PHYSIOLOGICAL CONTROL OF DIFFERENTIATION OF XYLEM ELEMENTS

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ABSTRACT

The physiological control of xylem differentiation is considered from holistic and reductionist perspectives. Cell type determination is identified as a central problem, but cell quality is also noted as being especially important from a utilization standpoint. The review focuses on biochemical and genetic controls and on cytological events during differentiation. The concluding section deals with the molecular assembly of xylem cells and suggests that a systems theory approach might be a powerful tool in understanding the physiology of xylem cell differentiation.

INTRODUCTION

The wood of trees is composed of a number of different types of cells, namely various types of vessels, fibers, tracheids, and parenchyma. The relative number, arrangement, and qualities of these different cell types determine the physical and mechanical properties of wood. Therefore, knowledge of the mechanisms that control the pattern of differentiation of xylem cells is of central importance as man attempts to maximize the productivity from forest ecosystems. This increased productivity must come from decreasing land areas allocated to forestry (because of population pressure, watershed requirements, and recreational use) and thus investigations on physiological and genetic controls of wood formation become ever more critical.

The physiological control of xylem differentiation has evolved since Silurian time some 425 million years ago. This represents perhaps as few as 4 million generations, an infinitesimal span in comparison to fruit flies or bacteria. However, this interval and the rapidity of tree evolution permit us to assume with some certainty that the control of wood formation in general will be adapted to the functions of the xylem and the tree as a whole. In fact, in some ways the entire forest is said to resemble a superorganism, and in this Clementsian concept the control of xylem cell differentiation within individuals of the forest represents an adaptation that evolved because it benefits the population as a whole.

As a basis for discussing the control of xylem cell differentiation (XCD), we will consider the overall functions of the xylem to be: (1) to transport large quantities of water, solutes, and gases from the roots to all parts of the tree including the foliage, meristems, the ground tissues, and the vascular tissue itself; (2) to provide structural support for the entire tree and to project the foliar organs into a competitive position for light reception; and (3) to serve as a reservoir of water, energy, minerals, and solutes. Any *pattern* of differentiation must serve these functions, and its control system must direct differentiation along channels that are consistent with this objective.

The xylem cells that we observe in the microscope are the structural manifes-

TABLE 1. *Factors in xylem cell differentiation (XCD).*

I. PHYSICAL
   Water, light, temperature, wind, gravity, pressure, stress

II. MINERAL
   Structural, stabilizing, and catalytic effects
   Nutrient-carbohydrate

III. HORMONES
   Types: auxins, cytokinins, gibberellins, ethylene, abscisic acid
   Effects: cell size, number and quality

IV. INTRA-PLANT INTERACTIONS
   Types: organs, meristems
   Effects: energy distribution and production, water relations, and hormones

V. STRESS
   Mechanical, physiological
   Elastic, plastic

VI. GENETICS AND METABOLISM
   Genome: nuclear and cytoplasmic components
   Regulator and feed-back systems
   Environmental sensing apparatus

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tations resulting from the space-time interactions of a genetic system with environmental parameters, i.e. they are cellular phenotypes; physiology is the connecting link between the genetic system and the morphology of the cell. As Klebs pointed out long ago, the external environment must first alter the internal environment of the tree, because it is the internal environment that directly impinges on the genetic system of the xylem cell. The primary factors that affect xylem cell differentiation are given in Table 1. Obviously, these categories overlap and this is one reason that the problem of determining control mechanisms is so difficult. Another reason is that the factors are usually studied individually and by different people.

Unfortunately, it is literally not possible, on the basis of current knowledge, to present a definitive account of the physiological control of XCD due to the fact that the basic processes involved are not fully elucidated. For example, until we know the functional mechanism of phytohormones and their interactions with each other and with the genetic system of the tree that programs XCD, we cannot hope to have a "complete" or "correct" understanding of the control of XCD. The next question is: what do we know about the effect of the control factors? There is now some information on the control of XCD at the biochemical-biophysical level, some at the whole tissue and tree level (Larson 1969), but very little at the intracellular level. For example, there is almost no information as to why one cambial derivative differentiates into a fiber tracheid and another into a vessel element. This cell type determination is the crux of the problem, but it alone is not the only determinant that must be considered, e.g. tracheids of a given coniferous species may vary in cell length, cell diameter, cell-wall thickness, wall density, and chemistry. Thus, cell quality determination is also part of the problem of XCD and from an applied point of view a very important part.

The multidimensional pattern of xylem cell differentiation obviously encom-

passes all aspects of tree biology from molecules to redwoods; however, because of space limitations this paper will consider only biochemical and genetic controls and cytological events during differentiation.

BIOCHEMICAL CONTROL

Quantitatively, the most important constituents required to make a xylem cell are cellulose, hemicellulose, and lignin, the main elements of the framework, matrix, and encrusting substances of the cell wall. Therefore, the control system for XCD must operate in some way on the biosynthetic pathways that result in the formation of these compounds.

