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Intrinsic Regulation of Cambial Growth

Rodney Arthur Savidge*

Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6C2

Abstract

Secondary growth in trees is an attractive system for explaining, through concerted research into mRNA, proteomics, and cell biology, how eukaryotic cellular differentiation is regulated. Differentiation pursuits by genetically uniform cambial derivatives are diverse, less than perfectly repetitive in time and space, and readily modified experimentally. Within each zone of both xylo- and phloiogenesis, competence for at least pluripotent, and not uncommonly totipotent, development evidently is retained. Thus, hypothetical concepts of cellular differentiation 'programs' and 'determined histogenesis' lack support beyond formation and continuing perpetuation of cambium as template for production of similarly shaped and sized daughter cells. The several distinct developmental zones of wood formation manifest metabolic plateaus, and their transitional regions indicate where equilibrium becomes overbalanced and cascades to the next step, changing cells biochemically, hence anatomically, into differentiated states. It remains unclear if differential gene expression during wood formation is strictly of a quantitative nature or if it also varies qualitatively. In addition to selective transcription, another plausible regulatory mechanism is quantitatively varying but still totipotent expression of so-called 'housekeeping' genes modulated through spatial information and changing environments, for example, at levels of mRNA supply and stability. The environment of fusiform and ray cells of the cambial region comprises, in addition to dynamic maintenance metabolism, fluxes in phytohormones, carbohydrates, water, O_2, root nutriment, and physical factors capable of influencing both gene expression and enzyme kinetics. In addition to phloem and xylem transport, intercellular communication is normal to cambium and its differentiating derivatives; thus, the procambium-cambium continuum appears to be a living 'fibre' communication network plausibly serving to integrate growth and development throughout the whole plant.

Key words: Cambium; Cellular differentiation; Commitment; Determination; EST; Gene expression; Specification

Introduction

Despite the underlying importance of secondary growth to terrestrial ecology, environmental science, forestry, and all of the varied industries dependent on woods and barks as raw resources, intrinsic regulation of cambial growth remains one of the least well-explained aspects of the biological sciences. The continuing ignorance is in part due to grossly oversimplified textbook treatments in deference to phenomena of primary growth, photosynthesis, and reproductive development. Acquisition of knowledge has also been thwarted by a long-

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Corresponding author; e-mail: savidge@unb.ca

standing attitude that research unable to solve "real world" forestry problems in the short term falls within the realm of "toy research" (Horn 1880; Sprague and Sprague 1976). Some research areas relevant to secondary growth nevertheless have begun to flourish, and a number of recent reviews have addressed questions of regulation (Fukuda and others 1998; Altmann 1999; Kost and others 1999; Lachaud and others 1999; Leyser and Berleth 1999; Thompson and Schulz 1999; Volkmann and Baluska 1999; Olsson and Little 2000; Sachs 2000; Savidge and others 2000; Wojtaszek 2000).

'Cambial growth' as used here includes formation of secondary xylem and phloem as well as formation, continuing areal expansion and maintenance of the vascular cambium itself (hereafter referred to simply as 'cambium'). Dendrochronology informs us that cambial growth in healthy trees on nutrient-adequate sites predictably occurs each year, provided environmental factors such as warmth, sunlight, soil water, O_2, and CO_2 are not limiting. In nature, cambial growth requires the presence of a root system capable of supplying water, nutrients, and organic metabolites to the above-ground parts, and living mature autotrophic organs are also essential (Büsgen and Münch 1929; Savidge and Wareing 1981b; Steeves and Savidge 2000). It is possible for springtime cambial reactivation to commence in some species of temperate-zone deciduous species, and also in experimentally defoliated conifers, by cambium drawing on storage reserves before bud scales separate or leaves mature, but ongoing cambial growth requires that mature leaves or needles, as well as roots, be present (Büsgen and Münch 1929; Savidge and Wareing 1981b; Steeves and Savidge 2000). How extrinsic conditions, roots, and photosynthetic organs foster the processes underlying secondary growth reduces to questions concerning translocation of energy and chemical compounds from sources, their allocation to and utilization by cells of the cambial region. Thus, to explain how secondary growth increments become variably distributed over tree axes, it is necessary to understand transport and unloading phenomena and how they are controlled (for recent reviews, see Savidge and others 2000).

At a finer level, there are many unanswered questions on how developmental phenomena known to occur within and on the inner and outer peripheries of the cambium may be regulated. Only some of those phenomena can be considered below, and it should be appreciated that there are limited biochemistry, cell, and molecular biology data on cambium. However, in the final analysis, my view is that the key generic questions needing answers can be stated as follows: Is the cambium epigenetically committed to vascular development? If so, is that commitment specified or determined? What is the genetic/biochemistry basis for commitment? How completely is the committed state transferred from cambial cells into cambial derivatives? How does the initial commitment translate into several possible pathways of cellular differentiation? If not committed, what are the inductive stimuli that promote cambium formation, maintain cambium, promote/inhibit cambial cell-division activity, promote/inhibit cellular differentiation? What regulatory mechanism is activated by each stimulus? What is the genetic/biochemistry basis for each of the triggered expressions? This review hopefully will stimulate ongoing research, but students should understand that the reflections offered below are my personal perspective, not necessarily attuned to the ideas of other researchers. Note that definitions and some other preliminary matters to what follows are dealt with in Appendix I.

Phenomena of Cambial Growth

Anatomical evidence across the Tracheophyta indicates that cambium formation and subsequent production of cambial derivatives are not obligatorily linked to the phenomenon of wood formation, nor is either development necessary for plants to become woody. For example, in storage organs of dicots such as carrot and beetroot (and many other species), cambium formation occurs and subsequent cambial activity facilitates expansion of cambial area (that is, increasing diameter and length of the 'cylinder' of cambium) and results in radial files of derivatives being produced, but if those derivatives ever become prosenchyma it is only much later, usually when the shoot bolts as a prelude to flowering. Similarly, at stem locations basal to the crowns of sapling and larger conifers, springtime cambial activity produces thin-walled derivatives (Figure 1a) which, in contrast to the rapid xylogenesis normally following cambial reactivation in hardwood species, remain in their enlarged nondividing state of secondary cellular differentiation over a protracted period before beginning to convert into tracheids (Savidge 1983b, 1996, 2000a). In conifers and dicots, prosenchyma arise in locations not produced by cambium (for example, in pith, bark, trichomes, stipules, cone scales, seed coats, and so on) by parenchyma differentiating into sclerenchyma (Fahn 1974; Janakowski and Golinowski 2000), indicating the ability for cells not of cambial origin to activate similar if not identical biochemical path-

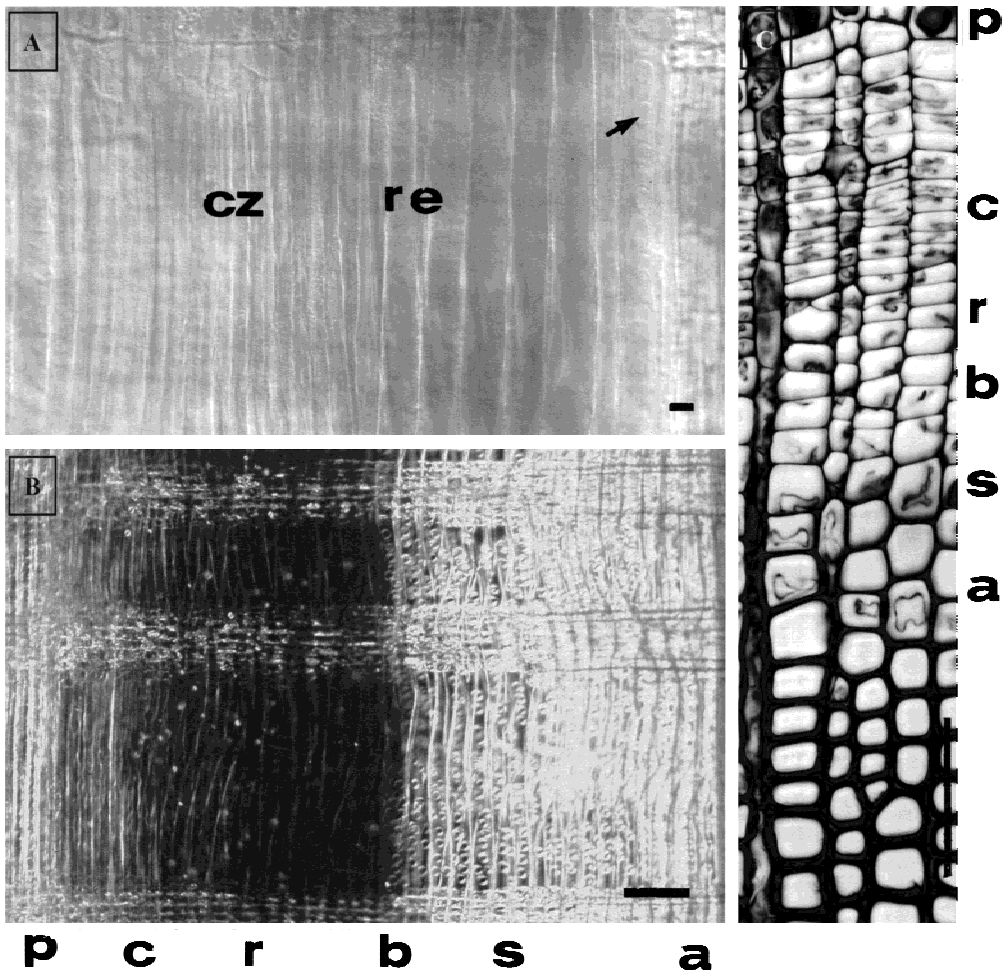

Figure 1. (**A**) Hand-cut unstained radial section of *Pinus strobus* cambial region as it appeared under interference contrast on May 9th, at the time of commencing the experiment. The last mature late wood tracheid is on the extreme right, phloem on the left. The cambial zone (cz) and zone of primary-wall radial expansion (re) are indicated. The cambial derivative adjoining the late wood has initiated bordered-pit development (arrow) but is still primary walled. Bar 10 µm. (**B**) Hand-cut unstained radial section showing mature phloem (p), cambial zone (c), zone of primary-wall radial expansion (r), zone of bordered-pit initiation (b), zone of secondary-wall formation and lignification (s), and zone of protoplasmic autolysis (a). The earlywood - latewood boundary is on the extreme right. Several rays traverse the zones. Bar 100 µm. (**C**) Microtomed cross-section (20 µm thickness) showing stages of differentiation of cambial region when collected in midsummer and fixed in FAA, embedded in paraffin, sectioned, and stained with safranin O -fast green FCF. Plasmolysis, so conspicuous here, is not evident when freehand sections are mounted in water, as per A and B. The zones explained above under B are indicated with the same designations. A ray traverses the view on the left side of the image. Bar 100 µm.

ways needed for attainment of the 'woodiness' attribute. It appears, therefore, that gene expression for prosenchyma formation cannot be attributed to an epigenetic change specific to cambium. In bamboo, palm trees, asparagus, and other woody monocots, prosenchyma also originate in the absence of cambium (Liese and Weiner 1996). Moreover, monocot sclerenchyma usually arise by cells that for an extended duration have been in a state of secondary cellular differentiation before commencing further differentiation. Looking briefly at the fossil record, it was not uncommon for Carboniferous 'trees' (for example, the giant horsetail *Calamites*) to grow 30 m and more in height, achieve large diameters, and become woody without forming cambium, whereas lycopods such as *Lepidodendron* and *Sigillaria* evidently produced a unifacial cambium supporting only wood formation (Kenrick and Crane 1997; Bateman and others 1998; Kenrick 2000). The above and additional observations point to distinct regulatory mechanisms operating, sometimes in concert and other times individually, to

promote or inhibit cambium formation, cambial cell-division activity, cell enlargement, and differentiation of cells into prosenchyma (Savidge 1983a, 1985, 1996).

