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THE ROLE OF THE HEMICELLULOSES IN THE NANOBIOLOGY OF WOOD CELL WALLS: A SYSTEMS THEORETIC PERSPECTIVE

ABSTRACT

The hemicelluloses have not received adequate attention in studies of wood cell walls because the complexity of their structures does not admit easy interpretation within the paradigms of polymer science. Two-phase composite models of the cell wall have led many to view their primary function as one of coupling cellulose and lignin to enhance the mechanical properties of the walls. But that is a *microscopic* interpretation based on macroscopic theories of reinforced structures. In contrast, recent studies have shown that hemicelluloses can participate in regulation of the nanoscale architecture of cell wall constituents. They influence the aggregation of celluloses, and they can also influence the pattern of inter-unit linkages in lignin analogs polymerized in their presence. It is clear that the hemicelluloses have higher functions than as mechanical coupling agents in a two-phase composite. The hemicelluloses are species and tissue specific, and they have also been shown to vary across the layers of cell walls in patterns that are similar for cells formed contemporaneously in the same tissue. Their structures are clearly *genetically encoded*. In this report it is proposed that the hemicelluloses have multiple functions in regulation of cell wall consolidation and in determining its properties. The hemicelluloses are viewed as part of a system whereby information encoded in the genes is communicated to regulate the assembly of the cell wall. In addition to their role in regulating the aggregation of cellulose and the formation of inter-unit linkages in lignin, they appear to act in concert with extra-cellular glycosidases, to accomplish gradual dehydration of the polysaccharide matrix within which lignin precursors are polymerized. These proposals are based on viewing the deposition and consolidation of the secondary wall as the result of multiple, simultaneous and sequential processes for synthesis of cell wall constituents wherein the syntheses are *distributed in both space and time*. The spatially distributed syntheses are considered essential to allow gradual modification of the molecules of the precursors of cell wall constituents. They begin as molecules optimized for solubility within the aqueous environment in the cell and are transformed into ones that can be consolidated within the secondary wall, which is more hydrophobic upon lignification. To add clarity to the analysis, a system theoretic approach to the discussion of the distribution of the biogenetic processes in space is adopted. The

overall system within each cell is regarded as three highly coupled subsystems. The nanoscale organization in the native state is then examined as a reflection of the processes of biogenesis of the plant cell walls. Issues associated with aggregation of the constituents are then considered and plausible pathways for the assembly of the matrix that are based on distributed synthesis of the major polymeric constituents are suggested. The molecular organization is regarded as the first stationary level of expression of phenotypic form and the implications of the similarity of this expression within contemporaneous cells in an annual ring of secondary growth are examined. It is concluded that all aspects of molecular organization must be governed by intracellular processes that can be orchestrated at levels well beyond those of the individual cells. It is proposed that the primary molecular carriers of organizing information, that is the mediators of the orchestration, are the hemicelluloses, and the manner of their action in the formation of the structure of lignin is suggested. The results of the analysis of hierarchic organization have a number of additional implications with respect to structure and its formation that are beyond the scope of the present report. One that is worthy of note at this time is that the organization of the deposition of the secondary wall, and its systematic variation within different tissues in higher plants, requires intimate coupling and orchestration of intracellular, membrane and extracellular processes, at levels higher than that of the individual cell in a manner that has not heretofore been considered.

1. INTRODUCTION

The hemicelluloses have become the orphan constituents of wood cell walls. They have been neglected for many years. One of the reasons is that the complexity of their structures does not admit easy interpretation within the paradigms of polymer science. These paradigms were originally developed in the context of investigations of the natural homo polymers cellulose, starch and natural rubber. However, since the 1950s further development of the paradigms for macromolecules has been based primarily on studies of synthetic polymers. Within these newer conceptual frameworks, the hemicelluloses have frequently been classified as random copolymers. The two-phase composite model of the cell wall has led many to view the primary function of the hemicelluloses as one of coupling celluloses and lignin to enhance the mechanical properties of the composite structures of cell walls. But that is a *microscopic* level interpretation growing from macroscopic theories of reinforced structures and theories of fiber-matrix composites.

In contrast, recent studies have shown that the hemicelluloses can participate in regulation of the nanoscale architecture of cell wall constituents. They influence the aggregation of celluloses, and they can also influence the pattern of inter-unit linkages in lignin analogs polymerized in their presence. It is plausible therefore to consider the possibility that the hemicelluloses have higher functions than as

mechanical coupling agents in a two-phase composite. It seems unlikely that evolutionary adaptation would have produced hemicelluloses as diverse, and as species and tissue specific as those that have been identified so far, for such a relatively simple function. Not only are the hemicelluloses species and tissue specific, but they have also been shown to vary across the layers of cell walls. Their structures are clearly *genetically encoded*.

In this report it is proposed that the hemicelluloses have multiple functions in regulation of cell wall consolidation and in determining its properties. It will be suggested that the hemicelluloses are part of a system whereby information encoded in the genes is communicated to regulate the assembly of the cell wall. In addition to their role in regulating the aggregation of cellulose and the formation of inter-unit linkages in lignin, they appear to act in concert with extra-cellular glycosidases, to accomplish gradual dehydration of the polysaccharide matrix within which lignin precursors are polymerized.

These proposals are based on viewing the deposition and consolidation of the secondary wall as the result of multiple, simultaneous and sequential processes for synthesis of cell wall constituents wherein the syntheses are *distributed in both space and time*. The spatially distributed syntheses are considered essential to allow gradual modification of the molecules of the precursors of cell wall constituents from molecules optimized for solubility within the fluid environment in the cell into ones that are eventually consolidated within the secondary cell wall that is essentially hydrophobic upon lignification.

To add clarity to the discussion, a system theoretic approach to the discussion of the distribution of the biogenetic processes in space will be adopted. The overall system within each cell will be regarded as three highly coupled subsystems. These are depicted in Fig. 1, which is an adaptation of a diagram presented by Terashima (1).