Lignin

Lignin is the last constituent to be formed, and the process of lignification begins after cell-type determination has been made, but the extent and distribution of lignin have a marked effect on cell quality. It will be considered first because it is the only cell-wall constituent for which there are actual data for portions of the biosynthetic pathway that are relevant to the control system, and it is the only system in which mutants have been isolated and partially characterized.

The initial step in lignin biosynthesis is the production of aromatic compounds from sugars formed by photosynthesis (for reviews see Berlyn 1970; Sarkanen and Ludwig 1971). The sugars are first transformed to erythrose-4-phosphate (from the pentose phosphate pathway) and phosphoenolpyruvate (glycolytic pathway) which then join to form deoxyarabinoheptulosonic acid phosphate. From here the pathway proceeds through shikimic acid to chorismic acid and thence to prephenic acid which is then aromatized and aminated to form the tyrosine and phenylalanine pools. These amino acids are shuttled into proteins or deaminated to cinnamyl alcohol derivatives that, purportedly, lead directly to lignin. Brown and Neish (1955) provided the first evidence for the involvement of the prearomatic pathway in lignification by showing that randomly labelled shikimic acid and phenylalanine were both extremely efficient precursors for lignins in wheat (*Triticum aestivum*) and boxelder (*Acer negundo*). Since that time considerable supporting evidence has accumulated (see Sarkanen and Ludwig 1971). In fact, Swain (1962) is of the opinion that shikimic acid is the key intermediate in the synthesis of lignins, hypothesizing that the lignin building stones are synthesized directly from shikimic acid and not from the aromatic amino acid pools. He rejects the Freudenberg coniferyl alcohol hypothesis because he claims that for all but a few species it is a "foreign" compound. In his view the lignin building stones are compartmentalized directly from shikimate and long before coniferyl alcohol is formed in the general pathway.

It has been shown in *Neurospora crassa* (Case and Giles 1975; Rines 1968 and 1969; Valone et al. 1971) that there is both an anabolic (synthetic) and catabolic (degradative) prearomatic pathway. However, the degradative pathway in fungi leads to the formation of protocatechuic acid and according to Sarkanen (1971) this is definitely not a precursor of lignins. If these two systems also operate in green plants, it would be logical to conclude that it is the synthetic system that is directly involved with lignification. Evidence has been accumulating (Case and Giles 1975; Chaleff 1974a, b; Valone, Case, and Giles 1971) that indicates that in fungi parts of the catabolic pathway are controlled by an operon type control

system such as those shown to function in bacteria by Jacob and Monod (1961). Genes for the synthetic pathway also have features similar to operons; the genes are clustered and transcribed as a polycistronic messenger, but unlike enzymes encoded by bacterial operons, these eukaryotic polyaromatic enzymes made up a multienzyme aggregate. However, this aggregation was not found in three species of green plants (*Nicotiana tabacum*, *Pisum sativum*, *Phaseolus aureus*) investigated by Berlyn et al. (1970), who showed that in these species the enzymes of the chorismic acid pathway are not associated as a multienzyme aggregate. So far no regulatory gene is known in the fungal synthetic *arom* pathway, but one has been demonstrated in the catabolic system (Case and Giles 1975).

After the establishment of the tyrosine and phenylalanine pools, the factor determining the extent of lignification is the activity of the enzymes phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL). PAL, which was discovered in 1961 (Koukol and Conn 1961; Neish 1961), catalyzes the removal of ammonia from the amino acid, and the formation of $\alpha-\beta$ double bonds in the three carbon side chain resulting in the formation of cinnamic acid. Rubery and Northcote (1968) were able to show that PAL was localized in differentiating xylem of sycamore stems and celery petioles. In coleus internode slices PAL activity was correlated with the differentiation of lignified wound vessel elements, and PAL was also associated with tracheary element formation in *Glycine max* (Rubery and Fosket 1969). The latter workers concluded that PAL is a rate limiting step in lignification because it represents a fork in a metabolic branchpoint, and its activity is closely correlated with xylem differentiation.

Among the cinnamic acids formed as a consequence of PAL activity are p-coumaric acid and ferulic acid. Ebel and Griesebach (1973), using an enzyme preparation from suspension cultured cells of soybean (*Glycine max*), affected reduction of these acids to their corresponding alcohols p-coumaryl and coniferyl alcohol. Mansell et al. (1972), using a cell free system from cambial tissue of *Salix alba*, were also able to reduce ferulic acid to coniferyl alcohol. The conversion required ATP, Mg^{++}, coenzyme A, and reduced pyridine nucleotides (NADPH and NADH); the system yielded a conversion of 40%. The authors speculated that ferulic acid was first converted to feruloyl-Co A. This speculation was confirmed by Gross et al. (1973) and Gross and Zenk (1974) who showed that feruloyl-Co A is indeed a first step in the reaction sequence and that it is subsequently reduced by NADPH to coniferyl aldehyde, which in turn is reduced by NADPH to coniferyl alcohol. One significance of these results is that this portion of the lignin pathway consists of an energy dependent sequence of reactions which suggests that the process of lignification is less random than heretofore believed (Gross 1977).