In Magnoliophyta and Coniferophyta, cambium is considered to have formed after interfascicular parenchyma differentiate into fusiform cells, the characteristically radially compressed appearance of cambial fusiform cells being generated mechanistically by periclinal divisions in parenchyma cells and intrusive elongation of their daughter cells (Larson 1994; Kalev and Aloni 1998; Barnett and Asante 2000). In cross-section, the physical changes occurring in interfascicular parenchyma generate a more or less closed ring of radially compressed cells, here and there traversed by medullary ray parenchyma. Thus, the absence or presence in an organ of a circumferentially extending zone of radially compressed fusiform cells is the principal criterion for deciding whether vascular development is at a primary or secondary stage, respectively. In addition, there is the general expectation (based on innumerable anatomical investigations) that 'primary' xylem elements associated with fascicular cambium will exhibit diagnostic types of secondary wall sculpturing (that is, annular, helical, reticulate, or scalariform secondary-wall thickenings), and that 'secondary' xylem elements generated by the cambium will have more massive and obviously laminated secondary walls (for example, with S_1, S_2, and S_3 layers) containing bordered pits. Yet another difference is that only tracheary elements and medullary parenchyma tend to be seen when viewing primary xylem, whereas additional types of elements are regarded as normal to secondary xylem. Elongated fusiform cells of cambium can reduce in length through asymmetric cell division to generate ray cells, and it has been found that cambial fusiform cells will de-differentiate into parenchyma if the apices of debudded defoliated cuttings are not provided a continuing supply of exogenous auxin (Savidge and Wareing 1981b; Savidge 1983b). Those observations combined with many observations in tissue culture and at graft junctions indicate that procambial, fascicular, and interfascicular fusiform cambial cells are first induced to form and then maintained by basipetally transported auxin moving preferentially through them (Savidge and Wareing 1981b; Savidge 1983b; Barnett and Asante 2000; Kalev and Aloni 1998). Multiple cambia within a single organ are not uncommon, and it is possible for a cambium to relinquish its meristem role as new cambium arises in the woody plant (Carlquist 1999; Rajput and Rao 1999; Rao and Rajput 2000).

In stems, branches, roots, and storage organs, formation of cambium appears, at least superficially, to be physiologically linked to extension growth. Thus, cambial growth might be considered as the plant's way of ensuring its stem and branches will have sufficient structural support and hydraulic conductivity to grow against gravity while also providing for the needs of the heterotrophic root system. However, the notion of a physiological tie between primary and secondary growth is not entirely in accord with observations of development. Torrey (1967) was successful in growing roots *in vitro* and getting the associated primary vascular tissues to form, but even with successive subcultures and continuing elongation of the roots, cambium formation was difficult to achieve. Similarly, etiolated long shoots produced by pine trees when grown in the absence of light failed to produce cambium or secondary xylem even at their bases, although they did produce primary vascular tissues (Savidge 2000a). Thus, it would appear that something more than auxin and pressure are needed for the primary to secondary transition. More fundamentally, such observations may be an indication that cambium formation is not specified as a developmental genetic program, but requires physiological feedback from other parts of the plant. If this is correct, it would seem to follow that secondary xylem and phloem do not arise in the tree to fulfill any specified or determined morphogenetic outcome, but that an outcome such as massive height growth becomes incrementally possible because cambium followed by secondary vascular tissues form in the tree. As considered in more depth elsewhere (Savidge 1985, 1996, 2000a; Steeves and Savidge 2000), cambial growth is not a high priority in terms of source-sink relations or the general physiology of woody plants, and when resources are allocated to cambium it seems to be an indication that the plant is enjoying good health.

Cambium is the source of cells that differentiate into what customarily have been considered as two very distinct 'tissues,' phloem and xylem. Cambial derivative production and subsequent phloem- and xylem-cell differentiation sometimes occur concomitantly on opposing sides of the cambial zone, but usually, when one side is developing the other appears to be awaiting its turn, and it remains unclear what controls whether the centripetal or centrifugal side is favored (Larson 1994). Both phloem and xylem are complex tissues, each containing more than one cell type. During development of phloem or xylem, cambial derivatives pass through successive stages of differentiation. As is evident in Figure 1a, it is normal in conifers (and also in hardwoods, but the zones are usually less pronounced) for cambial derivatives enriched in indolyl 3-acetic

acid (IAA, auxin) to undergo enlargement but thereafter to experience a protracted delay, sometimes extending over several weeks before commencing differentiation into tracheids (Savidge and Wareing 1981b, 1984; Savidge and others 1982, 1998; Savidge 2000a). Zones of dividing cells (cambial zone, CZ), primary-walled radially expanding cells (RE zone), enlarged cells with developing bordered pits or sieve areas, and the latter producing secondary wall lamellae (SL zone) can be readily distinguished (Figure 1b, 1c). The lag occurring between formation of enlarged thin-walled cells in the RE zone (Figure 1a) and commencement of terminal differentiation in the same, as well as the evident inability of cells within the RE zone to divide, indicates that cells within that zone are physiologically as well as structurally distinct from cells on either side of it. In conifers having less than 100% live crown, although the RE zone appears first in springtime near the base of the live crown, RE cells appearing later in foliated regions are first to commence differentiation into tracheids (Savidge and Wareing 1981b, 1984; Savidge and others 1982, 1998; Savidge 2000a). Thus, regulatory mechanisms operating within the cambial region evidently control which vascular tissue develops, where within the tree it first begins to develop each growing season, the rate at which cells pass through each developmental zone, and the end fate of each cambial derivative.

Cambium contains both fusiform and ray cells in almost all species (however, a number are known to be rayless; see for example, Rajput and Rao 1998, 1999); thus, cambium is distinct from apical meristems by having cells of variable size and shape as well as by having distinctive protoplasm within the fusiform cells (Bailey 1920; Savidge and Barnett 1993; Larson 1994; Chaffey and others 1997; Farrar and Evert 1997). Although it is well established that fusiform cells give rise to new ray cells through anticlinal and/or successive asymmetric periclinal divisions (Bannan 1950, 1957; Bannan and Bayly 1956; Hejnowicz 1961, 1964; Savidge and Farrar 1984), physical pressure (reviewed below) is evidently the best characterized factor regulating the plane of division; however, it is not clear what modulates dividing planes such that they vary under seemingly identical pressure relations. Also, little is known about factors inducing trans-differentiation of fusiform to ray cells as a primary cellular differentiation expression within cambium. Fusiform-cell to ray-cell ratio, the seriation value of rays (for example, uniseriate, biseriate, multiseriate), positioning of rays in relation to the apical and basal tips of fusiform cells, overall ray frequency, fusiform cell length, and so on, clearly are not rigidly determined because they vary with position and age of cambium (Bailey 1920; Büsgen and Münch 1929; Bannan 1951; Bannan and Bayly 1956; Gartner and others 2000). Hejnowicz (1961) expressed the opinion that "the elimination of the fusiform initials constitutes the mechanism accelerating the rates of the changes taking place in the cambium," but there undoubtedly are additional mechanisms.

The majority of tree species have non-storied cambia, but many exhibit greater organization with their cambial fusiform cells forming tangentially ordered ranks of storied cambium (Farrar and Evert 1997). Storied cambium can lose and regain its storied organization, but how organization may be induced and maintained is unknown. Whether storied or non-storied the orientation of the long axis of fusiform cambial cells can be parallel or at some angle between 0° and 90° to the stem (Bannan 1966; Hejnowicz and Zagorska-Marek 1974; Savidge and Farrar 1984; Kramer 1999). In conifers, the polarity of cambial fusiform cells most commonly is slightly upward to the left within the crown of the tree, axial near the crown base, and more or less upward to the right in stem regions below the live crown; however, exceptions to that general rule have also been reported. In hardwoods, the polarity of cambial fusiform cells is even less predictable. Some species show the fascinating dynamic of switching their orientation from left to right in successive years, resulting in the formation of interlocked grain within phloem and xylem (Panshin and de Zeeuw 1970; Larson 1994). As seen in tangential view, populations of cambial fusiform cells alternate from one polar orientation to another over short axial distances along tree stems, thereby establishing wavy 'domains' (Hejnowicz 1971). Microdomains, consisting of small numbers of adjoining fusiform cells that alter their polarity in advance of the larger population, have also been observed (Savidge and Farrar 1984). Fusiform cambial cells within bark bridges sloping across a girdle fairly rapidly change their polarity to become parallel to the long axis of the bridge (Savidge and Farrar 1984). Very little is known about the regulation of these polarity phenomena. Plausibly, they are somehow connected to waves of auxin moving basipetally through the cambium (Wodzicki and others 1987, 1999; Zajaczkowski and Wodzicki 1978a, 1978b; Zajaczkowski and others 1984). Although the control mechanisms remain uncertain, the fact that cambial cells change more or less in unison within microdomains, domains, and larger stem sectors appears to be unequivocal evidence that intercellular communication is normal to cells of cambium. As the cambium

extends from root to shoot as a sheathed network of living 'fibres,' presumably its ability for intercellular communication, hence propagation of messages, also extends to all parts of the plant, providing a mechanism for integrated control of whole plant growth and development.

Esau (1960) noted "that the course of differentiation of a cell is determined by its position within the general pattern." Barnett and Asante (2000) described how cambium of stock and scion accurately progress through callus cells to close the gap of the graft interface. Intercellular communication must be invoked to explain how complex structural organization is achieved and maintained and, as noted, research into the cambium and vascular development has provided convincing evidence for long-distance as well as cell-cell communication, beginning with the cambium forming a tightly organized sheath over the whole. Positional matching of half-bordered pits during differentiation of cells into tracheary elements, formation of perforation plates and sieve areas to link vessel and sieve members, respectively, into long-distance conducting systems, differentiation of cambial derivatives into lengthy resin canals, maintenance of ray continuity, and polarity phenomena over large areas of cambium are all additional evidence for communication occurring during cambial growth.

As a general rule in biological development, differentiation of cells by enlargement and/or additional changes does not occur concomitantly with cell division; that is, differentiation and division seem to be mutually exclusive, and differentiation always seems to be initiated at interphase. On the other hand, the population of cells within a meristem is not continually dividing, and those cells not dividing evidently are competent to enter secondary and terminal cellular differentiation pathways while still positioned within the meristem. If primary cellular differentiation events (such as conversion of cambial fusiform cells into cambial ray cells: Savidge 1983a; Savidge and Farrar 1984) are excluded from consideration, cambium conforms to the general rule. Thus, in nature, secondary and terminal cellular differentiation occur only on the margins of the cambium in derivatives that relinquish cell division activity in favor of primary wall radial expansion (Figure 1). On the other hand, both fusiform and ray cells within the cambial zone, whether active or dormant, differentiate directly into tracheids under the influence of a conifer needle factor (Savidge and Wareing 1981a; Savidge 1994, 1996, 2000b). Direct differentiation within the cambial zone probably occurs only rarely in nature, but however infrequent it nevertheless demonstrates that cambial cells are not determined as such.