The first subsystem is the intracellular environment A wherein the precursors to cell wall constituents are synthesized in a hydrophilic form suitable for a predominantly aqueous environment. This first subsystem is separated from the second one by the plasma membrane as envisioned in current plant cell theory. While multiple processes occur in the plasma membrane, for our purposes it is regarded as a boundary or interface between the intracellular A and the immediate extra cellular B environments or subsystems, and it is a boundary across which there is much molecular traffic as the biogenesis unfolds.

The second subsystem B is regarded as the highly hydrated extra cellular layer immediately adjacent to the membrane outside the cell during cell wall formation. It will be suggested that this subsystem is the context of additional modifications of the molecules that have emerged from the plasma membrane to prepare them for consolidation into the cell wall. The third subsystem C is regarded as the consolidating and lignifiying cell wall bounded by the primary wall and cell corners D on the outside and the less well defined interface between it and subsystem B on the inside. In the normal course of events the boundaries will move as the cell wall is deposited and the second subsystem B will disappear when the cell wall is fully formed.

We note here that this perspective has evolved in part from our collaborations with Professor Emeritus Noritsugu Terashima of Nagoya University and his extensive studies with his co-workers, focused on the processes of lignification during cell wall biogenesis. Many discussions with him have influenced our views of biogenesis. Two of the figures used herein are adaptations of figures that he has used to illustrate his views concerning the time sequence of deposition of lignin precursors and of lignification. However we assume responsibility for the adaptations and interpretations we use to complement those of Professor Terashima.

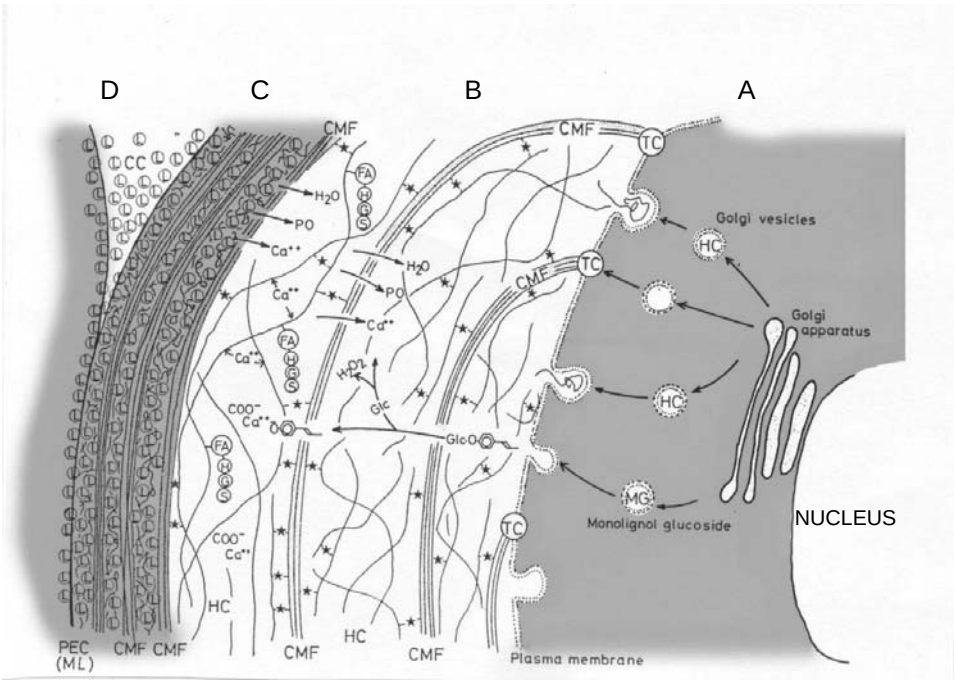

*Figure 1. Conceptual illustration of biogenesis of cell wall. Golgi apparatus participates in biosynthesis of cellulose, hemicellulose, and lignin. Cellulose microfibrils are laid separately in swollen gel of hemicellulose. Lignin deposition proceeds from intercellular region to inner part of the cell wall, and becomes hydrophobic. Water is removed from the swollen gel together with peroxidase (PO) and Ca toward inner side of the wall to cause an anisotropic shrinkage in the direction perpendicular to cellulose microfibril (CMF). This shrinkage brings about orientation of aromatic ring parallel to the cell wall surface and further growing of oligolignol to high polymer. *: hydrogen bond, PEC: pectic substances, ML: middle lamella, L: lignin, TC: terminal complex, FA: ferulic acid, H,G, and S: p-hydroxyphenyl, guaiacyl, and syringyl moiety of lignin (1).* <u>In adaptation for this report</u>, *the shading has been added to indicate the boundaries of the subsystems envisioned here. Thus subsystem A is the domain within the plasma membrane, which separates it from subsystem B, the highly hydrated polysaccharide matrix between the plasma membrane and the consolidating cell wall. The consolidating cell wall is subsystem C which is shaded somewhat darker than A.*

Subsystem D defined here as the cell wall corner, together with the primary wall provide the outer boundary of subsystem C.

While Fig. 1 depicts the spatial distribution of processes, Fig. 2, also developed by Professor Terashima and coworkers indicates the temporal sequence in the formation of the cell wall (2).