The only actual lignin mutants known, as far as I am aware, are the four described by Kuc and coworkers for Zea mays (Gee et al. 1968; Kuc and Nelson 1965; Kuc et al. 1968). These are called the brown midrib (bm) mutants and only one (bm₁) has been characterized to any extent. However, with the increasing emphasis on somatic cell genetics in plant agriculture it is hoped that in the future more lignin mutants will be isolated and analyzed by the methods of microbial genetics. The bm₁ mutants of maize have less p-hydroxycinnamic acid than wild type (Bm₁) and a greater resistance to nitrobenzene oxidation (implying lowered syringyl content). The mutant plants have less lignin at all developmental stages,

and at maturity they have 14% less lignin than Bm_1. The methoxyl content of bm_1 is 12.3% as opposed to 14.6% for Bm_1. Tracer experiments showed that uniformly C^{14}-labeled phenylalanine and tyrosine are incorporated much more rapidly in Bm_1 plants. Kuc and Nelson (1964) suggest that the primary action of the Bm_1 gene at the bm_1 locus is to exert a measure of control over the manner of incorporation of the phenolic building blocks into the lignin polymer; when only the recessive mutant gene is present, the lignin "core" has fewer sites at which p-hydroxycinnamic acid can be esterified. These results also imply that the lignin molecule is not polymerized as randomly as the Freudenberg hypothesis suggests.

Hemicellulose

The matrix constituents have a very specific association with cellulose microfibrils and comprise a supramolecular system with the cellulose even though the association is wholly or predominantly non-covalent. The main hemicelluloses of hardwood xylem are glucomannan and 4-O-methyl glucuronoxylan, while in conifers the main hemicelluloses are galactoglucomannan and arabino-4-O-methyl-glucurono-xylan (Dutton 1963; Lindberg 1962; Meier 1961, 1962, 1964; Northcote 1963a, b Perilä 1961; Schuerch 1963; Timell 1964, 1965; Wise and Lauer 1962). Albersheim and his coworkers have recently established that the main hemicellulose of the primary cell walls of suspension-cultured sycamore maple (*Acer pseudoplantanus*) and bean (*Phaseolus vulgaris*) is a xyloglucan (Albersheim 1974, 1975; Bauer et al. 1973; Wilder and Albersheim 1973). In sycamore maple the xyloglucan is the only hemicellulose present in suspension cultured cells. Albersheim concludes that the xyloglucan is a feature of all primary cell walls and he terms it "the cell wall hemicellulose." The existence of this xyloglucan was confirmed for intact plants by Jacobs and Ray (1975) and Labavitch and Ray (1974a, b).

The xyloglucan consists of a repeating heptasaccharide unit of 4 residues of β-(1,4)-linked glucose plus 3 residues of xylose. A single xylose is linked to carbon 6 of three of the glucose units by glycoside bonding. There is an additional unit that differs from the above by having a fucosylgalactose disaccharide attached to one of the xylose residues. Albersheim's analytical techniques employ purified polysaccharide-degrading enzymes coupled with gas chromatography-mass spectrometry. The suggested function of the xyloglucan hemicellulose is to provide a single layer coating for the cellulose microfibrils. It does this through the ability of its β-(1,4)-linked glucan backbone to hydrogen bond to the β-(1,4)-glucan chains of the microfibrils. The fucosylgalactose side chains prevent additional cellulose chain molecules from bonding to those xyloglucan chains that are already hydrogen-bonded to associated microfibrils. The reducing end of many of the xyloglucan chains is covalently attached to pectic polymers, and thus the xyloglucan serves as a bridge between the cellulose framework and the rest of the matrix and possibly the encrusting substances. The physiological control over a complex web of supramolecular structure such as this must be very complicated, but there is very little specific information on this control system.

