The Cell Walls that Bind the Tree of Life

KARL J. NIKLAS

Cell walls have evolved independently in many phyletically diverse clades, including the Eubacteria and Archaebacteria. However, a review of the available genetic and biochemical evidence indicates that the machinery responsible for synthesizing the cell walls of land plants, of their nearest algal relatives (the charophytes), and of some very ancient algal lineages (chlorophytes, rhodophytes, and phaeophytes) can be traced back to ancient primary endosymbiotic events involving Eubacteria (specifically cyanobacteria and proteobacteria). Lateral gene transfers attending secondary endosymbiotic events appear to be responsible for manufacturing the cell walls of more recently evolved photoautotrophic and heterotrophic lineages (e.g., euglenoids and tunicates). Recent research into the genetic basis of cell wall synthesis and chemical composition in bacteria, algae, and land plants continues to shed light on the phylogenetic relationships among a broad spectrum of evolutionarily and ecologically diverse organisms.

Keywords: algae, bacteria, cell walls, cellulose synthase, land plants, lateral gene transfer

The past decade has seen a renaissance in research on the evolution, chemical composition, synthesis, and functional roles of cell walls across a broad spectrum of organisms, ranging from bacteria to land plants (Ross et al. 1991, Saxena et al. 1995, Carpita 1996, Popper and Fry 2003, Matsunaga et al. 2004). This renewed interest is spurred by a greater awareness that cell walls have evolved independently in many different clades and that seemingly small modifications in their chemistry can have profound effects on the multifarious functions cell walls perform (Ligrone et al. 2002, O'Neill et al. 2003, Popper and Fry 2003). Research on cell walls is shedding more light on the phylogenetic and ecological relationships among organisms as diverse as bacteria, oomycetes, algae, slime molds, tunicates, and land plants.

Industrial applications and a heightened awareness of the fragility of Earth's biosphere have also fostered a renewed interest in plant cell wall synthesis. It is estimated that plants annually synthesize more than 10^{11} metric tons of cellulose, making this biopolymer one of the most abundant organic molecules in the biosphere (Hess et al. 1928). Yet, despite considerable progress in abiotically synthesizing cellulose in large quantities, deforestation driven in part by demands for paper products continues to imperil tropical ecosystems.

In this article, I review the current scientific understanding of cell wall chemistry, synthesis, and evolution. Two evolutionary characteristics revealed by the Tree of Life project (*http://tolweb.org/tree/phylogeny.html*) complicate this endeavor. First, the cell wall—here broadly defined to include any intra- or extracellular matrix made by the protoplast and adjoined to its cell membrane—has evolved independently in diverse bacteria and in a host of phyletically disparate unicellular and multicellular heterotrophs and photoautotrophs. Second, all eukaryotic lineages are believed to have evolved from either primary or secondary endosymbiotic events—that is, from the permanent establishment of previously physiologically independent prokaryotes or eukaryotes within host cells (Margulis 1981, Knoll 2003). The phyletic scope of any article treating cell wall evolution is thus exceptionally broad, because it can ignore neither the diversity of cell wall construction and chemistry nor the possibility that the machinery of cell wall biosynthesis has been laterally transferred from prokaryotic to eukaryotic organisms many times over the course of evolutionary history.

That this lateral transfer has figured prominently in life's history is nowhere more evident than in the phyletic distribution of cellulose biosynthesis. Cellulose not only figures prominently in the cell walls of all land plants, it is also a significant component in the walls of diverse algal lineages, which have separate evolutionary origins (Niklas 2000, Knoll 2003). Many other life forms, ranging from slime molds and oomycetes to heterotrophic protists and tunicates, are capable of synthesizing cellulose as well; indeed, many of the evolutionary branches shown on the Tree of Life have been grafted by threads of cellulose. For this reason, cellulose serves as one of the overarching themes of this article.

Karl J. Niklas (e-mail: kjn2@cornell.edu) is a professor in the Department of Plant Biology, Cornell University, Ithaca, NY 14853.

A diversity of walls

The terminology and nomenclature for cell surfaces and walls is confusing, because the same structures have been given different names by workers in different disciplines and because the same term is often used to describe structurally nonhomologous structures. For example, the structurally flattened vesicles underlying the cell membranes of ciliates, dinoflagellates, and apicomplexans have been called the *amphiesma* (by protozoologists), the *alveolus* (by phycologists), and the *inner membrane system* (by mycologists), whereas the term *theca* has been used to describe the walls of dinoflagellates, diatoms, and some prasinophytes. The production of large amounts of extracellular mucilage by some species adds to this confusion, because it remains unclear whether this material is an integral metabolic part of the cell. For example, many cyanobacteria produce large amounts of mucilage external to their well-defined walls, whereas the protoplasts of some euglenoids, such as *Trachelomonas lefevrei,* are enclosed in anastomosed strands impregnated with manganese.