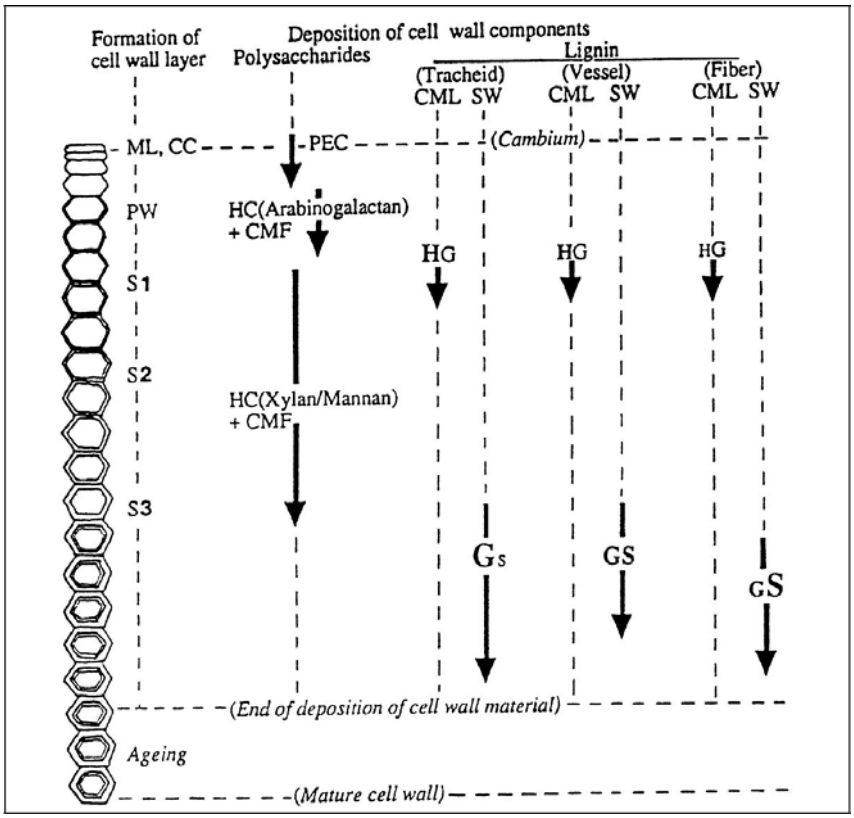

Figure 2. A conceptual illustration of the relationship between formation of cell wall layers and incorporation of polysaccharide and lignin. Cell wall layers are formed successively in the order of middle lamella (ML/cell corner (CC), primary wall (PW), followed by the outer, middle, and inner layers of the secondary walls (SW)(S1, S2 and S3 layers). Monolignol units are incorporated in the order of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, and incorporated thus in three distinct stages. The first lignification takes place in the CC and compound middle lamella (CML) after the PW is formed and just before S1 formation starts. Thereafter slow (or no) lignification takes place during the deposition of cellulose microfibrils (CMFs) and hemicelluloses (HC) in S2. The most extensive deposition of lignin takes place in the S2, after S3 formation has started (2).

In order to establish the perspective that has informed our discussion, we begin with an overview of our findings concerning molecular organization in secondary walls of woody tissue. We then provide a brief account of prevailing views concerning biosynthesis of major cell wall polymers; this is necessarily brief and limited in scope. We next examine issues of aggregation and distributed synthesis. We follow with a discussion of relationships between adjacent cells, the degree to which processes of biogenesis in neighboring cells are coupled, and the extension of the coupling to cells further removed within an annual ring of secondary growth. Finally, we consider the combined implications of information at different levels of structure and present some proposals concerning biogenesis of the cell wall matrix and the role of the hemicelluloses and cellulose as primary factors in its organization.

2. BACKGROUND

Studies of biogenesis of plant cell walls have, most often, addressed biosynthesis of the major constituents individually. In general the investigative approach has been reductive and there have been only limited efforts at integration. The focus in studies of polysaccharides is usually on synthases responsible for their formation, and on organization of the synathases within the Golgi apparatus or on the plasma membrane. In studies of lignin, the focus has been on enzyme systems responsible for synthesis of precursors and on processes of polymerization in the extracellular matrix. Issues of aggregation and tertiary structure formation have in general received less attention.

Our own studies have been concerned with molecular organization in wood cell walls where aggregation and consolidation of the cell wall matrix become paramount, particularly in secondary walls. Our findings required us to examine development of tertiary structure. We found that questions of tertiary structure cannot be separated from questions of assembly of the matrix, and that, in turn, assembly of the matrix cannot be separated from questions of biosynthesis of the constituents. We have thus been motivated to seek a unifying model for biogenesis and assembly of the major constituents of cell walls. In our search, the *role of the hemicelluloses was given special attention*. Though this first grew out of the differences between wood secondary wall hemicelluloses and those that have been investigated in primary walls of non-woody plants, it eventually led us to a system theoretic approach to understanding the processes of cell wall formation. The purpose of this report is to present such a model which evolved from our contemplation of the factors that must be considered from a system theoretic perspective.

In our search for a unifying model, we have been guided by two classes of considerations associated with spatial organization of cellular systems. The first class includes those arising regarding aggregation of the constituents and of their precursors in the different solvating environments represented by the cytosol, the extracellular matrix and the consolidating walls. These led us to examine the possibility that the processes of biogenesis require *distributed synthesis*, that is, a

sequence of synthetic steps that are distributed in space as well as separated in time. Hence the three subsystems proposed above, each representing a different environment wherein different categories of reactions can occur.

The second class of considerations are those flowing from recognizing that the molecular architecture of cell walls represents the first level of stationary expression of phenotypic form, and from examining the degree to which both the architecture and the assembly of the individual cell reflect its similarity to other cells within a particular tissue type. The symmetry of some structures at higher levels of organization, as in annual rings, for example, suggests a synchrony of molecular processes removed from each other in space, though not in time.

The considerations outlined above led us to explore the system and information theoretic perspectives set forth in the Introduction. We would emphasize that the intent of our proposals is not to negate the import of prior efforts in this arena, but rather to complement and integrate them on the basis of information derivable from examination of secondary wall structures and the potential role of the hemicelluloses. Where our proposals depart from previous models, we intend them as hypotheses that can rationalize observations, expecting that they will lead to new directions in research concerning the hemicelluloses, the results of which will allow discrimination between alternatives. We favor our own proposals primarily because we find them more plausible from both physical chemical and system theoretic perspectives.