There is general agreement now that the sugar monomers of cell-wall polysaccharides are donated by nucleoside diphosphate sugars (see review by Delmer 1977). Kauss and his coworkers have studied the biosynthetic systems involved

in "hemicellulose" synthesis (see Kauss 1974). Most studies to date have used particulate preparations, and it has not been possible to eliminate glycosyl interconverting enzymes from the particles. For example, exogenous UDP-glucose may be added to the system, but the particle can change it to UDP-galactose or UDP-arabinose before incorporation into a polymer. However, Odzuck and Kauss (1972) were able to selectively inactivate the xylose and arabinose transferases. They found that the arabinose transferase required an optimal concentration of Mn^{2+} for activity, while the xylose transferase had no Mn^{2+} requirement. Presumably the metal has catalytic and/or conformational function. Kauss has also determined that the glucuronic acid donor for hemicellulose is UDP-glucuronic acid, and that the methyl groups are derived from S-adenosyl L-methionine (SAM). The latter donation is mediated by a hemicellulose methyl transferase whose activity is enhanced by Co^{2+} (4-fold stimulation) or Mn^{2+} (2-fold stimulation). The enzyme is not affected by Mg^{2+}, Ni^{2+}, or Zn^{2+}. This is in marked contrast to the situation for the transfer of methyl groups from SAM to the carboxyl groups of the polygalacturonic residues of pectin. The transferase in this case (pectinmethyl transferase) is not stimulated by Co^{2+}, Mn^{2+}, or Mg^{2+}, and is inhibited by Zn^{2+} and Ni^{2+}. The metal co-factors may well be involved in the biochemical regulation of cell-wall synthesis, but their role is not clear at this time. Ligand functions may also be involved since EDTA inhibits the hemicellulose methyl transferase but stimulates the pectin methyl transferase. Methylation, at least in the case of pectin, occurs after polymerization, but Kauss indicates that the two processes occur in a closely linked structural compartment that contains both the substrate and the required enzymes. Thus, the particulate enzyme particles isolated by the biochemists are either contained within the structural compartment or closely associated with it. Present evidence would support that the structural compartment is identical with the Golgi vesicles.

Cellulose

The synthesis of cellulose is also mediated by sugar nucleotides that serve as glycosyl donors (Delmer 1977). Particulate enzyme fractions have been isolated that form alkali-insoluble polymers *in vitro* using either guanoside diphosphate glucose (GDPG) or UDPG. However, alkali insolubility *in vitro* is no guarantee that cellulose is present, since it is possible to produce insoluble galactan *in vitro* using UDP-Gal and no such polymer is known as a natural plant product (Panayotatos and Villemez 1973). Villemez (1974) outlines a number of problems in using *in vitro* cell-wall enzyme systems. By careful studies of polymer size, Villimez (1974) concluded that cellulose synthesis probably utilizes UDPG as the glucose donor because of the high degree of polymerization (DP) of the polymer produced. He also stated that GDPG is most probably involved in glucomannan synthesis because the polymer produced in this case had a peak DP of about 80 and a molecular weight of less than 28,000. These latter data agree quite well with Timell's (1960) determination of glucomannan size. This role of GDPG was recently confirmed by Hinman and Villemez (1975) for *Pisum sativum* as well as *Phaseolus aureus*. However, Hopp et al. (1978) claim that there are both lipid and protein intermediates in cellulose synthesis and that both UDPG and GDPG are required in the pathway. In their view UDPG donates a glucose molecule to

the lipid (dolicholphosphate), which in turn links up with an oligosaccharide that participates in the formation of a glucoprotein. Cellulose is formed by the donation of glucose residues from GDPG to the glucoprotein.

As the primary wall is formed, the DP of the cellulose molecules ranges from 2,000–6,000, but the cellulose in the secondary wall has a DP of 14,000 or higher and is remarkably monodisperse (Marx-Figini 1971; Marx-Figini and Schultz 1966). Thus, in the case of the secondary wall we have an enormous linear macromolecule whose DP is precisely controlled to yield a monodisperse product. Villemez (1974) points out that among other high DP biopolymers only nucleic acids and proteins are monodisperse and these polymers are formed on templates through the mediation of a code. Mühlethaler (1967) suggested that the wide range of DP of the primary cell-wall cellulose makes a template mechanism doubtful. However, there are rather stringent time restrictions on primary wall formation, and the polymerization of cellulose in this case could either be a wholly time-dependent process or a time-limited template process. A template mechanism for formation of cellulose in the secondary wall is appealing and the stereospecific hypothesis of Stanley and coworkers (Des et al. 1969) may provide some evidence for this. They found that glucan synthesis from UDPG was stimulated by different sugars and glycols and that D-arabitol enhanced the rate 6.5 times, while L-arabitol increased it only 2.1 times over the control.

Evidence has accumulated to suggest that lipid intermediates may be involved in cellulose formation (Alam and Hemming 1971; Colvin 1961, 1964; Elbein and Forsee 1973; Hopp et al. 1978; Kauss 1974; King and Colvin 1976; Pinsky and Ordin 1969; Van Der Woude et al. 1974; Villemez et al. 1968). However, Kauss (1974) states that present evidence suggests that a mannosyl-lipid is involved only with side chain formation in glucomannan synthesis, and he sees no evidence that the concept of lipid intermediate can be applied to other cell-wall hemicelluloses or to cellulose. Leppard et al. (1975) have recently reported that in *Acetobacter xylinum* and *Acetobacter acetigenum* there is a nascent form of the cellulose microfibril which is wrapped in an amorphous sheath consistent with the concept of an intermediate polymer or polymers in cellulose biosynthesis. They did not determine the chemical nature of the sheath, and existence of such a sheath in higher plants also remains to be demonstrated.