Wood anatomy investigations have made a significant contribution to the biological sciences by recording that secondary xylem as a 'tissue' is not at all constant, rather highly variable in its anatomical, chemical, and physical properties. By the early 1800's researchers had amply recorded that wood under the microscope was a mixture of heterogenous types of "elements," indeed so complex and diverse that it was necessary to develop specialized terminology and sophisticated schemes to systematically classify woods based on their distinguishable structural differences (Panshin and de Zeeuw 1970; Wheeler and Baas 1998). With ongoing wood anatomical research, it emerged that considerable variation in the nature of wood existed not only in different organs of the individual tree but also within any one type of element, resulting in terminology for many different subtypes of fibres, parenchyma, and subcellular features. "Abnormal wood" is nevertheless a term commonly found in the wood anatomy literature for types still remaining outside of the classification scheme. Sieve cells within xylem constitute "included phloem," and sclerenchyma are xylem fibres when found in wood but become "phloem" or "bast" fibres on the other side of the cambium.

The elongated fusiform shapes of phloem and xylem elements owe their origin primarily to the same shapes existing in cambial fusiform cells, the latter dividing periclinally from tip to tip to yield the fusiform character in daughter cells. Excepting their fusiform shapes, parenchyma and sclerenchyma elements of xylem and phloem are not unlike the same cell types found in isodiametric or less elongated cells. Thus, as a first hypothesis, it may be supposed that the biochemical pathways underlying formation of the cell wall structures of fusiform cells are closely related, if not identical, to those operating in non-fusiform cells.

Excepting sieve area/plate, simple-pit, bordered-pit, and perforation-plate development, the subcellular processes underlying differentiation of cells into all of the many kinds of vascular elements appear to involve closely related if not identical biochemical pathways. There is evidence that a novel organelle, possibly of vacuolar origin, serves to protect cell-wall locations from modification as the fundamental step underlying both simple and bordered-pit development (Savidge 2000a). The major difference distinguishing non-woody and woody tissues appears to be that relatively few cells remain exempt from secondary wall formation and lignification once cells have begun to differentiate into prosen-

chyma; nevertheless, ray cells and axial parenchyma somehow resist lignification but not other changes during secondary xylem formation. Thus, the question of what regulatory mechanism prevents cells from becoming lignified, or woody, appears to be worthy of equal consideration to that concerning what causes woodiness during xylogenesis. Differentiation of the gelatinous or tension wood fibre is an example of how biochemical pathways can be differentially regulated in a seemingly qualitative manner: development of S_1 and S_2 layers proceeds more or less normally, but then pathways underlying hemicellulose and lignin deposition evidently are deactivated enabling pure cellulose to be deposited in the G layer. It deserves repeating that the biochemistry of cambial cells has been researched only at coarse 'tissue' resolution.

Although some proportion of prosenchyma in all woody species evidently is always the type known as tracheary elements, in angiosperms it is not at all uncommon for the bulk of xylem to consist of non-tracheary elements, for example, sclerenchyma, parenchyma (ray and axial), and specialized cell types (Panshin and de Zeeuw 1970). *Fraxinus* spp. are an example; they normally produce one to a few tiers of predominantly vessel members in their early wood, with the vast majority of cells within each annual ring being sclerenchyma and parenchyma (Panshin and de Zeeuw 1970; Chalk 1970). Cambial fusiform cells of some species, for example, within the genera *Populus* and *Salix,* produce derivatives within radial files which differentiate in fairly repeatable sequences as equi-diameter vessel members and libriform fibers, and the only major deviation from this recurring theme arguing against a complex kind of committed xylogenesis is a line of 'marginal' axial parenchyma at the earlywood - latewood boundary. On the other hand, many species (*Castanea dentata, Castanopsis chrysophylla, Celtis occidentalis, Fagus grandifolia, Fraxinus americana, Gleditsia triacanthos, Maclura pomifera, Ostrya virginiana, Platanus occidentalis, Robinia pseudoacacia* and *Ulmus rubra,* to name just a few) exhibit less predictable variation. Wavy tangential bands of vessel members arise through more or less concomitant 'pulses' of homologous differentiation occurring in cambial derivatives within numerous neighboring radial files, and such clusters are separated tangentially by intervening radial files of non-vessel elements (for example, *C. occidentalis, M. pomifera, R. pseudoacacia, U. rubra*). Vessel members may occur more or less serially within particular radial files to create radiating chains of vessels of variable number and proximity but usually extending from earlywood to latewood. Radial chains of vessels rarely are systematically repetitive, in terms of either spacing or cell number within radial files, and usually there are very few or no vessels produced in the intervening radial files (for example, *F. grandifolia, G. triacanthos, I. opaca, O. virginiana, P. occidentalis*). Randomly scattered cambial derivatives of *C. chrysophylla* differentiate into singular large earlywood vessels at the beginning of each growing season, followed in the same and neighboring radial files by derivatives differentiating into smaller diameter vessels, tier after tier, and thus generating V-shaped vessel clusters extending from earlywood to latewood as seen in cross section. Those V-shaped clusters are separated by areas entirely devoid of vessels and containing fibre tracheids with abundant parenchyma.

Woods of the above-mentioned species also show remarkable flexibility in their number and distribution of living, nonwoody elements. Some hardwood species have few parenchyma other than those making up their rays, and that ray component can vary from relatively small (for example, *Populus, Salix,* and *C dentata* have only uniseriate rays) to very large (for example, *Quercus* spp. having both uni- and multiseriate rays). As already noted for *Populus,* it is not unusual for hardwood species to exhibit a line of nonwoody 'marginal' parenchyma as the last cambial derivatives differentiate at the end of the growth season. In *G. triacanthos,* marginal parenchyma form, but they do not delineate a distinct annual ring boundary, cambial derivatives continuing to differentiate as earlywood parenchyma in the succeeding annual ring. Major tracts of parenchymatous conjunctive tissue are produced between earlywood vessels, or between vessels and rays (for example, *M. pomifera, U. rubra*). Clusters of adjoining cambial derivatives differentiate into sheaths of parenchyma to enclose vessels (for example, *M. pomifera*). Large numbers of cambial derivatives differentiate into tangential lines, or bands, of axial parenchyma, particularly during latewood formation (for instance, *G. triacanthos, M. pomifera, O. virginiana, U. rubra*). Finally, xylem parenchyma do not always occur in groups , Sheaths, or clusters, nor are they preferentially always localized to earlywood or latewood; they may be abundant in the xylem but occur as randomly scattered cells or in short lines exhibiting no obvious regularity (for example, *C. dentata, P. occidentalis*).

Both cambium and its differentiating derivatives are competent to de-differentiate or pursue differentiation pathways atypical of normal wood formation when nearby regions are wounded (Bannan 1933, 1934; Savidge and Wareing 1981b; Savidge 1996, 2000b; Nagy and others 2000). Moreover, there is convincing evidence that the kind of wood

that develops can be readily altered by manipulating environmental factors such as photoperiod, gravity, soil water potential, nutrient availability, as well as phytohormones (Kennedy and Farrar 1965; Savidge and Wareing 1981b; Zhong and Savidge 1995; Jiang and others 1998; Savidge 1996, 2000a; Yoshizawa and others 2000).

In addition to the fascinating anatomical differences among wood, the chemistry of wood is known to be highly variable (reviewed in Savidge 2000a). Variation between element types is well known, but there can also be variation in chemistry of the secondary walls of the same type (Mansour and DeFay 1998).

Regulation by Auxin and Other Transmissible Agents

When the existence of auxin was first discovered in the late 1800's, it provided hope for explaining the intrinsic regulation of seasonal diameter growth in an appealingly simple way, in terms of a promoter of cambial activity and wood formation issuing from buds to cambium and diffusing basipetally through the cambium, but only after bud scales had separated and new shoots were actively extending (historical review: Savidge and Wareing 1981b). From that early concept, a substantial body of semiquantitative bioassay-based research data permitted the interpretation that auxin does in fact diffuse out of elongating shoots and expanding leaves as well as the basal ends of stem segments (Wodzicki 1978; Zajaczkowski and Wodzicki 1975). Although the older literature indicates the probable existence of more than IAA as a component of 'auxin' diffusates, IAA has become synonymous with auxin. That molecular definition enabled definitive isotope dilution combined gas chromatographic-mass spectrometric (GC/MS) methods and immunospecific assays to be developed for greatly enhanced accuracy and precision in quantitative estimation of endogenous IAA, but it in no way excluded the possibility of revisiting earlier studies, discovering additional components (if any) of the original 'auxin', and then applying similarly rigorous quantitative analysis to those compounds. Thus, although the traditional bioassays of plant physiology have seldom been the focus in recent years, they are by no means outdated and may well be the only way forward, particularly in terms of sorting out the true physiological relevance of so-called 'quantitative trait loci' to diameter growth of trees.

Cambium has been used for a variety of bioassays (Savidge and Wareing 1981b; Savidge 1983b, 1993, 1996), including gene modification studies aimed at distinguishing regulatory mechanisms operating within the cambium from those operating at a distance (Bossinger and Leitch 2000). The bioassay approach was crucially important for the discovery of auxin waves (Zajaczkowski and Wodzicki 1978; Zajaczkowski and others 1984; Wodzicki and others 1987) which were subsequently corroborated as polar IAA waves moving through conifer cambium (Wodzicki and others 1999). As detailed elsewhere (Torrey 1967; Savidge 1985, 1996), bioassays may enable a number of as-yet-uncharacterized transmissible regulators to be elucidated.

Cambium and derivatives at distinct developmental stages (Figure 1) can be obtained as large masses of cells by the straightforward procedure of bark peeling (Savidge and others 1998; Savidge 2000a), but only rarely has the sophisticated quantitative methodology supporting contemporary analysis of phytohormones and other metabolites been combined with the anatomical precision needed to ensure high resolution analysis of specific cell types, or even of singular 'tissues'. For example, phytohormone content usually is expressed as moles or grams of phytohormone per unit mass of plant cells investigated, but crude anatomical resolution can negate or misrepresent correlations between phytohormone content and development. As shown in Figure 1, cambium is an extremely thin zone (typically less than 30 μm in radial thickness) of relatively low density cells which, when carefully isolated, yields very little mass per unit area, much less than do the same areas and thicknesses of adjoining phloem and xylem elements with their thickened walls. Thus, if any phloem and/or xylem are included when cambial scrapings or sections are being weighed at the start of phytohormone analysis, the true phytohormone content of the cambial zone cannot be accurately estimated. Given that the cambial zone, or any of its derivative zones, can be isolated as a 'pure' tissue, in relation to its content of whatever molecule happens to be under investigation, there remains the difficulty that every zone contains both fusiform and ray cells. To discover how the molecule may be differentially distributed will require development of some clever techniques. Protoplast isolation and separation may be a useful approach to this problem (Leinhos and Savidge 1993). The behavior of cambial fusiform cells and their nuclei can also give some indication of IAA distribution, at least in conifers. Cambial fusiform cells undergo repeated transverse divisions in response to limiting auxin; that is, they dedifferentiate into axial parenchyma (Savidge and Wareing 1981b; Savidge 1983b). Nuclei of cambial fusiform cells characteristically are

drawn out into rod-shaped bodies, but when auxin becomes limiting they become spherical in association with dedifferentiation of the fusiform cells (Savidge 2000a).