We do not minimize the importance of cell wall proteins by excluding them from discussion of wall structure, for they are the primary agents of change in the synthetic processes in all subsystems. Rather, we focus primarily on the major constituents of the consolidated cell walls because they are the primary determinants of properties and because their native structures reflect the processes of assembly. They become in a sense terminal boundary conditions for the processes of assembly. The key role of the proteins in the dynamic geometry of the primary wall during morphogenesis is beyond the scope of this report.

3. OBSERVATIONS OF TERTIARY STRUCTURE IN SECONDARY WALLS

The primary objective of our work has been to understand structure in the *native state,* that is, the organization of constituents of secondary walls prior to disruption by the relatively harsh processes needed for isolation of individual constituents. More recently, we recognized that the structures of the individual polymers of the cell wall are interdependent. It became necessary to integrate knowledge of the assembly of cellulose with knowledge of the formation and deposition of the hemicelluloses, and to reconcile our understanding of the polymerization of lignin with our models of the polysaccharide matrix where the polymerization occurs.

Our focus on the structure of secondary walls in woody tissues is motivated by their dominance in such tissue and by the fact that their deposition is not complicated by changes in geometry. A deeper understanding of formation of the secondary wall can contribute information complementary to that derived from studies of primary walls. We regard primary walls as more complex due to their

dynamic geometry, their thinness, and the high degree to which proteins participate in their development (3).

Within secondary walls, our concern is with molecular organization at the nanoscale level, that is, domains of the order of 1 to 10 nm. This focus is motivated by the observation that collective properties of oligomers of cell wall constituents converge to those of the polymers within this size range. Structures that give rise to collective properties of the intimately integrated constituents are well defined by organization at this level; the spectral signatures of oligomers of homopolymeric polysaccharides typically converge between the hexamer and the decamer, and for hemicelluloses, branching patterns are well defined within a backbone interval of 10 to 15 units.

3.1. Observations in summary

When we commenced our work, the accepted models of cell wall structure were those described by of Preston (4) and Frey-Wyssling (5). The cell wall was regarded as two phases, the cellulose fibrils, considered crystalline and homogeneous, and a surrounding matrix, seen as a poorly defined blend of hemicelluloses and lignin. Since then our early work (6-9) showed that native cellulose is a composite of two distinct forms, I_α and I_β, which occur in different proportions in different species; I_α is dominant in bacterial and algal celluloses while I_β is dominant in celluloses from higher plants. Later work revealed a variability in distribution of the hemicelluloses within wood cell walls (10). Pentosans are concentrated in the primary wall and the S_1 and S_3 layers, while hexosans are predominantly in the S_2 layer, which is by far the largest fraction of matter in the walls. More recent work has shown that hemicelluloses are intimately blended into the structure of cellulose (11-13). The hemicelluloses co-crystallize with cellulose, within elementary fibrils, to a degree that brings into question the validity of extending to higher plants a two-phase model of cell walls. That model may have been plausible for some of the primitive marine algae that were the focus of the early studies of cell wall celluloses, and which had nanofibrils that were approximately 20 nm in lateral dimensions, but it certainly cannot be regarded as adequate in systems wherein the elementary nanofibrils are of the order of 3 to 6 nm. The hemicelluloses also have been show capable of influencing the balance between I_α and I_β in cell wall celluloses.

Regarding the structure of lignin, our early work (14-16) pointed to a higher degree of orientation than previously recognized and more recent results point to coupling between the organization of lignin and the structure of polysaccharides (17-20). More specifically, early studies, using the Raman microprobe, demonstrated orientation of lignin relative to the plane of the cell wall and the cellulose within it, and also revealed variability in the ratio of lignin to cellulose in different locations within the wall. More recent results suggest that the cellulose and the hemicelluloses play an important role in the organization of lignin. The observation of photoconductivity in wood reflects a coherence of order in lignin that is sufficient to allow coupling between the lowest unoccupied molecular orbitals of the phenyl propane structural units (19). That coherence of order can arise from

association with a polysaccharide surface possessing some regularity has been demonstrated through molecular modeling studies (20).

The influence of association between precursors and the matrix is also demonstrated in preparations of lignin analogs within cellulolse-hemicellulose matrices by dehydrogenative polymerization; the synthetic lignins are designated DHPs (17, 18). The DHPs were found to be closer approximations to milled wood lignin (MWL) than any previously prepared. The implication of this finding and some earlier ones (21,22) is that an association between the matrix and lignin precursors, *strong enough to influence the pattern of interunit linkages*, can play an important role in assembly of lignin.

The observations summarized, when considered together with earlier findings concerning variability of the hemicelluloses within secondary walls (10), suggested that hemicelluloses may be the key to a new model for assembly of lignin, one that could reconcile much of what is known about the polymerization of precursors with the requirements of intracellular control of overall assembly of the cell wall.

4. MODELS OF BIOSYNTHESIS

It is not within the scope of this report to provide an extensive discussion of biosynthesis of constituents of the secondary wall. Perusal of the literature reveals dominance of distinctive paradigms regarding each of the major constituents. This is clear from examining some recent overview volumes dealing with both biogenesis and structure (23-26). The paradigms have considerable value in that they provide organizing principles for information in specific areas. When considering the cell wall as a whole, however, it is necessary to ask how well the processes can mesh together in an overarching model for assembly of cell walls. While the complexity of the assembled walls has been recognized (27, 28), the processes involved in the consolidation of the three major constituents have received limited attention. But the biosynthetic pathways for constituents must be integrated in the process of biogenesis of the walls, and the integration must be consistent with what is known of biological regulatory processes that govern phenotypic expression of genotypic information.