SITES OF SYNTHESIS

The main cytological components thought to be involved in or associated with cell-wall synthesis are the wall itself, the Golgi apparatus, microtubules, the endoplasmic reticulum (ER), vesicles, and particles located at the cell wall/cytoplasm interface (i.e. outer surface of the plasmalemma). Radioautographic studies have indicated that cellulose is the constituent deposited by apposition outside the plasmalemma, while matrix substances are intussuscepted into the existing wall (Ikenberry and Berlyn unpublished; Ray 1967).

Cell wall

Lignification begins at the cell corners (middle lamella area) and moves centripetally through the primary and then the secondary wall (Berlyn 1964, 1972; Berlyn and Mark 1965; Larson 1969; Wardrop 1957, 1965, 1976). Therefore, at least the final polymerization process actually occurs within the wall. The walls,

especially the primary wall, may not be nearly as inert as is often assumed, e.g. considerable cytoplasmic material may be incorporated in the wall especially at the cell corners (Berlyn 1970) and a number of hydrolytic enzymes (viz. esterases, lipases, and acid phosphatase) have been localized in the wall (Olszewska et al. 1966). The differentiation and growth of the wall are also intimately associated with hydroxyproline-rich glycoproteins (Chrispeels 1972; Dougall and Shimbayashi 1960; Lamport 1965, 1967, 1969; Lamport and Miller 1971; Lamport and Northcote 1960; Monro, Bailey, and Penny 1974; Sadava and Chrispeels 1973; Vaughan 1973; Winter et al. 1971). These glycoproteins have been termed "extensins" (Lamport 1965) because they are thought by some to be involved with extension growth of cells. The proteins cross link polysaccharides, i.e. carbon one of arabinose is glycosidically linked to carbon four of the hydroxyproline units of the protein. According to the Albersheim model 3,6 linked arabinogalactans are attached to serine units of wall protein (Keegstra et al. 1973). Thus, the glycoproteins of the wall have a polypeptide backbone with oligosaccharide side chains (Lamport 1969). Presumably "extensin" changes in conformation upon the addition of indole acetic acid (IAA) giving rise to increased wall plasticity; this conformational change is postulated to be due to reduction of disulfide bridges in the glycoproteins (Lamport 1965) although the initial effect of auxin is hydrogen ion secretion. Cleavage of disulfide bonds in yeast has been shown to require the enzyme disulfide reductase (Nickerson 1963) and this would mean yet another active protein function in the primary cell wall. Cleland (1968) suggests that the role of hydroxyproline proteins may be to determine the correct orientation of hemicellulose molecules as they are being incorporated into the supermolecular structure of the wall. The possibility also exists that extensin itself may have enzymatic function (Lamport 1965; Ridge and Osborne 1971; Vaughan 1973). Mühlethaler (1967) presented evidence that particles external to, but associated with, the plasmalemma are responsible for cellulose synthesis, and he postulated that these particles contain extensins. In his scenario, extensin has a role in both the bonding of the microfibrils and in their orientation. The involvement of plasmalemma glycoproteins in cellulose synthesis has recently been proposed by Franz (1976), but he did not examine its relation to extensin.

Microtubules

These structures were once proposed as possible organelles of cell-wall synthesis (Hepler and Newcomb 1965; Ledbetter and Porter 1963, 1964; Murmanis 1971). Microtubules are present in both plants and animals, and the extensive literature has been the subject of several reviews (Hepler and Palevitz 1974; Newcombe 1969; Soifer 1975). Microtubules range in diameter from 180–300 Å. The wall of the microtubules is ca. 70 Å thick and is composed of 13 globular protofilament subunits (each about 40 Å in diameter) that are equally spaced (ca. 45 Å center to center) around the circumference of the tubule. The structural subunits are α- and β-tubulin, proteins whose structural subunit is a dimer of 110,000 to 120,000 molecular weight. However, the 40 Å protofilament seen in the electron microscope must be the monomer with a molecular weight of 55,000–60,000 because the heterodimer would not fit into the 40 × 40 × 50 Å space of the morphological structure. The tubulins contain nucleotide binding sites that could function in polysaccharide assembly as recognition and/or orientation sites.