Anatomically focused GC/MS investigations, such as those reported by Savidge and others (1982), Eklund and others (1998), and Sundberg and others (2000), provided quantitative evidence confirming that IAA content is substantially elevated in, and clearly localized to, the cambial region. However, there is less than perfect agreement concerning which zone of the cambial region contains more IAA. Data based on serial tangential sections of frozen tissue indicate the cambial zone (Uggla and others 1998; Sundberg and others 2000) and data based on bark peeling indicate the enlarged cambial derivatives that are developing into xylem (Savidge and Wareing 1981b; Savidge and others 1982; Eklund and others 1998). Possibly, the different interpretations have to do with the different species or ages of stem investigated. The entire database of truly quantitative estimates of cambial IAA content remains quite limited and addresses only a paucity of species (Savidge 1996).

There is no doubt that cambium is abundantly enriched in IAA, that IAA moves basipetally through cambium, and that IAA is a factor inducing and maintaining cambial fusiform cells, but there is also little doubt that IAA promotes cambial derivatives to differentiate into both xylem and phloem elements, as further described below. Thus, there is the logical difficulty that cells are induced and perpetuated in states of primary cellular differentiation by the identical transmissible factor that evidently can also induce them to undergo secondary and terminal cellular differentiation. Sundberg and colleagues (2000) considered that differential expressions across the cambial region (for example, Figure 1b) may be due to IAA concentration being distributed radially as a concentration gradient, the maximum being centered over the cambial zone with declining concentrations toward both phloem and xylem, with the developmental response being a function of IAA molarity. Thus, IAA in that scheme acts as a positional signal (Uggla and others 1998; Sundberg and others 2000).

Metabolic mechanisms for modulating a cell's IAA content, for example by conjugation or oxidation, are well known, but another possible mechanism for controlling IAA content and distribution is compartmentalization or 'packaging' of IAA within cells on the outer margins of the cambial zone. This scheme agrees with the endogenous IAA distribution observed by Savidge and others (1982) and Savidge and Wareing (1981b). When exogenous auxin is applied to apical ends of stem cuttings or tops of debudded conifers, cell division in cambium invariably precedes enlargement of cells on the cambial zone's centripetal periphery, and although slight enlargement occurs during interphase within the dividing population, only cells on the periphery of the cambial zone undergo massive enlargement with concomitant cessation of cell-division activity (Savidge and Wareing 1981a; Savidge 1983b, 1993). Similarly, exogenous auxin, when applied to the apical ends of dormant hardwood cuttings or debudded trees, also promotes immense radial expansion of centripetal cambial derivatives, as the first stage of vessel member differentiation, and that enlargement also occurs exclusively in nondividing cells (Aloni and others 2000). Dose response curves in auxin bioassays have been extensively investigated, and it is well established that auxin effectively inhibits, and even kills, as well as promotes cell division and expansion activity (thus, there exist herbicides such as 2,4-dichlorophenoxyacetic acid). Export of IAA from the inner region of the cambial zone to its peripheries would not only serve to maintain the cambium's interior level of IAA relatively low, conducive to cell division, but higher levels on the peripheries presumably would inhibit division and promote expansion. In addition to explaining how zonation within the cambial region is regulated (Figure 1), such a mechanism would serve the unobvious purpose of cleansing the auxin transport corridor (that is, cambium) of inhibitory auxin levels (Savidge 1993) which might otherwise kill its cells.

Considerable evidence has indicated that the sources of auxin in cambium are buds and leaves (Wodzicki and Wodzicki 1973; Zajaczkowski and Wodzicki 1975; Wodzicki, 1978; Savidge and Wareing 1981b; Wodzicki and others 1987; Little and Pharis 1995; compare, however, Sundberg and Uggla 1998; Sundberg and others 2000). That evidence, together with the ability of exogenous auxin to promote cambial growth when applied to the apical ends of young cuttings, evidence for auxin waves, and steep gradients in auxin distribution within the cambial region, has led to schemes where auxin is portrayed as the primary or only signaling mechanism determining every aspect of vascular development (Zajaczkowski and Wodzicki 1978; Zajaczkowski and others 1984; Wodzicki and others 1999; Sundberg and others 2000).

Limitations of auxin regulation that are well established but have not been sufficiently emphasized (perhaps because they tend to be negative results) are that 1) auxin induction of cambial growth in isolated conifer stem segments decreases with cambial age, ultimately becoming ineffective as a cam-

bial growth promoter, but nevertheless being needed to maintain the structure of cambial fusiform cells (Zajaczkowski 1973; Savidge and Wareing 1981b; Savidge 1983b, 1993); and 2) under ideal culture conditions where nutriment is not limiting, the volume of cambial growth that can be induced experimentally *in vitro* is invariably much less than would occur if the cambium had been left *in arbor* (Zajaczkowski 1969; Savidge 1993; Leitch and Savidge 2000). When the physiology of secondary growth is well understood, it should be possible to reproduce *in vitro* what occurs in nature, and development of a fully defined system allowing such simulations would seem to be imperative for progress in physiological genomics. Auxin so far has not satisfied that expectation; however, the fact that isolated stem segments from 3-year-old or older stem regions of conifers do not respond developmentally to exogenous IAA (or other auxins) in the same manner as younger stem regions is an indication that cambium in relation to wood formation is not determined. If somehow specified by IAA, cambial cells clearly require something more to achieve full xylogenic expression.

It may be that normal ongoing growth and functionality of the cambium will be achievable *in vitro* only when both root and shoot sources are simultaneously simulated, perhaps using a modified experiment approach after that developed by Brown and Wodzicki (1969). A probable role for the root system in regulating cambial growth in the stems of trees is evident by the responses shown in Figures 1 and 2 (original data of the author). Three replicate 10-m-tall *Pinus strobus* saplings with 70% live crowns were selected on May 9th on the basis that each was growing in a shaded location (to minimize water loss during the course of the experiments) and that each had active cambium but none had yet produced any earlywood tracheids. As shown in Figure 1a, at 1.6 meters above ground level the cambia in those trees had become vacuolated, commenced cell-division activity, and had 6–10 primary-walled expanding and expanded derivatives but no new earlywood tracheids on their inner peripheries on May 9th. On that date, the trees were sawn transversely to produce 1.6-meter-tall rooted and shaded stumps having neither branches nor living foliage. A 10-cm-long segment was cut from the apical end of each rooted stump, first marking North at positions above and below the saw cut. At five equi-spaced positions around the circumference of each rooted stump and also its removed 10-cm segment, filter papers (1 × 2 cm Whatman no. 1) containing known but varied masses of IAA (recrystallized from stock provided by Sigma Chemicals, applied in acetone to the paper and allowed to air dry) were inserted into the cambial region at the apical ends by separating a 1 × 2 cm 'tab' of bark from the wood at the top, momentarily bending that tab back, and sliding the lower end of the filter paper to the basal position of the tab where bark and wood had not been separated. To minimize misinterpretation of the data due to possible preexisting physiological variability around the circumference, axially corresponding positions of rooted stump and 10-cm segment were matched up and provided equal masses of IAA. Parafilm was wrapped firmly around the stems to hold the separated bark tabs firmly in place and sandwich the IAA-containing paper between wood and phloem. The 10-cm upper segments were stood upright in buckets having distilled water reaching to a height of 5 cm on the segments. The rooted stumps and their top segments were left until June 1st, at which time specimens (1 cm wide by 2 cm axial) for microscopy were dissected such that the apical end of each was 2 cm axially below the basal end of the inserted filter paper. After fixing in 70% ethanol, the sampled specimens were hand sectioned at their apical ends (that is, at 2 cm below the source of IAA), mounted in water, and examined in the microscope.

Comparing Figure 2a with Figure 1a, it can be seen that preexisting, radially enlarged cambial derivatives in rooted stumps differentiated into mature tracheids when no exogenous IAA was provided, but little if any continuing cambial cell-division activity accompanied that differentiation. Cambial activity and tracheid production were both promoted in rooted stumps receiving 0.2 mg to 2.0 mg IAA (Figure 2b). At 5.0 mg IAA, the nuclei of the cambial fusiform cells appeared more dense and some cell-division activity evidently occurred, but there was no differentiation of cambial derivatives into tracheids (Figure 2c), in agreement with previous observations that hyperphysiological amounts of IAA inhibit differentiation (Savidge 1993). In nonrooted 10-cm stem segments, cells remained living throughout the 23-day period, and controls (treated with filter paper having no IAA) were still undergoing cyclosis on June 1st. However, no evidence was seen for cambial activity being promoted or for preexisting cambial derivatives completing differentiation into tracheids, regardless of the mass of IAA provided (Figure 2d). The results were consistent across the three replicates and confirmed similar findings made in the preceding year.

Such qualitative differences between rooted and nonrooted stems are an indication that enlarged cambial derivatives, even after commencing bordered pit development (see Figure 1a), can experience developmental arrest in their differentiation