4.1. The Hemicelluloses

The area of greatest consensus is that of formation of hemicelluloses; it is generally agreed that they are assembled in final form within the Golgi apparatus and transported in vesicles for extracellular deposition by exocystosis. The processes involved have been reviewed in the context of wood formation (28, 29), as well as in the broader context of plant cell wall development (30, 31). A recent discussion of the function of the Golgi apparatus also addresses its role in the synthesis of complex cell wall polysaccharides (32).

4.2. Cellulose

The degree of consensus is more limited with respect to biogenesis of cellulose (29). There is evidence that membrane bound metabolically active entities are associated with assembly of cellulose microfibrils (33, 34). Furthermore, cellulose synthase enzymes have been isolated from cellulose-forming bacteria (35), but the isolation of similar activity from higher plants remains elusive and controversial (36-38). Different interpretations of the phenomenology of cellulose biogenesis have arisen. Some evidence supports a coupling of synthesis and crystallization in bacterial systems and for a number of eukaryotes (39). On the other hand, it is observed that there is a high degree of coordination between cytoskeletal architecture and patterns of cellulose deposition (40). Many studies have shown correlation between the organization of microtubules and the orientation of cellulose microfibrils. Also, treatments which disrupt organization of microtubules result in disruption of patterns of deposition of cellulose microfibrils (40). Thus, many questions remain concerning cellulose biogenesis (41).

4.3. Lignin

Least well defined is the process of assembly of lignin, where formation of precursors is intracellular and polymerization is extracellular. With the exception of the work by Terashima and coworkers cited above, research on biosynthesis of lignin has focused on formation of precursor monolignols (42). In addition, many studies have sought to understand lignification by investigating DHPs formed from monolignols under conditions that simulate different aspects of the microenvironment in the wall matrix (17,18,43). Implicit in all studies of lignification is dominance of free radical coupling reactions described by Freudenberg (44). It is assumed that the distribution of linkage types is determined by the relative reaction rates between free radicals under conditions prevailing in the cell wall matrix; variations in the microenvironment are thought to influence electron density distributions within the radical intermediates.

5. ISSUES OF AGGREGATION AND THE NEED FOR DISTRIBUTED SYNTHESIS

The question of aggregation arises because consolidation of the cell wall matrix implies driving forces that result in association of the constituents simultaneously with progressive dehydration. The association is irreversible and the levels of hydration are well below those in subsystem B and the intracellular environment A. The aggregation cannot be regarded as a separation of phases; it is more akin to formation of insoluble aggregates in polymeric systems. In the primary wall, consolidation reduces the degree of hydration but, prior to onset of lignification, the coupling between constituents remains weak enough that they can be separated. It is possible to separate pectic substances and hemicelluloses from the cellulose, although it is not always certain that the cellulose is free of small amounts of hemicelluloses.

Since research on cell wall matrix formation has focused on primary walls, issues of aggregation have required little attention. In secondary walls, in contrast, particularly in woody tissue, consolidation of the polysaccharide matrix involves a higher level of coupling of hemicelluloses with the cellulose. This is evident in holocelluloses prepared by mild oxidative delignification of woody tissue. The dominant hemicelluloses in secondary walls cannot be separated from cellulose without use of strong caustic solutions. Yet all of these constituents have their origin in intracellular environments where they are highly solvated in aqueous media. It is necessary, therefore, to consider transformations that alter the relationships of the constituents to their environments. In particular, it is important to consider the likelihood that constituents that are inherently insoluble in water, or are of very limited solubility, are first synthesized in glycosylated forms to facilitate their solvation in the intracellular environment, and that they are later deglycosylated by action of one of the many glycosidases that occur in the extracellular environment in the vicinity of the plasma membrane (45). The possibility that such transformations are central to assembly of the cell wall matrix arises with respect to all three major components of the cell wall. Such transformations can be regarded as a form of *distributed synthesis,* that is, a progression of synthetic steps that, in addition to being sequential in time, are distributed in space with respect to the domains within which they occur. Distributed synthesis is well established among intracellular processes; one instance is the synthesis of glycoproteins, which is thought to begin at the endoplasmic reticulum and to be completed within the Golgi apparatus (32).

5.1. Lignin Precursors

Distributed synthesis is most obvious with lignin precursors. These occur as monolignol glucosides within the cytoplasm and have been isolated by extraction of thin layers of differentiating xylem from a number of woody species. Significant quantities of coniferin and syringin have been isolated in this manner (46). As glucosides, these precursors of lignin are soluble in water to a level approaching 30%; upon separation of the glucose solubility declines to less than 1%. It has been recognized for some time that extracellular glucosidases cleave the glycosidic linkages to liberate monolignols (46). This has been confirmed by recent observation of a coniferin-specific glucosidase in lignifying tissues (47,48). Upon deglycosylation, monolignols can penetrate readily into the polysaccharide matrix which, when considered as a solvating medium, is similar to a mixture of water, alcohol and ether. Thus, the precursors of lignin represent an instance of a distributed synthetic process where the constituents are transformed along the synthetic pathway to alter their aggregative properties. They originate in subsystem A, are modified in subsystem B and polymerized in C.

5.2. The Hemicelluloses

The principle of distributed synthesis also provides a basis for understanding the phenomenology of hemicelluloses, though the need for transformation is not

obvious. In studies of primary walls the majority of constituents, with the exception of cellulose, can be isolated using mild extractive procedures. This possibility has led to a substantial literature on organization of the primary wall as well as its primary non-cellulosic polysaccharides, the xyloglucans and the pectins (3,49,50).

In secondary walls of woody tissues, in contrast, the presence of major hemicellulose fractions would be difficult to comprehend apart from the possibility of distributed synthesis. The xylans in hardwoods and the glucomannans in softwoods include subsets that can be separated from cellulose only by extraction with strong caustic solutions. And they cannot be kept in solution upon dilution or neutralization; indeed progressive dilution and neutralization are the bases of methods for their fractionation. If these hemicelluloses are also synthesized in the Golgi apparatus, it is very likely that they are assembled first in a soluble form, and are later modified prior to aggregation with cellulose.