The spindles of dividing cells consist of bundles of microtubules that are often aggregated in specific patterns in animal tissue. However, recent evidence does not support a biosynthetic role for the microtubules, although many workers now postulate that they function in the orientation of microfibrils, because in a number of studies the microtubules were shown to mirror the orientation of the adjacent microfibrils (Cronshaw 1965; Gantt and Conti 1965; Ledbetter 1965; Marx-Figini and Schultz 1966; Robards and Kidwai 1972). In other studies, however, there appeared to be no correlation between microtubular and microfibrillar orientation (e.g. Mühlethaler 1965, 1967; Newcomb and Bonnett 1965; Pickett-Heaps 1967; Preston and Goodman 1968).

Recent evidence obtained through the use of high-voltage (10^6V) electron microscopy and fluorescent antibody techniques suggests that microtubules are only the largest of at least three classes of cytoplasmic filaments. These structures form a three-dimensional network that comprises the framework for the metabolic activities of the protoplast. The smallest elements, termed microfilaments (50–60 Å), are also postulated to function in moving organelles around in the cell. The intermediate microfibers (70–100 Å) are not well characterized (Miller 1977).

Golgi

The first evidence of wall differentiation is the development of the cell plate after mitosis, and Golgi apparatus is the primary organelle involved (Brown 1969; Mollenhauer and Morré 1972). The Golgi have also been implicated in the differentiation of the primary and secondary wall by numerous investigators in recent years, although there is still controversy as to which wall components are synthesized and/or processed by the Golgi complex (Berlyn 1970; Northcote 1974; Ray 1967, 1975; Ray et al. 1969; Shore and Maclachlan 1975; Van Der Woude et al. 1974; Van Der Woude and Suarez 1975; Whaley et al. 1972).

The individual Golgi complex (or apparatus) consists of a central stack of flattened sacs in the shape of a shallow cup and associated vesicles, which are profuse along the margins of the stack and also can be seen internally in various states of development. The term Golgi complex is used to refer to both the individual assemblage and the cell's entire complement of Golgi. In the past the term dictyosome was used to refer to individual assemblages in both plants and animals or as a distinctive term for the Golgi of plants. Each flattened sac of the central stack is disc-shaped and consists of a membrane enclosing an interior space of about 150–200 Å except that the margins of each disc are swollen so that the interior space in this area is ca. 600–800 Å. The central stack usually consists of 4–8 flattened sacs that are spaced about 200 Å apart and are slightly cupped. The spacing of the cisternae (sacs) is regulated by "binding substances" of unknown structure (Mollenhauer and Morré 1972). The binding substances occupy some 20–40% of the total volume of the Golgi complex. The binding substances may be rapidly cleared (5 to 15 seconds) with phosphotungstic acid (PTA) or more slowly removed with monovalent salts. Amino acid crosslinking substances retard the unstacking of the Golgi and thus phospholipids and proteins appear to be constituents of the binding substance (Mollenhauer and Morré 1972). The binding substance is thought to be related to the "zone of exclusion" that exists around Golgi bodies as a sort of morphogenetic field. Along the concave face and periphery of the Golgi complex are numerous small vesicles ranging in

size from 200 to 1,000 Å in diameter. The vesicles continually bud off from the complex and seed out into the cytoplasm. They may continue to enlarge and may reach diameters of 1 μm or more (Berlyn 1970) and contain fibrils, granules, and tubules. The site of vesicle loading is probably in the Golgi complex itself (Caro and Palade 1964; Northcote and Pickett-Heaps 1966) although some loading may occur in the "transition elements" between the ER and the Golgi. In plant cells the Golgi complex appears to be more or less evenly distributed in the cytoplasm; they are more numerous (up to several hundred per cell) in differentiating tracheids, fibers, and vessel elements; all cells that are actively forming cell walls. Secretory cells of animals (e.g. salivary gland cells) may contain thousands of Golgi per cell. The classic study of Caro and Palade (1964) confirmed the previously postulated secretory function of the Golgi. They administered radioactive amino acids to pancreas cells and by sequentially fixing cells, were able to follow the path of the label. After three minutes the label was localized over rough ER and from there it moved to vesicles of the ER; within 20 minutes it was localized in the Golgi sacs. From there is moved into Golgi vesicles, which were observed to discharge their contents to the cells exterior while the vesicle membranes fused with the plasmalemma. These results have been amply confirmed by other investigators (see review by Favard 1969).

Northcote and his colleagues have fed labelled carbohydrates to a number of plant tissues and have observed the label to move to the Golgi and subsequently to the cell walls (Harris and Northcote 1971; Northcote and Pickett-Heaps 1966; Wooding 1968). When labelled glucose was presented to cells and the labelled products from the walls were analyzed, it was found that the label was located in many other monomers besides glucose, e.g. galacturonic acid, glucuronic acid, galactose, mannose, arabinose, xylose, fucose, ribose, and rhamnose (Northcote 1974). Membrane fractions (Golgi and ER) also showed this transformation; however, the Golgi fraction had at least twice the activity of the rough ER, and thus Northcote (1974) concluded that the Golgi apparatus is an extremely important focal point in the synthesis and transport of plant polysaccharides. However, no glucans could be isolated from the Golgi of higher plants and Northcote postulated that the Golgi was primarily involved in the synthesis of the matrix components of the cell wall, i.e. pectin and hemicellulose. This view has been supported by a number of other investigators (Berlyn 1970; Ray 1975; Van Der Woude and Suarez 1975; Van Der Woude et al. 1974).