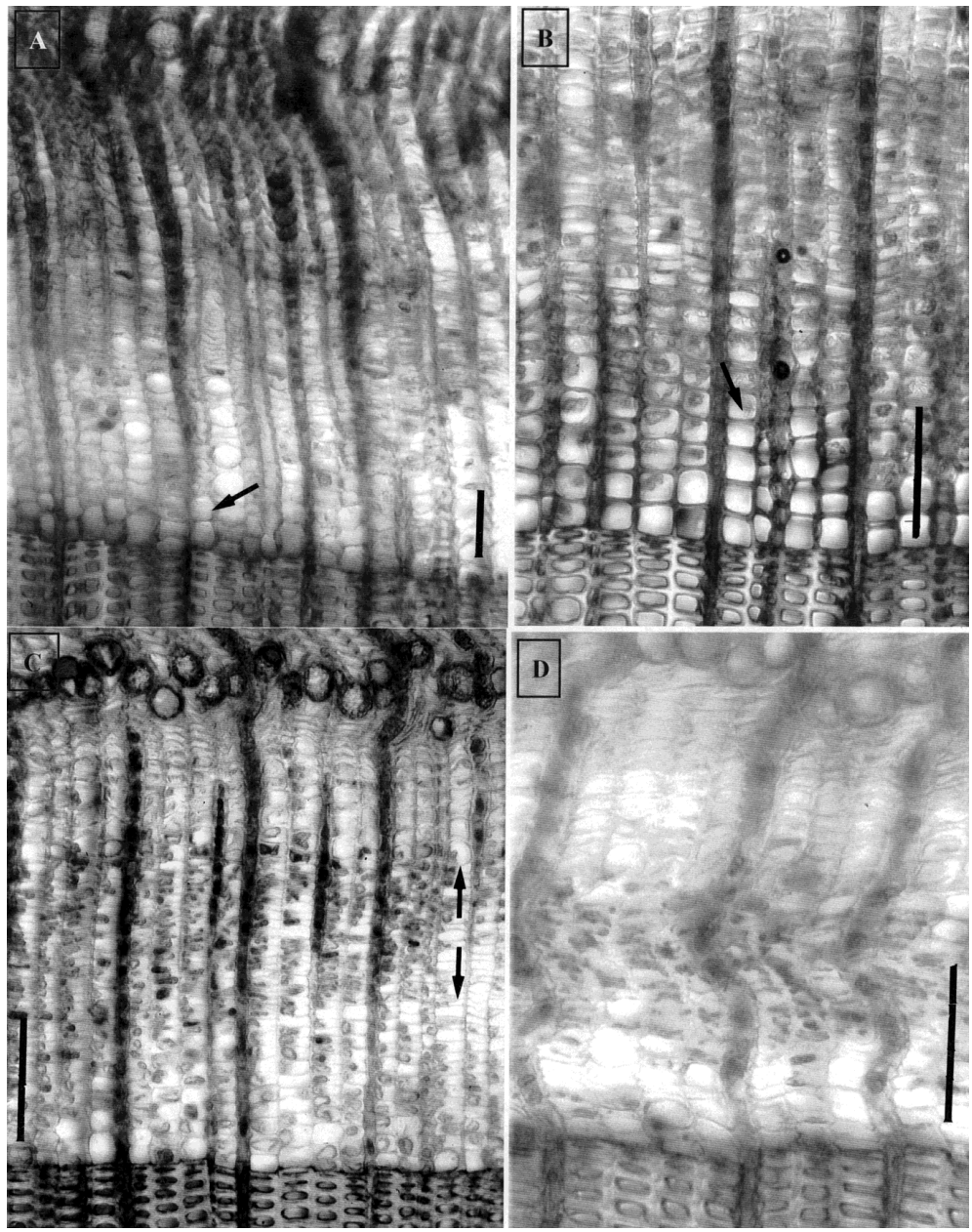

Figure 2. Hand-cut unstained brightfield cross-sections of *Pinus strobus* stems following IAA treatment from May 9th to June 1st (see text). Phloem is at the top and late wood at the bottom of each section. Bars = 100 μm. (**A**) IAA (0.0 mg) to a rooted stump: 1–2 earlywood tracheids (arrow) per radial file are evident. (**B**) IAA 2.0 mg to a rooted stump: 5–7 earlywood tracheids (arrow) per radial file induced by that treatment. (**C**) IAA (5.0 mg) to a rooted stump: no tracheid matured but periclinal division activity widened the cambial zone (arrow). (**D**) IAA (2.0 mg) to a nonrooted stem segment 10 cm above the location shown in (**B**). Neither cambial activity nor tracheid production occurred. The same absence of differentiating of mature earlywood tracheids was noted at all IAA concentrations in nonrooted stem segments.

pursuit, that an intact root system is essential for enlarged cambial derivatives in the stem to complete their differentiation, and that IAA of itself (that is, in the absence of other regulatory factors) is not sufficient to promote cambial derivatives to complete their differentiation into tracheids nor to induce cells to enter that differentiation pathway. The same conclusions have been arrived at through various other experimental observations (Savidge and Wareing 1981a, 1981b; Savidge 1983b, 1993, 1994; Savidge and Barnett 1993), although the present evidence is the first to indicate that something more

than water from the roots is needed for cambial derivatives to enter and complete terminal differentiation.

Auxin regulation of cambial growth has held center stage over the last century, but exogenous gibberellic acid (GA_3) when applied to hardwood stem cuttings, is usually more effective than exogenous IAA in promoting cambial cell division activity (Wareing 1958; Digby and Wareing 1966; Zhong and Savidge 1995; Jiang and others 1998). GA_3 stimulates periclinal cell divisions in the cambial zone, but its application does not lead to massive radial enlargement of cambial derivatives, nor does it promote vessel or other types of prosenchyma development. GA_3 alone does, however, effectively promote elongation of fusiform cells (Stant 1961), and it may well be the primary mechanism regulating the variable fibre lengths found in hardwoods (Chalk 1970). Exogenous IAA + GA_3 applied to hardwood cuttings typically promote vigorous cambial growth and enable cambial derivatives to differentiate into fibres, as well as maintaining the IAA effect of inducing differentiation into vessel members. Thus, depending on their molarity ratio and the absolute number of moles provided, combinations of IAA + GA_3 can be used to manipulate vessel to fibre ratios (Digby and Wareing 1966; Zhong and Savidge 1995). Based on preliminary time studies, IAA + GA_3 synergism also includes an enhanced rate of hardwood cambium proceeding from dormancy into reactivation and xylogenesis in *Populus spp.* (N. A. Forneris and R.A. Savidge, unpublished data). Although IAA + GA_3 clearly promote cambial growth in Magnoliophyta, the induced response in cuttings or *in vitro* cultures rarely if ever equals that occurring *in arbor.* In agreement with findings from one-year-old and older stem material (Savidge and Wareing 1981b; Savidge 1983b, 1994), Kalev and Aloni (1998) working on young pine seedlings observed that gibberellin did not promote wood formation in conifers, whereas auxin did promote both cell division and wood formation.

There is also considerable evidence that ethylene, abscisic acid, cytokinins, and brassinolide regulate one or more aspects of cambial growth (Savidge and Wareing 1981b; Savidge 1988, 1989; Little and Pharis 1995; Moritz and Sundberg 1996; Savidge 1996; Altmann 1999; Eklund and Little 1998; Little and Eklund 1999; Sitbon and others 1999; Yamamoto and others 1997; Fukuda and others 1998). In addition, there is evidence for a transmissible regulator associated with mature conifer needles which, in contrast to known phytohormones, promotes secondary-wall formation and lignification directly within dormant as well as active cambial cells (Savidge and Wareing 1981a; Savidge 1983b, 1994, 1996, 2000b). There evidently is a link between the needle regulator and calcium concentration in the cambium (Savidge 1994; R.A. Savidge and Z. Wu, unpublished data), but because the identity of that 'tracheid-differentiation factor' is still uncertain it remains outside of the bounds of both biochemistry and molecular biology.

Proceeding radially across the cambial zone, from phloem to xylem, there are gradients in soluble carbohydrate, pH, superoxide anion, various types of enzyme activity, water potential, inorganic ions, and probably many additional factors including hormones (Weiler and Ziegler 1981; Catesson and others 1994; Kuhn and others 1997; Dunisch and others 1998; Savidge and others 1998). I have discussed radial transmission in more depth elsewhere (Savidge 1996).

Physical pressure is well established to influence the plane of cell division and induce zones of radially narrow cambial-like cells (Barnett and Asante 2000); thus, positive pressure may be the primary reason why the cambial zone always occurs between two constraining tissues, and for cells within that zone dividing principally in the periclinal plane. On the other hand, auxin also appears to be a factor determining the plane of cell division, because when auxin supply to the cambium is experimentally interrupted, cambial fusiform cells divide in the true transverse plane despite the continuing presence of the physical constraints (Savidge and Wareing 1981b; Savidge 1983b). In addition to planes of cytokinesis, pressure also influences the nature of xylem-cell development (Savidge and Wareing 1981b; Savidge 1985; Bauer and Eschrich 1997; Barnett and Asante 2000).

Water potential changes are the explanation for most physical stresses that arise in plants. For example, pressure potential determines how much force adjoining cells exert against one another, tensional stress normally operating in the tangential or circumferential field and compressional stress in the radial (Hejnowicz 1980). In general, both cell division and cell expansion are more sensitive to decreasing water potential than are photosynthesis and stomatal closure, and variation in water availability has profound effects on seemingly every aspect of cambial growth (Paul 1963; Zahner 1968; Bissing 1982; Savidge 1996; Abe and Nakai 1999). In addition to turgor-driven cell division and expansion, water relations undoubtedly also influence each cell's vacuolation, transport, diffusion, unloading, synthetic, and hydrolytic reactions. Based on the preceding, the cambium's water potential during the growing period probably acts as a versatile regu-

lator, promoting and inhibiting cell-division activity, determining radial dimensions achieved by cambial derivatives, and influencing the nature of secondary and terminal cellular differentiation. Water as a regulator of cambial growth appears to be on an equal footing with both phytohormones and transcription factors.

Except for its contribution to mass, the role of water in relation to gravity-induced responses remains unclear. However, tissue stresses due to gravity not only accompany cambial growth but serve to regulate the nature of development (Kennedy and Farrar 1965; Hejnowicz 1997; Allona and others 1998; Jiang and others 1998; Wu and others 1998; Little and Eklund 1999; Wilson 1998, 2000; Yoshizawa and others 2000).

Inner stem tissues, both bark and wood, are often not considered as harboring living microorganisms; however, this appears to be the case more often than not (Purcell and Hopkins 1996). On the other hand, the cambial zone itself, when grown *in vitro,* rarely yields microbial colonies (Savidge 1983b, 1993; Leitch and Savidge 2000).

The above brief considerations suggest that the 'environment' of protoplasm and apoplasm of the cambial region includes a multitude of physical and chemical factors and is by no means restricted to fluxes in metabolism generated internally, as might be expected in a closed system. Rather, it is readily and continually modified by long-distance axial and shorter-distance radial transmission of messages originating outside of the cambial zone. Mature xylem has long been used as a permanent record of the planes and frequencies of cell division activity that previously occurred in the cambium (for example, Bannan and Hejnowicz references), and with increased knowledge it is likely that the variable anatomical features locked up in wood will also be useful as a record of the changing physiological states (for example, the IAA + GA_3 ratio) existing in the cambium's enlarging and differentiating derivatives. Knowledge of the full spectrum of transmissible ions, molecules, and physical signals of the cambial region cannot be given its due in this limited space, and in my view that spectrum remains far from exhaustively characterized. Nevertheless, the numerous known factors leave no doubt that the 'E' of each cell's G X E interactions has the capability of being highly complex in both time and space. Another way of thinking of 'E' regulation is as gene expression in reverse. Forward, or 'normal' gene expression at distant sources results in proteins and consequent production of metabolites (some of which are designated as hormones and others are not) which, upon arriving in the cambium, not only serve as nutriment but modify the biochemical environment, influencing catalysis and mRNA stability and ultimately affecting initiation of transcription within cambial cells. The biochemistry basis for metabolites and symplastic enzymes modulating gene expression appears to be well established (Voet and Voet 1990).

Committed versus Noncommitted Development

Viewed at the level of the whole plant, persistent presumably genetic components operate to determine each plant's overall developmental pathway (Wilson 1998, 2000). Unusual tree phenotypes clearly exemplify this concept. For example, cuttings taken from dwarf, prostrate, weeping, bushy, strongly divaricate, or main-axis-overarching trees of species that otherwise commonly exhibit dominant vertical stems and persistent fixed-angle branching, when propagated or grafted, develop such that they continue to exhibit the unusual phenotype of the source, even when grown in varied environments. Thus, overall form commitment within apical meristems appears to be the starting point for the existence of morphological and ecological distinctions within, as well as between, species. Similarly, it may be supposed that systemogenetic commitment arising in early embryogenesis is the reason for shoot and root systems being faithfully maintained as such. Systemogenetic commitment must require that literally thousands of distinct aspects of gene expression are both orchestrated and channeled, and for such all- encompassing control to be indefinitely perpetuated, as it is in trees, surely requires fundamental and tenacious epigenetic changes. On the other hand, considering that shoot cuttings can yield roots, root cuttings shoots, and explanted tissues somatic embryos, epigenetic constraints determining systemogenetic development evidently are fixed only in apical meristems, somehow weakening and/or disappearing as cells become spatially removed or re-dissected from their source meristems.

The initiation of systemogenesis arguably has its origin in a single cell immediately following syngamy, when opposing poles of the fertilized ovum have their systemic fates (that is, root versus shoot system) decided. If this is correct, it is possible that every aspect of organogenesis which attends embryogenesis and post-germinative organogeneses also has its origin in singular cells. Although supracellular commitment is also a possibility, there is no obvious reason why commitment cannot arise in a single cell and then spread to neighboring cells.