The modification that suggests itself is analogous to that recognized for the precursors of lignin. If hemicelluloses are first synthesized with some limited substitution of mono- or disaccharide branches, they would be soluble in the intracellular environment. The action of extracellular glycosidases would then strip them of branches to facilitate aggregation with cellulose and with other β-1,4 linked glycans of limited substitution. Though occurrence of extracellular glycosidases has been recognized for some time, the function proposed here has not been considered among their roles (45). Thus, not unlike the lignin precursors, they originate in subsystem A, are modified in subsystem B and aggregate with cellulose in C

An alternative rationalization of the problem of aggregation has been proposed by Bolwell (27). He suggested that hemicelluloses may be assembled in the extracellular environment from oligosaccharides. While this proposal addresses the issue of aggregation in the intracellular environment, it is not clear that such a process is consistent with the regularity characteristic of the structures of hemicelluloses. This regularity suggests that the enzymes enabling assembly are organized in space relative to each other; such organization is implicit in the topology of the Golgi apparatus. If similar organizing structures occur in the extracellular matrix, they have not been reported. The kinetic implications and requirements of Bolwell's proposal have not been fully developed.

5.3. Cellulose

The possibility that a distributed synthetic process may be involved in the production of cellulose must also be considered. While such a proposal departs from accepted models, it is not at all inconsistent with much of the data available on cellulose synthesis, and it may provide a basis for rationalizing coupling of cytoskeletal organization with cellulose deposition. It could also explain the considerable difficulty encountered in efforts to isolate cellulose synthases from higher plants.

The process of distributed synthesis proposed for cellulose is similar to that proposed for hemicelluloses. Cellulose would be assembled first as a gluco-glucan, with a β-1,4 linked backbone and glucose branches, not unlike xyloglucan, but with

glucose branches instead. Such a polymer would have escaped detection in studies that rely on hydrolysis and sugar analysis to validate the isolation of cellulose. Colvin *et al*. isolated a gluco-glucan with a glucose substituent at the 2 position of every third backbone unit from cultures of *Acetobacter xylinum* and proposed it as an intermediate in the synthesis of cellulose (51,52). They withdrew that proposal when they were unable to demonstrate that extracellular enzymes in cultures of *Acetobacter xylinum* could convert this polymer into cellulose (53). They did not consider the possibility that removal of the sidegroups might occur at the membrane during secretion of the polymer. In addition, Delmer has discussed observations by a number of investigators wherein radiolabeled soluble polymers associated with cellulose eventually appeared to have the radiolabel incorporated into the cellulose (54). Delmer notes that it was not possible to develop convincing evidence that the soluble fractions were precursors to cellulose.

In our speculation, we envision the de-branching of the precursor polymer as occurring at the sites of assembly complexes that have been visualized in association with the deposition of cellulose. The particulate complexes are viewed as sites where side branches are removed and cellulose molecules are aligned and organized for deposition as fibrils with the lattice structure of cellulose I. The microtubules of the cytoskeleton could then be regarded as part of the system for transport of the cellulose precursor polymer to the membrane, and for coordinating movement of the assembly complexes at the surface to regulate the direction of deposition of cellulose fibrils. The question remains as to where the synthesis of the proposed cellulose precursor can occur. The most likely location is in Golgi systems within the cell. These would be topologically more simple than the Golgi producing the heteropolysaccharides, and would have to be coupled, in some way, with the microtubules.

5.4. Distributed Synthesis

From a systems theoretic perspective, the proposals concerning the three major constituents of the cell wall matrix presented above have two common elements. The first is *transformation of the solvation characteristics* of precursors or intermediates in the synthesis. The second is introduction of *multiple points of regulation* of the processes of biogenesis.

Transformation of hydrophobic structures into soluble ones by glycoslylation is well recognized in other biological systems. In the present study, it is most obvious for the monolignols, which occur as the glycosides in the intracellular environment and are deglycosylated prior to polymerization in the course of lignification. It is not as obvious in the case of polysaccharides, but the β-1,4 linked homopolymers of all of the common pyranoses are essentially insoluble in water beyond the heptamers or octamers. It is also well known that limited amounts of neutral substitution, can dramatically increase solubility of the homopolymers in aqueous media; the xyloglucans are an example of this effect. With cellulose and many of the secondary wall hemicelluloses, it is obvious that their function requires them to be insoluble, for that is the form in which they occur. It is quite plausible that the same

mechanism as the one used for the solubilization and subsequent modification of hydrophobic species would also be adapted for the insoluble structural polysaccharides.

With respect to susceptibility to regulation, the availability of multiple points of control is important. To the extent that processes of distributed synthesis are based on activity of multiple enzyme systems at different points in the cycle of synthesis, the opportunities for regulation of assembly through expression of encoded processes are expanded. This is important because in the formation of tertiary structure many degrees of freedom at the molecular level of each of the constituents must be controlled. That is, many aspects of structure at the nanoscale level must be coordinated. This is obvious from examination of walls in tangentially adjacent cells in a particular tissue; what they have in common goes beyond the primary structures of the constituents of the walls. The common features include, among others, the lateral dimensions and orientation of cellulose fibrils in their walls, the patterns of inter-unit linkages in the lignin and the sequence of deposition of the hemicelluloses in the layers of the secondary wall. The regulation of these aspects of structure argues for activity of agents of expression of encoded characteristics. The entry into distributed synthesis of multiple enzyme systems, separated with respect to activity both in time sequence and in location, is the most plausible mechanism for expression of this encoding.