In the alga *Pleurochrysis scherffelii*, Brown et al. (1969) and Brown and Romanovicz (1976) have shown that cellulose scales are synthesized by both the membranes of ER and the Golgi apparatus and in fact can be seen in the Golgi vesicles before transport to the wall. The scale is a special structure in a special taxon and contains many substances besides cellulose, but it is known that the Golgi of higher plants also possess glucan synthetase (Ray 1975; Van Der Woude et al. 1975). However, Ray (1975) postulated that Golgi bound glucan synthetase (glucosyl transferase) of higher plants functions in concert with xylosyl transferase to mediate the synthesis of hemicellulosic xyloglucan for export to the cell wall. Northcote (1974) describes the Golgi apparatus as a one-way valve that develops from the ER and shuttles material along the entire membrane system, beginning with the nuclear envelope and culminating in the plasmalemma. The Golgi vesicles not only provide pectic and hemicellulosic constituents but also

fuse their membrane with that of the plasmalemma and thereby increase its girth. This view is consistent with autoradiographic data. Lysosomes are also postulated to develop from the Golgi vesicles, and thus the Golgi may exert control over both anabolic and catabolic processes in the cell. They may, for example, control the degeneration of the cytoplasm in maturing tracheary cells of the xylem.

Plasmalemma

The plasmalemma is thought to be the primary site of cellulose synthesis and deposition by a number of workers (Berlyn 1970; Franz 1976; Mühlethaler 1965, 1967; Northcote 1974; Ray 1975; Van Der Woude and Suarez 1975; Van Der Woude et al. 1974). Van Der Woude and Suarez (1975) suggest that cellulases are positioned on the outer surface of the membrane and that cytoplasmic microfilaments are involved in vectorially moving the enzymes during ordered deposition of microfibrils.

Recently, additional evidence has been presented that supports the plasmalemma particle or granular hypothesis of cellulose microfibril synthesis (Ruiz-Herrera and Bartnicki-Garcia 1974; Ruiz-Herrera et al. 1975). In this case the authors studied the synthesis of chitin microfibrils of fungi. They found that these microfibrils arose from 350–1,000 Å granules and that they were formed by end-synthesis. Particles have also been implicated in microfibril synthesis by many previous authors (Moor and Mühlethaler 1963; Mühlethaler 1965, 1967; Preston 1974; Robards 1969), but the evidence produced by Ruiz and coworkers is particularly striking. Murmanis (1971) reported particles ranging in size from 130 Å to 160 Å in *Pinus strobus*.

A number of investigators (Berlyn 1970; Fowke and Setterfied 1969; Mahlberg et al. 1974) have observed several types of membrane-bound bodies depositing material into the cell wall (for review, see Roland 1974). In the case of Berlyn's type 2 lomasome, the deposited material consisted of particles of ca. 1,000 Å in diameter. These particles were present in both pine and lentil and are in the same size category as those observed by Ruiz-Herrera and co-workers.

The plasmalemma could function as a control site in several ways. The membrane contains proteins and some of these proteins are enzymes. A growth hormone from the leaves can attach to membrane-bound enzymes from either the symplast or the apoplast. This can be an allosteric effect whereby the enzyme changes in conformation to expose or create an active site that can function in the differentiation process of the cell wall. This concept provides for the required amplification, because a single hormone molecule may bind to a single enzyme, but the enzyme can catalyze the transformation of a large number of substrate molecules to product molecules. Other growth substances might also bind to specific proteins in the plasmalemma and thereby alter its transport properties, which in turn might initiate or accelerate part of the differentiation process. This is post-translational control.

On the other hand, the proteins on the membrane might change quantitatively and/or qualitatively during development and differentiation and these space-time sequences could be controlled at the transcriptional and/or translational level. Another possibility is that when membrane transport properties are altered, the environment of the nucleus becomes changed in a manner that induces and/or inhibits gene action. Goldstein (1976) has presented evidence that small nuclear

RNAs (snRNAs) play a role in the control of gene action. These metabolically stable snRNAs are normally contained within the nucleus during the non-mitotic portion of the cell cycle, but they are not bound to the chromosomes to any extent. The snRNAs are freed to the cytoplasm at metaphase and by late anaphase they associate with the chromosomes to an extraordinary degree. As mitosis continues, the snRNAs lose their association with the chromosomes but are contained within the newly-formed nuclear membrane. This occurs as the acidic nuclear proteins (ANPs) re-enter the nucleus (about 3 h). At this point the cell plate is forming and both transcription and translation are proceeding at a rapid rate. Hormone mediated changes in membrane transport properties might also be important in this sequence. It is controversial as to whether plant cells require a quantal mitosis before differentiation of their cellular phenotype following an inducing state change in the cell. Torrey et al. (1971) claimed that they do, and if this is in fact the case, then, according to Goldstein (1976), the primary event could be the removal of old ANPs from the chromosomes and their replacement or partial replacement with new ANPs that would differentially displace snRNAs.