Given that systemogenetic determination has a genetic basis, organogenetic expressions clearly must be activated within the constraints of determined systemogenetic expression. Predictable phyllotaxis in a species indicates that positional information underlies initiation of leaf primordia. In tomato and mutant *Arabidopsis thaliana,* IAA is sufficient to induce organogenesis within apices, indicating that the position of the IAA source is a controlling factor (Reinhardt and others 2000). However, it remains unclear whether early organogenesis is specified in a cell(s) that then commences its development involving, in the first instance, cell-division activity or, alternatively, if random cell-division activity occurring in fully competent cells is what (perhaps combined with other factors, such as physical pressure) enables or induces some to become organogenetically committed subsequently. Once commitment to an induced pathway of organ development has occurred, it somehow is transmitted through mitosis and cytokinesis to all successive somatic replications of the genome, until the mature organ has been formed. Findings of organ identity mutants in *Arabidopsis* appear to support the latter interpretation, but the earlier conclusion (Goethe 1790) that no real boundary exists between cotyledons, foliage leaves, bracts, and the organs of the flower is another way of saying that organogenetic commitment appears not to be determined, rather open to specification, inductive stimuli regulating competence such that a variety of different organs can arise from a single primordium.

Morphogenesis frequently is considered reductionistically in terms of organs, tissues, and cells, and this undoubtedly has influenced how intrinsic regulation of cambial growth and other aspects of plant development have been investigated. Within cell theory an organism can be seen, both phylogenetically and ontogenetically, as comprising a complex of cells each of which fulfills an independent role in determining the nature of the organism. However, once a cell has achieved a particular fate that contributes to the organization of the whole, although still living and potentially totipotent, it tends to retain that physiological role as well as its position, evidently because the whole continues to exert influence on each of its unit cells. Again, adventitious root formation rarely occurs in intact stems, but when stem pieces are cut from the whole, it becomes possible for them to produce new roots.

Although Schmidt (1924) divided the dicot apex into two regions, the tunica (or outer dome-shaped layer of the apex), distinguished by its anticlinal divisions as well as its superior position, and the corpus (immediately basal to the tunica) characterized by cells that divide in all planes, attempts to demonstrate that particular tissues developing basal to the apex are specified or determined in cells derived from only the tunica, or only the corpus, have in general been unsuccessful. Although tunica and corpus have been perceived to contain initial and derivative cells, Esau (1960) cautioned that apical initials are not inherently different from, and may become supplanted by, their derivatives. Thus, systemogenetic determination evidently is either generally distributed among apical cells and not dependent on positional information, or it can be readily passed on as a cell assumes the initial role. Following induction of differing ploidy levels in discrete layers of the shoot apices of fruit trees, Dermen (1953) found that vascular cambium originated from the layer serving as pith progenitors as well as the layer serving as cortex progenitors.

Some developmental biologists have questioned cell theory, and especially the concept that individual cells are the building blocks of plants (Cooke and Lu 1992; Hagemann 1992; Kaplan 1992; Sitte 1992), but an alternative view (Savidge 1983a, 1996) is that plants can produce virtually any form, or multicellular shape, through incremental growth by employing mechanisms of primary cellular differentiation, provided that cells remain turgid. During both systemogenesis and organogenesis, secondary and terminal cellular differentiation events must follow if the fashioned, or marginally fashioned, structures generated through primary cellular differentiation are to take full shape and be maintained against variable forces of nature, as for instance when water becomes limiting. The mechanisms operating within the definition of primary cellular differentiation (See Appendix I) are modulated through biophysical and biochemical changes enabled by selective gene expression occurring within individual cells. Such selective, primary cellular differentiation-specific gene expression attends, for example, the cell cycle (Doerner 2000). In other words, the hypothesis here is that systemogenetic and organogenetic commitments reside exclusively in genes underlying primary cellular differentiation, specifically, cell-division activity. Kaplan (1992) expressed the view that "patterns of cell division have no significance for form generation in plants," and while it remains to be seen if that is correct, if the point is conceded it could still be that there is more to gene regulation/expression attending cell cycling, in relation to developmental commitment, than is overtly apparent.

Pfeffer (1903) reasoned that the successive steps in growth and development are not independently controlled, attainment of one stage providing a

mechanism to control which of the alternative pathways is selected at the next developmental step. Fahn (1974) considered that the specialization of the different cells and tissues in the plant, and the sites where the various types of cells and tissues appear, can only be explained in terms of the organism *as a whole* regulating the nature of its cells. Thus, in agreement with the discovery by Nehemiah Grew (1682) that the whole body of a tree is "truly continuous by means of the parenchyma," organismic theory stresses the unity of the protoplasmic mass (Fahn 1974). Grew (1682) did not consider the tree to be networked by its cambium because he considered cambium (a term he coined from the Latin *cambire* or possibly *cambio,* meaning change or barter) to be noncellular, rather a kind of sap that could form either xylem or phloem. However, had Grew had the resolving power of modern light microscopy, there can be little doubt that he would have included cambium in his statement!

The concept of 'tissue' as a biological entity, placed through reductionism between organ and cell, has been given its authenticity primarily by promulgation of the term throughout the biological sciences. Following Sachs (1875), Haberlandt (1914) attempted to classify and characterize tissue systems of plants on a functional basis, and Esau (1960) further grouped tissues of vascular plants into three tissue systems: dermal, vascular, and fundamental (or ground). Foster and Gifford (1974) explained that "The wide and continued use today of vascular patterns in morphology is based upon the fundamental assumption that the vascular system is more stable, or conservative, in a phylogenetic sense, than other tissue systems and hence is reliable as a criterion in morphological interpretation."

Given that systemogenesis begins through cellular determination, that committed organogenesis occurs within determined systemogenesis in conjunction with the ability of the whole to 'bend' phenotypic expression into particular developmental pathways, and that organs comprise tissues which themselves comprise cells, the simplistic deduction is that histogenesis, whether it be formation of tissues during primary growth or subsequent formation of cambium, secondary phloem, secondary xylem, or other tissues within an organ, must also be committed development. On the other hand, the preceding logic hinges on tissues being, both structurally and functionally, genetically encoded multicellular entities, as opposed to chance aggregations of cells in states of secondary and terminal differentiation forced into the status of 'tissue' because of their position and the imagination of science.

Arguments for Committed Histogenesis

Cambial growth can be seen in at least two distinct ways—as committed histogenesis or as physiologically regulated differentiation of noncommitted pluri- or totipotent cells. In relation to the development and complexity of the stele, the concept of determined histogenesis was implicitly embraced more than a century ago (Van Tieghem and Douliot 1886). The usefulness of the pattern of vascular development as a phylogenetic criterion seems, of itself, to be a strong argument that histogenesis and particularly vascular system development must be genetically encoded. On the other hand, during embryogenesis of conifers, procambium is formed but elements of xylem or phloem have not been detected (RA Savidge, unpublished data), possibly an indication that commitment of the vascular system does not extend beyond the procambium (although developmental arrest is also a possibility). There are differences in anatomy and physiology between vascular systems of shoots and roots (Cutler 1976; Cutler and others 1987; Wilcox 1964; Panshin and de Zeeuw 1970), but cambial growth occurs in both shoot and root systems. An argument for determined xylogenesis is that the anatomical makeup of each piece of wood is diagnostic of the genus (Panshin and de Zeeuw 1970; Wheeler and Baas 1998).

During organogenesis, some tissues require more time than others to emerge, but the existence of a temporal progression of histogenesis within a developing organ no more contradicts the possibility of developmentally specific gene expression operating in concert at a multicellular level than does sequential 'building' of the overall predictable form of the system or of its organs. On the other hand, assuming that histogenesis is a committed aspect of organogenesis, and that committed expressions arise principally through gene regulation, the occurrence of successive changes in the developing organ clearly does require the genome to be organized at several levels of commitment—that is, primary systemogenetic commitment, secondary organogenetic commitment, tertiary histogenetic commitment for tissue formation within the primary axes, quaternary histogenetic commitment for evocation of secondary tissues, and so on. Within this scheme, commitment evidently would require chromosomal modifications to occur more or less synchronously in adjoining populations of cells, from one level of commitment sophistication to another in a succession that, although flexible both in timing and spatial extent, would ultimately generate a particular structural and functional outcome.

Given that shoot-root systemogenetic determina-

tion is activated with the first division of the zygote, it is conceivable that individual cells of the cambial zone could also specify at division the ensuing nature of development. Larson (1994) reviewed the history of the concept of the cambial "initial" as the determined progenitor of xylem and phloem "mother cells," themselves also perceived to be *ab initio* committed to generate xylem and phloem elements. The logical difficulty of having a cambial fusiform 'initial' competent to oscillate between two distinct types of determination (that is, production of both phloem and xylem mother cells) has sometimes been an argument for the existence of two cambial initials within each radial file, although a single initial continues to be favored (Larson 1994). Other difficulties with 'initial' and 'mother-cell' terminology have been considered elsewhere (Savidge 1985, 1996, 2000a). The use of 'initial' and 'mother cell' has become so widespread that it is unlikely that they are consistently used to mean committed development, although that is the implicit meaning.

In conifers, the biosynthesis of coniferin within the cambium only during the period of cambial growth appears to be evidence for some type of specification operating (Savidge 1989; Savidge and others 1998; Savidge and Förster 1998); however, it appears to be specific to the annual cycle of growth and dormancy as opposed to secondary or terminal cellular differentiation events associated with histogenesis.

Arguments for Noncommitted Histogenesis

The readily observable phenotypic plasticity evident in xylem anatomy and chemistry indicates that the cambium genome is not determined to produce rigid outcomes. However, the possibility that specification (for example, for prosenchyma) operates as a committed but reversible expression cannot be excluded with current knowledge (Savidge 1996). As detailed above, the dilemma exists in relation to cambial commitment that the plant 'tissues' known as xylem and phloem are far from uniform and, in fact, exist in abundantly varied forms even within the individual organ as well as within and between species. The existence in secondary xylems of waves, bands, clusters, lines, V-shaped groups, and pockets of specialized more or less randomly distributed cellular phenotypes clearly indicates that intercellular communication occurs, not as a general signal affecting all cambial derivatives equally, rather as a number of distinct, tangentially separated signals spaced less than predictably around the circumference of the cambium and shifting position as organ diameter increments. Under experimental conditions, it can be seen that these signals have cellular resolution even within intact cambium (Savidge 1994, 2000b).

The fact that the pattern of vascular development at the level of primary growth has proven highly useful as a phylogenetic criterion does not constitute proof that histogenesis *per se* is committed. It seems equally possible that histogenetic patterns owe their origin and character to commitment operating at higher levels (systemogenesis, organogenesis), those committed expressions concomitantly generating intercellularly transmissible signals which serve to induce secondary and terminal cellular differentiation events. What has been perceived as committed tissue development within organs could also be viewed as individual cells independently but nevertheless collectively undergoing more or less similar types of secondary cellular differentiation, sometimes followed by terminal differentiation, in response to changing environments.

The reality of each species tending to have unique physical, chemical, and anatomical properties in its wood does not necessarily point to the conclusion that each of the approximately 80,000 species of woody plants on earth has a specific regulatory mechanism controlling its xylogenesis. However, it certainly does indicate that histogenetic commitment, if it has any reality at all in relation to cambial growth, is not uniform across species. The fact that the cellular differentiation pursuits of cambial derivatives in the Magnoliophyta can be readily manipulated by varied phytohormone treatments indicates that, whatever the regulatory mechanism, cell-type specification must involve a highly versatile mechanism. What would be most informative but is currently lacking is an *ex arbor* fully defined and environmentally controlled secondary growth system which can be manipulated to provide predictable, variable outcomes *in vitro* (Leitch and Savidge 2000).