In presenting this perspective we do not minimize the importance of inherent self organizing characteristics of polysaccharides in the development of tertiary structure (55). Rather we note that such self organizing tendencies must be modulated by a higher level of coordinating information if self organization of the constituents in adjacent cells is to proceed in the coherent manner reflected by similarities in the molecular architecture of adjacent cells in particular tissues.

6. HIERARCHIC ORGANIZATION IN SECONDARY THICKENING

The hierarchic nature of structure in plants is reflected in anatomical descriptions and in studies of differentiation and morphogenesis at the cellular level (56). The hierarchy of structures has not been examined, however, from an information-theoretic perspective with respect to the constraints that one level of organization provides with respect to structures and processes at lower levels. In the present work our concern is with the constraints that the symmetry of an annual ring implies with respect to processes of assembly of the individual cells. The cross-section of an annual ring in a tree trunk, in the absence of any reaction wood, possesses a cylindrical symmetry that points to synchrony of the processes of assembly within all cells at the circumference. This, in turn, suggests a coupling of the processes within the different cells.

The symmetry is expressed at two levels of organization. The more obvious one is reflected in the geometry of cells as established during morphogenesis, and is an integral part of discussions of organization in plant anatomy (57,58). The next level of symmetry, and the one that is central in the present study, is that governing the synchrony of processes of deposition of cell walls *after their final geometry has*

been established during earlier phases of morphogenesis. This level of symmetry is not precisely geometric; it is defined by similarity in the molecular architecture of cell walls when specified in terms of patterns of deposition of cellulose, the composition of hemicelluloses, and patterns of interunit linkages in lignin.

It has been suggested that morphogenetic processes proceed as though each cell has a map and an internal clock (59) to regulate its development in relation to its function and location. In annual rings it appears that the clocks of cells formed at the same point in time are kept in synchrony by some external mechanism.

The result is symmetry along the circumference. This in turn suggests that radial growth proceeds with very similar regulation of the cell wall deposition along the perimeter in the cambial zone. The consequences are readily visualized with the aid of Fig. 3, which shows an SEM image of the corner of a sample of loblolly pine sectioned horizontally, radially and tangentially.

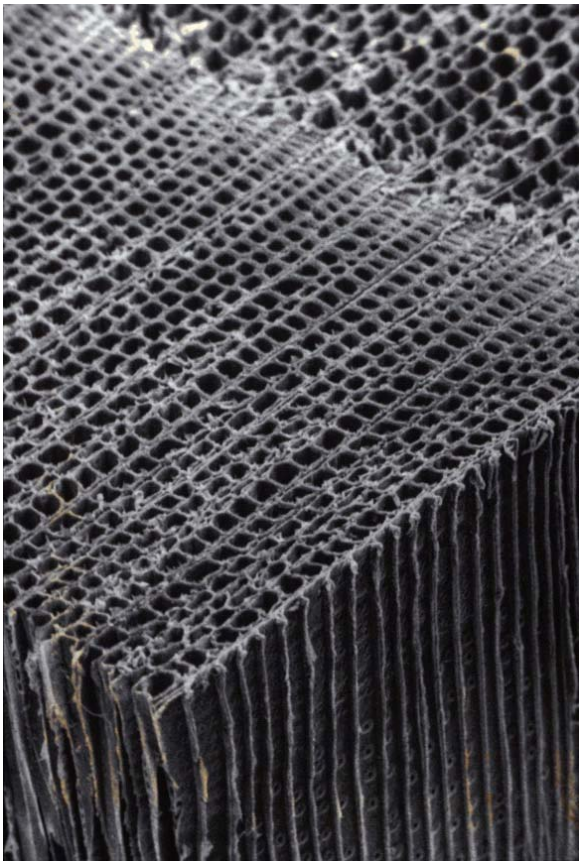

Figure 3. An SEM image of a corner of loblolly pine tissue sectioned horizontally, radially and tangentially. The radial similarity of cells reflects that they are daughter cells of the same

initial in the cambial zone. The transition to latewood and the early wood beyond reflects the simultaneity of changes in cell wall deposition along the perimeter.

Fig. 3 shows that though there may be minor differences between cells deposited along the perimeter, these subtle differences are preserved within the series of daughter cells from the same initial. Thus it is clear that the formation of cell walls must be regulated through the response of intracellular processes that reflect the expression of different parameters for the cell wall in response to signal molecules released through the action of environment-sensing systems in foliage and meristems. Moreover, these processes that appear to be synchronized must be the primary determinants of molecular organization in the cell wall. That this is so is reflected by the fact that no difference has ever been reported in the molecular organization of wood on the basis of northern or southern solar exposure; clearly the side of the tree with the highest solar exposure would be expected to have, on average, a higher temperature during daylight hours. If microenvironment and its influence on kinetics were primary determinants of structure, such differences should have been observed decades if not centuries ago. The conclusion then must be that the organization of the cell wall is regulated by intracellular functions that are synchronized.

In studies of secondary thickening in woody species reviewed by Steeves and Sussex (56), it was shown that mechanical pressure has a significant influence on cell wall organization. However, pressure cannot act alone in regulating morphogenesis because its modulation cannot possibly carry the level of information that is necessary to orchestrate the many processes involved in the patterning of molecular architecture.

There are many aspects of structure that could be examined with respect to synchrony of their development within an annual ring. In relation to secondary walls, three come immediately to mind; the fibril angles within different layers, the radial distribution of hemicelluloses across the layers, and the organization of lignin within the polysaccharide matrix.

The variation of fibril angles within different layers of secondary walls and the relative uniformity of this variation within an annual ring are well recognized. So also is some gradual variation of fibril angles from one annual ring to the next as the tree matures. The implications for cellulose synthesis and deposition are broad and well beyond the scope of the present report. It can be said, however, that any mechanism proposed for the formation of cellulose must allow for synchrony of expression of genetically encoded aspects of structure, even as it also allows for variation of this expression at different stages of development. A high degree of orchestration of intracellular, membrane and extracellular processes within adjacent cells is also implied.

