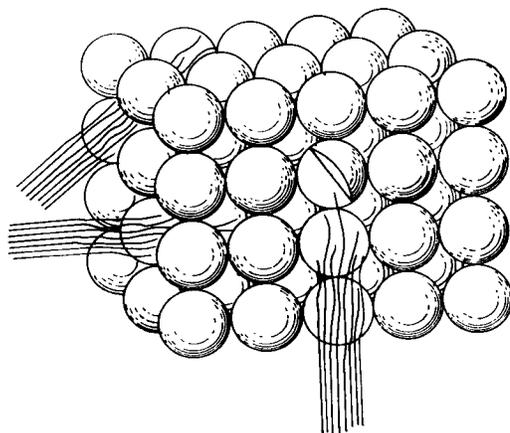
Fig. 13. Diagrammatic representation of a model proposed by Preston (1964) for synthesizing and orienting microfibrils by end growth.

plasmic membrane, we merely transfer our question as to the underlying cause of orientation from the cell wall to the plasma-lemma. I conclude by quoting Mühlethaler (1965) who, after pondering this question, offered the following: "It seems that this and related questions can be answered, but it will take some time."

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