Differential Gene Expression in Relation to Diameter Growth

Within the hypothetical scenario that the genotype is constant among the population of cambial cells and their derivatives, and also is totipotent, hence devoid of epigenetic commitment to any particular type of histogenesis, type of secondary or terminal cellular differentiation, those cells by virtue of being alive and undergoing so-called 'housekeeping' or maintenance metabolism would nevertheless remain capable of having their homeostatic condition overbalanced through G X E interactions. Once the anabolic - catabolic equilibrium has been pushed too far either way, subsequent cascading and self-

inducing changes in catalysis, hence substrate supply, conceivably could enable alterations in gene expression such that cells become progressively and irreversibly different. The several distinct developmental zones of wood formation (Figure 1) clearly manifest metabolic plateaus, and their transitional regions indicate where equilibrium becomes overbalanced and cascades to the next step, changing cells biochemically, hence anatomically, into differentiated states. The concept that master regulatory genes amplify and/or orchestrate expression of families of structural genes is not incompatible with this interpretation (Savidge 1996). Loss of equilibrium induced by changing 'E' could result in differential rates of production, binding activity, or degradation of regulatory proteins.

Both the nature and control of gene expression in relation to formation, perpetuation, cell-cycle/dormancy activity, and trans-differentiation within the cambial zone, and also in relation to the alternative pathways of secondary and terminal differentiation of cambial derivatives, remain to be fully elucidated. Whether any particular aspect of gene expression or biochemistry is specifically turned on, or merely amplified from a basal level, during any type of secondary or terminal cellular differentiation, appears to be the key issue in relation to identifying the underlying genes and understanding cellular control mechanisms. It is not yet possible to say with any real confidence if differential gene expression associated with developmental changes occurring in the cambial region involves selective (that is, qualitative) expression with attending complete turn off of some genes and turn on of others, as appears to occur in animals (Voet and Voet 1990), or alternatively, if it is more akin to what happens in prokaryotic systems where reduced but still significant basal levels of totipotent expression are maintained. Significant basal levels of plastid-encoded expression, some of which supposedly are relevant only to photosynthesis, have been observed in both etioplasts and roots (Gruissem and Schuster 1993), possible support for the prokaryotic model. Lignification is the pivotal event for cells being sorted by anatomists into parenchyma or prosenchyma, and monolignol biosynthesis has been considered to be the first committed step in the formation of lignin. There is abundant quantitative data indicating that monolignol-glucoside synthesis occurs within the cambium in the complete absence of attending lignification (Savidge and others 1998) and, more recently, that monolignols are synthesized even in dormant cambium (Savidge and Förster 2001). Another aspect that may be important in altering gene expression, as well as metabolism, is the cambium's evidently normal ability to function under conditions of both ambient and low O_2 concentrations. Acetaldehyde and ethanol are abundantly present in cambia of both hardwoods and conifers (MacDonald and Kimmerer, 1991), and using GC/MS we have confirmed that those indicators of anaerobic metabolism are actually synthesized in vitro by cambial tissues (N. Forneris and R.A. Savidge, unpublished data). Biochemical evidence for gene expression specific to the annual cycle of cambial growth and dormancy in perennial woody plants has been reported (Savidge and Förster 1998), and as aerobism - anaerobism also appears to fluctuate with the annual cycle (Eklund and others 1998), there may be a link between the two.

Are 'housekeeping' genes different from genes underlying secondary and terminal cellular differentation? Processes identified to be essential for differentiation of cambial derivatives into prosenchyma, such as cell-wall biosynthesis, clearly are dynamic and may never actually be 'switched off' within living cells; their kinetics varying in both magnitude and proportion as the course of differentiation changes. By their nature, none of electrophoresis, *in situ* hybridization, or immunolocalization appears to be an unequivocal method for excluding the possibility that totipotent gene expression continues to 'tick over' at a low rate as cells differentiate. Subtractive expressed sequence tag (EST) libraries are being used to distinguish developmentally specific gene expression from that needed for "housekeeping"; however, again, it remains problematic whether any aspect of structural gene expression is specific to secondary or terminal cellular differentiation. EST homologue data are insufficient to establish that any particular reaction, biochemical pathway, or other mechanism dependent on gene expression is actually operating in the cells investigated (Appendix II), but such data certainly do provide bases for formulating hypotheses and undertaking quantitative biochemistry research to test them. To obtain unequivocal evidence will require rigorously quantitative highly precise analysis (for example, by combined liquid chromatography - mass spectroscopy) of, at least, polypeptides and ideally of physiologically mature proteins, comparing cells before and after they commence differentiation. There is the added difficulty that increasing evidence indicates that mRNA, and possibly also proteins, are transported intercellularly (Ruiz-Medrano and others 1999). Thus, the cambium in isolation needs to be investigated, separate from external sources of transmissible mRNA and/or protein, before drawing any conclusions on what aspects of gene expression are "specific" to the development under study.

By comparing an EST with sequences of known genes in data banks, homology analysis permits deductions about which genes are actively being transcribed. Recently, 5,692 genes associated with cambial growth and wood formation in *Populus* spp. (Sterky and others 1998), and 1,097 EST obtained from developing xylem of *Pinus taeda* were reported (Allona and others 1998). Those were the first two major efforts to characterize the multifaceted gene expression underlying vascular development in trees, and at this time approximately 43,000 gene sequences have been registered (http://www.ncbi.nlm.nih.gov/dbEST/ searching under phloem cambium, cambial zone, and xylem).

Comparative investigation of EST and/or proteins in reaction vs. normal wood has been done (Baba and others 2000; Wu and others 1998; Allona and others 1998), and similar work could be attempted with ray cambial cells (and their derivatives) and fusiform cambial cells (and their derivatives) from each zone, after separating them as protoplasts (Leinhos and Savidge 1993; Savidge 2000a). *In situ* hybridization and immunolocalization will also be essential for achieving cellular resolution. Successive dates of tissue collection (for example, during periods of springtime cambial reactivation or autumn inactivation) enable zonation-based protein or EST analysis, and such research is underway in my laboratory.

Although, as considered above, phytohormones, physical pressure, and numerous additional transmissible factors undoubtedly contribute to the 'E' component of the cambial region's G X E interactions, it is still unclear how any interacts, either directly or indirectly, with the 'G' to influence gene expression. IAA evidently has a general effect of transcriptionally activating Aux/IAA genes (Fujii and others 2000; Worley and others 2000), and it may seem reasonable to expect the same effect on cambium, but woody plants remain to be investigated. *A. thaliana* is a short-lived annual that typically exhibits only primary vasculature, although secondary xylem is produced in aged or flowering-circumvented plants, (Lev-Yadun 1996; Zhao and others 2000). Thus, *A. thaliana* genomics/proteomics research undoubtedly will contribute to identifying the genetic and biochemistry bases for formation of procambium, primary xylem and phloem, vascular cambium, and secondary xylem and phloem.

Plausible mechanisms of gene regulation in the cambium that are worthy of investigation include the following. Based on other eukaryotic systems, the favored hypothesis may be variation in competence for transcriptional initiation (Voet and Voet 1990). That competence can be deactivated through nucleotide derivatization (for instance, cytosine methylation), or activated by transcription factors, or activated/deactivated by facultative changes in heterochromatin content and position. Rates and efficiencies of transcription can be modified by changes in and relative abundances of histone and/or non-histone proteins, also by derivatization of promoter or enhancer elements, and by splicing mechanisms involving interchanging exons and introns (Voet and Voet 1990). The abundance of rDNA, hence rRNA, and ribosomes can vary, a consideration perhaps particularly relevant to differentiation of cambial derivatives into prosenchyma because ribosome numbers appear to increase greatly during that type of differentiation (Savidge and Barnett 1993; Lloyd and others 1994, 1996). It deserves mention that nucleoli are surprisingly numerous but variable in nuclei of cambial ray and fusiform cells (Savidge 2000a; Larson 1994). Differential rates of translocation of mRNA-protein complexes to the cytosol represent another mechanism for differential gene expression, as do variable rates of translational initiation (Voet and Voet 1990). mRNA degradation rates may vary depending on the sequence and cellular environment (Belasco and Brawerman 1993). Proteolytic cleavage and covalent modification of polypeptides are important mechanisms for regulating enzyme activity (Iliev and Savidge 2000), and posttranslational derivatization (for example, glycosylation) operates to target proteins to their final cellular destinations. Plastids presumably make a major contribution to lignification by housing the Shikimate pathway, and those organelles appear to place considerable emphasis on posttranscriptional mechanisms that regulate mRNA stability and processing (Gruissem and Schuster 1993).

Elsewhere the regulation of xylogenesis was examined from the standpoint of what evolutionary changes had to occur in the primitive plant genome, and I elaborated a 'continuum hypothesis' aimed at reconciling the anatomical reality with the developmental potentiality (Savidge 1996). Variable masses of secondary-wall formation during tracheary element differentiation were suggested to be a function of the duration of continuing homeotic regulation of the underlying structural gene expression, and it was considered that the fibre tracheid arises as an intermediate between tracheary elements and sclerenchyma, and that the differences between parenchyma, collenchyma, sclerenchyma, and tracheary elements arise not through cell-fate determination but by one biochemical pathway being in a better environment to operate than another during cellular differentiation. If regulatory genes/proteins govern independent structural pathways, such as

those underlying production of cellulose or lignin, rather than cell type programs, then differentiation becomes highly versatile in a modular sense, as it indeed appears to be during xylem formation (production of the gelatinous layer during differentiation of cambial derivatives into tension wood fibres is a persuasive example). Although homeobox genes and their proteins appear to be components of organogenetic determination in animals (for example, in *Drosophila:* Voet and Voet 1990), gene regulation by homeotic proteins in plants does not necessarily imply either determination or specification, as homeobox transcription conceivably can be initiated in response to transmissible factors.

Conclusions

Formation of vascular tissues and their subsequent functioning in service to the whole plant depend on initiation of determined shoot and root systems followed by specified organogenetic expressions. Morphogenesis attending both systemogenesis and organogenesis occurs through cell division, cell expansion, and making or breaking of intercellular bonds, and those primary cellular differentiation activities are distinct from the secondary and terminal cellular differentiation events that follow the early formative processes, particularly by the absence of cell division. The same applies to cambial activity vis-á-vis phloem and xylem development. The evidence indicates that in every case developmental commitment does not arise spontaneously, but is induced and linked to genes regulating the cell cycle, diminishing with increasing distance from meristems but becoming reinstated under conditions inducing cell division activity. Thus, cells of the cambial zone evidently are committed in their primary cellular differentiation, but observations that the overall nature of the vascular system is phylogenetically and ontogenetically highly conserved appear to have their explanation in signals generated at the levels of shoot and root systemogenesis and organogenesis, rather than in committed development of vascular tissues *per se*. Cambial fusiform cells as a polar auxin transport corridor serve as an axial communication network linking shoot and root systems, and rays serve as a radial communication network linking exterior and interior conducting cells to cambium. In addition to 'feeding' the cambium, it is proposed that those networks enable three-dimensional monitoring, integration, and regulation of growth and development throughout the plant at all stages. Because the cambium is evidently committed only at the level of primary cellular differentiation, considerable plasticity exists in the anatomical, chemical, and physical properties attained by cambial derivatives, and that plasticity combined with genetic variability influences the whole plant phenotype. The prevalent assumption that genes are selected during secondary and terminal cellular differentiation, and that "housekeeping gene" expression is qualitatively distinct from structural gene expression during vascular development, is based on comparative semiquantitative as opposed to precise quantitative analysis; thus, the assumption remains to be substantiated. Secondary and terminal cellular differentiation evidently are induced and the extent of differentiation modulated through repeated expression of generic structural genes regulated by a combination of long-distance signals and cell-cell messages, that is, as genotype X environment interactions. It is proposed that homogeneous clusters of vessels, tracheids, sclerenchyma, sieve cells, or axial parenchyma form in xylem when populations of differentiating cambial derivatives receive and themselves propagate uniform signals, whereas groupings of heterogeneous elements develop when differentiating cells receive and/or generate variable messages.