The distribution of hemicelluloses across secondary walls in woody tissue has been investigated for a number of species (60). It is implicit in all of these studies that the distributions are uniform within the same tissue types and within annual rings. In relation to the hemicelluloses, the question of uniformity has not arisen because their basic identity is established within the Golgi apparatus. Further limited modification in the extracellular environment does not depart from intracellular

control, since the population of extracellular glycosidases is the result of intracellularly regulated processes.

The organization of lignin is the one aspect of structure that has heretofore been assumed to be dominated by chemical microenvironment within the polysaccharide matrix. The variables defining microenvironment include the levels of peroxidase and/or laccase activity, the rate of generation of hydrogen peroxide or the availability of oxygen, the local pH levels, and the rates of infusion of precursors into the matrix. But the dominant parameter in any system governed by the kinetics of chemical reactions is temperature. The symmetry of the annual ring, in spite of significant temperature variations during daylight hours, essentially excludes the possibility that temperature is a determinant of structure. This, in turn, suggests that, while microenvironment may influence the progress of formation of covalent bonds between phenyl propane units, it cannot control patterns of inter-unit linkages. These are clearly regulated by intracellular processes that are sufficiently coupled that they can be synchronized among the cells undergoing lignification simultaneously. Regulation and synchrony are most likely to occur through the provision of similar templates for assembly of lignin in cells that are forming their cell walls contemporaneously. A question then arises as to the nature of the templates. The considerations presented in the next section point to hemicelluloses as the most plausible carriers of the information.

7. THE HEMICELLULOSES AS TEMPLATES FOR THE ASSEMBLY OF LIGNIN

In biological systems the primary carriers of information are molecular. It is therefore necessary to consider which molecules involved in the process of wood cell wall assembly are the most plausible carriers of structural information. Cellulose is the key skeletal component in cell walls and, although its pattern of association varies among species and among different tissues within the same specie, it is a constant in cell wall tissue. The hemicelluloses, on the other hand, vary from one tissue type to another, and, within an individual cell wall, can vary with radial position (10,60,61). Furthermore, they are modified whenever the tissue is subjected to external stress, and this modification is correlated with differences in the structure of lignin formed under the same conditions (62). Finally, their assembly within the Golgi apparatus is governed by precisely encoded information. Thus, they are capable of providing the information linkage between intracellular and extracellular processes.

The next question is whether they have the capacity to induce organization in the structure of lignin. Much information has been accumulated to support an affirmative answer to this question. The most significant is the effect of polysaccharide matrices on synthetic lignin analogs. Molecular modeling studies also have shown that the monomers and oligomers of lignin can associate with polysaccharide surfaces and confirm findings of Komamine *et al.* (63) and Haigler *et al.* (64,65) who observed an association of lignification with cellulose and xylan in differentiating tracheary elements from cultures of *Zinnia elegans*.

The remaining question concerns the manner in which information is carried and transferred. Since oligosaccharides are known to be involved in biological recognition processes, and since the side branches of most hemicelluloses tend to be mono-, di- or tri-saccharides, it is plausible to regard them as the key. When one also considers that oligomers of lignin, formed by membrane bound enzymes, occur in the extracellular environment (66,67), it does seem likely that specific oligosaccharide branches are programmed to bind specific monomers or oligomers of lignin and to organize them in space in preparation for their polymerization by radical coupling or propagation reactions. The β-1,4 linked backbones of cellulose and the hemicelluloses may provide sites for binding of the monomers of lignin into ordered arrays, the evidence for which was outlined above.

We would note here that our observations of the influence of hemicelluloses on the structure of cellulose formed in their presence indicate that hemicelluloses also play a role in regulation of the aggregation of cellulose. They appear to be key factors in governing the distribution between I_α and I_β forms. Thus, the role of the hemicelluloses appears to be definition of the overall character of the polysaccharide matrix. This is another instance where extracellular aggregation is under regulation of intracellular processes through control of the identity of the regulatory molecules. It is viewed as an intermediate point in the formation of the cell wall, prior to onset of lignification, but in preparation for it. The influence on the structure of lignin is then regarded as the second stage in the regulatory function of hemicelluloses. Further consideration of these questions is well beyond the scope of the present report.

8. CONCLUSIONS

In conclusion, we believe that analysis of the molecular organization of the constituents of plant cell walls in their native state is an important key to advancing understanding of biogenesis of the plant cell wall matrix. We have examined issues associated with aggregation of the constituents and suggested plausible pathways for the assembly of the matrix that are based on distributed synthesis of the major polymeric constituents. We then considered molecular organization as the first stationary level of expression of phenotypic form and examined the implications of the similarity of this expression within contemporaneous cells in an annual ring of secondary growth. We concluded that all aspects of molecular organization must be governed by intracellular processes that can be orchestrated at levels well beyond those of the individual cells. We have proposed that the primary molecular carriers of organizing information, that is the mediators of the orchestration, are the hemicelluloses, and we have suggested the manner of their action in the formation of the structure of lignin. The results of our analysis of hierarchic organization have a number of additional implications with respect to structure and its formation that are beyond the scope of the present report. One that is worthy of note at this time is that the organization of the deposition of the secondary wall, and its systematic variation within different tissues in higher plants, requires intimate coupling and orchestration of intracellular, membrane and extracellular processes, at levels higher than that of

the individual cell in a manner that, to our knowledge, has not heretofore been considered.

It is also our view that after consolidation of the cell wall, the hemicelluloses continue to play a very important role within the walls. That is to regulate and maintain hydration of the cell wall constituents and act as a counterbalance to the many hydrophobic secondary metabolites that are formed as wood matures. However, this function of the hemicelluloses is beyond the scope of this report.

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