ASSEMBLY OF XYLEM CELLS

The preceding portions of this paper have dealt with the basic biochemical and cytological systems that serve as loci for control of xylem cell differentiation (XCD). The general dogma is that XCD at any point in the plant is controlled by substances that are produced in the leaves (or buds) and transported through the vascular system of the shoot to the vascular cambium and differentiating xylem cells. These substances (hormones, sugars, and possibly others) initiate (and terminate) the mitotic phase and also fuel the subsequent phases of differentiation. However, the pattern of differentiation is modulated at every point in space and time by the physical and chemical interactions between cells and tissues. Hormones are of course also produced in roots (e.g. cytokinins) and nearby cells (e.g. ethylene) and these may also function in the XCD system.

Restricting our consideration to fibers and vessel elements, differentiation may be summarized in the following six phases: (1) mitosis and cytokinesis; (2) primary cell-wall formation; (3) cell enlargement; (4) cell-wall thickening; (5) lignification; and (6) cytoplasmic senescence and elution.

These complex phases are controlled by a cybernetic system. The system consists of a sequence of subsystems that form an interactive complex with the environment, which is here defined as the set of all objects and influences that can change the state of the system. Biological systems such as this have three unique properties in that they are: (1) open (exchange energy and material with the environment); (2) adaptive (exhibit differential sensitivity); and (3) homeostatic (exist in dynamic steady states with feedback controls). The XCD system requires essential biochemical components and also a series of regulatory components. According to Amen (1966) the advantage of the systems theory approach is that it achieves an integration of data and delineation of significant relations and relates cell biological processes to common physical reference points.

The enzymatically catalyzed assembly of xylem cells is powered directly by ATP energy, but the ultimate energy source is the negentropy of the sun which flows through the leaves and (in variously transformed manifestations) into the sites of xylogenesis. Some of this energy is in the form of information because

the adaptive and homeostatic functions of the XCD system require information in order to be operational. Even at the turn of the century, the paradox of Maxwell's demon suggested that there was a relationship between entropy and information, but the relationship was not finally quantified until 1948 (Shannon 1948). It requires 10^{23} bits (binary digits) of information to reduce the entropy of a system by 1 cal/mole-deg (Lehninger 1965). Thus, one calorie is equivalent to an enormous amount of information and this is one reason why communication and storage of information is a relatively cheap process economically and thermodynamically.

According to Shannon (1948) entropy or uncertainty, S, in information terms can be expressed as follows (Tribus and McIrvine 1971):

$$S(Q|X) = - K \sum P_i \ln P_i$$

where Q = a question of interest
     X = knowledge about Q
     P_i = probabilities assigned to the possible answers to Q. $0 \leqslant P \leqslant 1$
     K = arbitrary scale factor

If $P = 1.0$ for one answer (and therefore zero for all other answers), S = 0. That is, if you know the right answer you have no uncertainty. If your information does not permit you to distinguish one answer from another, then all possible answers have equal probability and S is maximized. This is a state of great ignorance that is only surpassed when you do not even know the Question, Q. Shannon's concept is independent of the means of communication and is therefore applicable to biological systems, such as the XCD system. It has the added property of also being useful in determining the codes used in the system.

Information, I, in bits can be expressed as $\ln_2 P_r/P_s$ (Morowitz 1970) where P_s is the probability that a signal was sent and P_r is the probability that the signal was received by the target cells. In the XCD system $P_r = 1.0$ and $I = -\ln_2 P_s$. For example, consider a wall polysaccharide composed of 4 common monosaccharide monomers: $I = +\ln_2 4 = 2$ bits. This means that in the polysaccharide synthesizing subsystem, two correct binary choices would be needed to select the correct sugars. If the DP is 200, the information required per polysaccharide is 4×10^2 bits. On this basis the cell wall alone probably requires more than 10^{15} bits, but such enormous information requirements are circumvented to some degree by preprogrammed self-assembly systems, wherein the specification of one element of a system also specifies several other attributes of the system such as, for example, shape and orientation. Structural compartmentalization may also reduce the number of choices available for a specific process.

In conclusion, it can be said that the reactions that control xylem differentiation are of great interest, but that they are as yet unknown. In such a system, where the microstates and structures are unknown or partially unknown a classical thermodynamic and/or information theory analysis may be very useful.

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