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Appendix I. Definitions and Other Preliminary Considerations

Perhaps the paramount difficulty remaining in plant biology's effort to explain how growth and development are regulated has to do with the developmental biologist's promulgation of 'big', usually esoteric terms which rarely are given even a mention in biochemistry and molecular biology treatises (for example, Buchanan and others 2000). Although developmental terms generally convey what appear to be valid hypotheses, they implicitly embody multiple and not uncommonly poorly defined assumptions about gene regulation, biochemistry and biophysics, making it difficult to test them within the scientific method. Molecular biology has begun to come to developmental biology's rescue by providing definitive evidence that diagnostic types of gene expression and/or aspects of gene regulation are associated with particular types of development. That is a starting point, but neither association nor the strongest correlation is necessarily equivalent to

causation in complex, multi-cellular, multi-organ, perennial, phenologically varying eukaryotic systems, and it is easy to lose sight of the whole when the focus is on individual genes found in small numbers of cells at one moment in time. Moreover, progress to date has been almost exclusively of a qualitative or, at best, semiquantitative, nature. Immense research efforts in proteomics and physiological genomics still appear to be needed if the big considerations of morphology are to be meaningfully addressed. Because I found it necessary to use many of the traditional 'big' terms in this paper, I offer the following definitions.

Phenotype is the visible expression and is used to designate the product of interactions between the genome (or genotype) and its environment (physical and chemical phenomena, also other biological species); in other words, P = G X E. **Morphogenesis** refers to developmental processes and changes generating phenotypic expressions, in particular shapes, at all visibility levels (for example, whole plant, shoot vs. root systems, organs, tissues, cell associations or 'sub-tissues', individual cells, and subcellular components) and all developmental stages throughout a plant's life cycle, beginning with the zygote. **Embryogenesis** is morphogenesis from syngamy to development of competent-to-germinate seed embryo, and **somatic embryogenesis** is the same except that it appears to begin with a single somatic cell rather than a fertilized ovum. **Systemogenesis** is morphogenesis of the shoot or root system. **Organogenesis** refers to any spatially localized and surficially distinguishable aspect of embryogenesis or systemogenesis involving a multicellular population of adjoining cells and displaying, in the final product if not also during development of the organ, specialized structure and/or biochemistry. **Histogenesis** refers to a spatially localized population of adjoining cells, whether within or on the surface of an organ, developing a phenotype and/or having biochemistry which distinguishes it from neighboring cellular populations. Histogenesis occurs either concomitantly with or following upon organogenesis. **Cambial activity** refers to cambium undergoing cell division activity, but it should be understood that not all cells in the cambial zone divide synchronously, either within or between radial files.

Cellular differentiation refers to an individual cell becoming different from what it was. Traditionally, cellular differentiation has been based on anatomical discrimination, but biochemical changes undoubtedly precede and facilitate all visible changes. Thus, differentiation includes biochemical specialization whether or not there are detectable changes in overt phenotype. As per previous usage (Savidge 1983a, 1985, 1996), **primary cellular differentiation** refers to changes occurring within or near active meristems, and those changes include cell division, cell expansion, and making or breaking of intercellular bonds, but dormancy, homeostasis, and other interphase phenomena are not excluded from consideration. As discussed, not all cambial fusiform cells are derived directly from apical meristems, but also from parenchyma, and it is possible for cambial fusiform cells to **dedifferentiate,** or revert, into parenchyma (Savidge and Wareing 1981b; Savidge 1983b). **Secondary cellular differentiation** is used to designate any state achieved by a still-living plant cell (for example, chlorenchyma, collenchyma, parenchyma) after it has ceased primary cellular differentiation activities and achieved mature functionality. Unless determined (see below), a cell in a state of secondary differentiation can revert to primary differentiation activities under appropriate inductive influence (for instance, wounding followed by tissue culture), and it also remains competent for pluri- or totipotent expression (see below). Thus, the living cell in a state of secondary differentiation can **transdifferentiate** to another secondary state, undergo senescence, or become terminally differentiated. **Terminal** or **tertiary cellular differentiation** refers to the loss of the nucleus (for example, during differentiation into sieve cells) or complete protoplasmic autolysis. In relation to xylogenesis, it should be understood that despite many papers published in recent years about 'programmed cell death' or 'apoptosis', protoplasmic autolysis does not necessarily follow secondary wall formation and lignification when cambial derivatives become prosenchyma, and when it does occur the duration can range from hours to years (Dumbroff and Elmore 1977; Nix and Villiers 1985). Death of xylem parenchyma during heartwood formation evidently does not involve protoplasmic autolysis (Hillis 1985; Yang and others 1994).

Competence refers to the capacity of a cell or tissue to respond to an inductive stimulus by following a particular developmental pathway, different from that which it would follow in the absence of induction (Bird and others 1982). **Epigenesis** refers to orderly physical or chemical changes occurring to the structure or expressability of genetic information, relative to the zygote's original competence, and persisting through DNA replication and nuclear division (Savidge 1983a, 1985). **Commitment** refers to an epigenetic change such that gene expression can yield only one outcome (Bird and others 1982; Wareing 1982; Graham and Wareing 1984). Slack (1991) defined commitment as an "aspect of the in-

trinsic character of a cell or tissue region which causes it to follow a particular pathway of development or fate," and he distinguished three connotations, **specification, determination,** and **potency,** which with minor modifications are used here to refer to cells derived from meristems. A cell or tissue explant is **specified** if, when isolated under conditions where the physiological environment is maintained constant (except for the wounding needed for isolation), that explant develops autonomously into a predictable phenotype. A cell or tissue explant is **determined** if it will achieve a predictable phenotype autonomously even when the physiological environment is altered. **Potency** is a term to express the range of possible phenotypes into which a cell or tissue can develop under changing environmental conditions. Determined cells are strictly **unipotent.** Specified cells also exhibit unipotency but they nevertheless retain competence to respond to environmental change by expressing alternative phenotypes; thus, in reality they are **pluripotent.** If the cell or tissue is able to become any type found in the mature organism, it is **totipotent.** In other words, determined cells can be considered as fully committed, specified cells partially committed, and totipotent cells not at all committed. Cambium has been reported to be totipotent (Kumar and others 1991; Jouira and others 1997), but it remains to be seen whether such potency is generic.

Presence or absence of lignin provides a useful dichotomy for distinguishing phenotypes of both xylem and phloem arising through secondary and terminal cellular differentiation. Excepting meristem-associated cells, **parenchyma** comprise all nonlignified living cells whether with or without obvious secondary walls. **Prosenchyma** is a term rarely seen anymore and when encountered its context unfortunately may be in relation to any elongated cell having tapering ends (Committee on Nomenclature 1964); however, prosenchyma in the older literature was used as a collective term for all woody, or 'hard' (as opposed to 'soft' parenchyma), cell types (Committee on Nomenclature 1964), and that is how it is used here. Thus, prosenchyma denotes all cells, whether living or protoplasmically autolyzed, having lignified secondary walls. Prosenchyma therefore comprise both sclerenchyma and tracheary elements (see below). **Sclerenchyma** comprise living cells or dead elements having lignified secondary walls but lacking bordered pits, and the group includes more or less isodiametric **sclereids** as well as fusiform and more complexly shaped **fibres. Tracheary elements** include tracheids (including ray tracheids and fibre tracheids) and vessel members, types which in addition to being lignified must be capable of water conduction; therefore, in secondary xylem they are diagnosed by the presence of bordered pits, perforation plates enabling vessel members to be distinguished from tracheids (however, see Aloni and others 2000). The term **initial** in relation to cambium is frequently seen in the literature, dating from Sanio (1873), although Sanio met strong resistance to the concept (see Larson 1994) and earlier researchers advocated "mothercell" rather than initial (Hartig 1853, 1878). As discussed elsewhere (Larson 1994; Savidge 1996, 2000a), use of ' initial' implies that cambial cells are committed to producing xylem and phloem, and because the evidence for that kind of commitment remains unconvincing, the term is used by me only when considering that possibility.

Brief comment on 'qualitative' vs. 'quantitative' is also warranted. Mathematics is often described as the language of science, and there can be no doubt that quantitative treatment of biological development is appropriate and important for progress. On the other hand, quantitative treatment in advance of having discovered, characterized, and clearly defined the parameters under investigation is antithetic to integration and unlikely to yield other than fuzzy solutions having unacceptable errors. Theodor Hartig (1878), the discoverer of sieve tubes in the phloem of trees as well as the Hartig-net of ectomycorrhizae, considered that in living organisms no reactions take place other than the forces of matter ("daß auch im lebenden Organismus keine anderen als die Kräfte des Stoffs arbeiten"), and D'Arcy Thompson (1917) wrote that "the things which we see in the cell are less important than the actions which we recognize in the cell." In both of those early reflections was the vague appreciation that energy transformations must be understood in order to explain how living systems change, and this of course is now a well appreciated fundamental principle of biochemistry. However, it is equally well appreciated that reactants, products, catalysts, and other conditions should be defined before attempting quantitative analyses in biochemistry. Unambiguous definition requires making qualitative distinctions, whether the consideration be a gene sequence, an mRNA, a protein, a hormone, a metabolite, a cell structure, a cell type, an organ, or a whole organism. Although much has been published on plant morphology and anatomy, it would be supercilious or naive to think that all of the distinct physiological phenomena underlying cambial growth are already known, or that the contribution of any to the functioning of the whole has been fully and clearly defined. Once a molecule or physical phenomenon has been sufficiently well character-

ized that it can be accurately and precisely distinguished from other phenomena, it becomes appropriate to quantify it. At that point, the detection limit attending the quantitative procedure becomes the primary issue.

Appendix II. Enzymatic Catalysis vis-a-vis Est Analysis

Discovery of an EST (expressed sequenced tag), which evidently encodes an enzyme, does not constitute proof that a catalytically functional enzyme is either already present or being produced, rather, merely circumstantial evidence for impending production of a polypeptide that, if it is actually synthesized at the ribosome, could remain to be spliced, folded, and variously derivatized before (or after) it has been transported to a location in the cell where it can function in catalysis. cDNA homologues may have semblance in both structure and origin, but it does not necessarily follow that they share the same function. Enzymes having very similar polypeptide primary structure can catalyze quite different reactions. Moreover, it is not uncommon for enzymes to exhibit versatility, the compound(s) actually serving as substrate at any moment being determined by the physical and chemical environment in which the enzyme functions, as well as by the relative abundance of that compound in relation to alternatives. Subcellular compartmentation, various types of enzyme inhibition, limitations in water, temperature, light, and co-factors, and other regulatory mechanisms can all operate to modulate, or totally suppress, as well as activate, enzymatic catalysis. Thus, even if the needed precision in tissue and cellular isolation (for example, ray vs. fusiform cambial derivatives) were brought to bear and assumptions about gene expression based on EST data were verified, it seems unlikely that such gene expression data in isolation can provide unequivocal information on the regulation of cambial growth. The need for quantitative mRNA and proteomics data is apparent.

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