Preisig and colleagues (1994) reported 34 synonyms among 100 terms collected from the primary literature treating cell surfaces and walls; they attempted to codify and simplify this terminology, but with limited success. More recently, Becker (2000) proposed a classification scheme that has the merit of employing only five basic types of cell surface or cell wall: simple, albeit externally extended, plasma membranes (type I); cell surfaces bound to internal materials (type II); surfaces with external materials (type III); surfaces with internal vesiculated materials (type IV); and surfaces with materials both internal and external to the plasma membrane (type V). Types II and V are unique in terms of their phylogenetic appearance; type II occurs exclusively among euglenoids (in the form of a pellicle), whereas type V is unique to the cryptomonads (in the form of a periplast) (figure 1). Interestingly, each of these two groups is composed of relatively few species, and each is believed to be the evolutionary byproduct of a eukaryotic photoautotrophic endosymbiont and a eukaryotic heterotrophic host cell. Species within other, much more species-rich lineages also share the same type of cell surface or cell wall architecture (figure 1). For example, all Archaebacteria have a type I cell surface, which in some species has a thick coating (the glycocalix) made up of cell membrane glycoproteins and glycolipids. Likewise, all land plants (embryophytes) and the majority of their closest algal relatives (the charophytes) have type III cell walls.

It is evident, nevertheless, that organisms currently nested in some large groups manifest very different types of cell surface or cell wall. For example, the stramenopile, or chromophyte, clade includes lineages with type I or type III cell surfaces or walls (e.g., the eustigmatophytes [type I] and the xanthophytes, phaeophytes, and diatoms [type III]) as well as the unique cryptomonad type V. Furthermore, the same cell surface type can have different chemical compositions, suggesting that some large clades are polyphyletic. For example, diatoms and xanthophytes possess type III cell walls that are composed, respectively, of silica and of cellulose and runic acids. Conversely, structurally dissimilar types of cell surface or wall may share similar or identical chemical components. For example, the cell surfaces of euglenoids, ciliates, and dinoflagellates are reported to be immunologically related (Becker 2000).

Additional problems exist for any cell surface classification scheme. Convergence among some types of cell surface or wall may result from evolutionary reduction and loss. Likewise, some species produce temporary cell walls differing from their more perennial protoplast investitures, whereas other species have two life forms that differ in their cell wall compositions. The same species, therefore, may have a chemically or structurally diverse cell wall repertoire. Clearly, in the absence of detailed chemical or immunological information, classification schemes based solely on the architecture of cell surfaces or walls run the risk of reducing terminological confusion at the cost of conflating analogous with homologous structures. Nevertheless, cell surface classification schemes, like that of Becker (2000; see also Okuda 2002), draw sharp attention to the structural diversity underwritten by seemingly well-defined phrases such as "extracellular matrix" or "cell walls." When superimposed on recent phylogenetic trees, these classification schemes illuminate the phyletic distribution of chemically similar cell surfaces and walls (e.g., those containing cellulose).

Deep roots

Cell walls have remarkably deep roots in the Tree of Life, roots that have translated chemical differences across the canopy of taxa they support (figure 1). The two major bacterial domains of life, the Archaebacteria and Eubacteria, which are distinguishable in many ways, differ profoundly in the chemistry of their cell walls. The Archaebacteria evince substantial diversity both in terms of the presence of cell walls and in terms of their chemical composition. Some species have naked protoplasts, but most have either a rigid sacculus cell wall composed of proteinaceous or glycoproteinaceous polymers or a cytoplasmic membrane reinforced with glycocalyx (Kandler 1994, Kandler and König 1998). This diversity in chemical composition suggests that the first archaeal protocells probably radiated before the cell surface chemically diversified. In contrast, the Eubacteria are characterized by a single sacculus wall-forming substance: a murein, or cross-linked peptidoglycan composed of glycan strands linked by short peptides that form a covalently closed net completely surrounding the bacterial protoplast. With few exceptions (e.g., *Mycoplasma, Planctomyces,* and *Chlamydia*), a chemical variant of this polymer is present in every species of the Eubacteria, including those in the oldest recesses of the eubacterial domain (Schleifer and Kandler 1972, Ross et al. 1991, Kandler and König 1998). The evolution of murein thus probably preceded the radiation of the first eubacterial protocells.

The apparent chemical uniformity of eubacterial cell walls is misleading, however, because some species have structurally complex cell walls composed of a wide array of polysaccharides, including cellulose. For example, the sheath

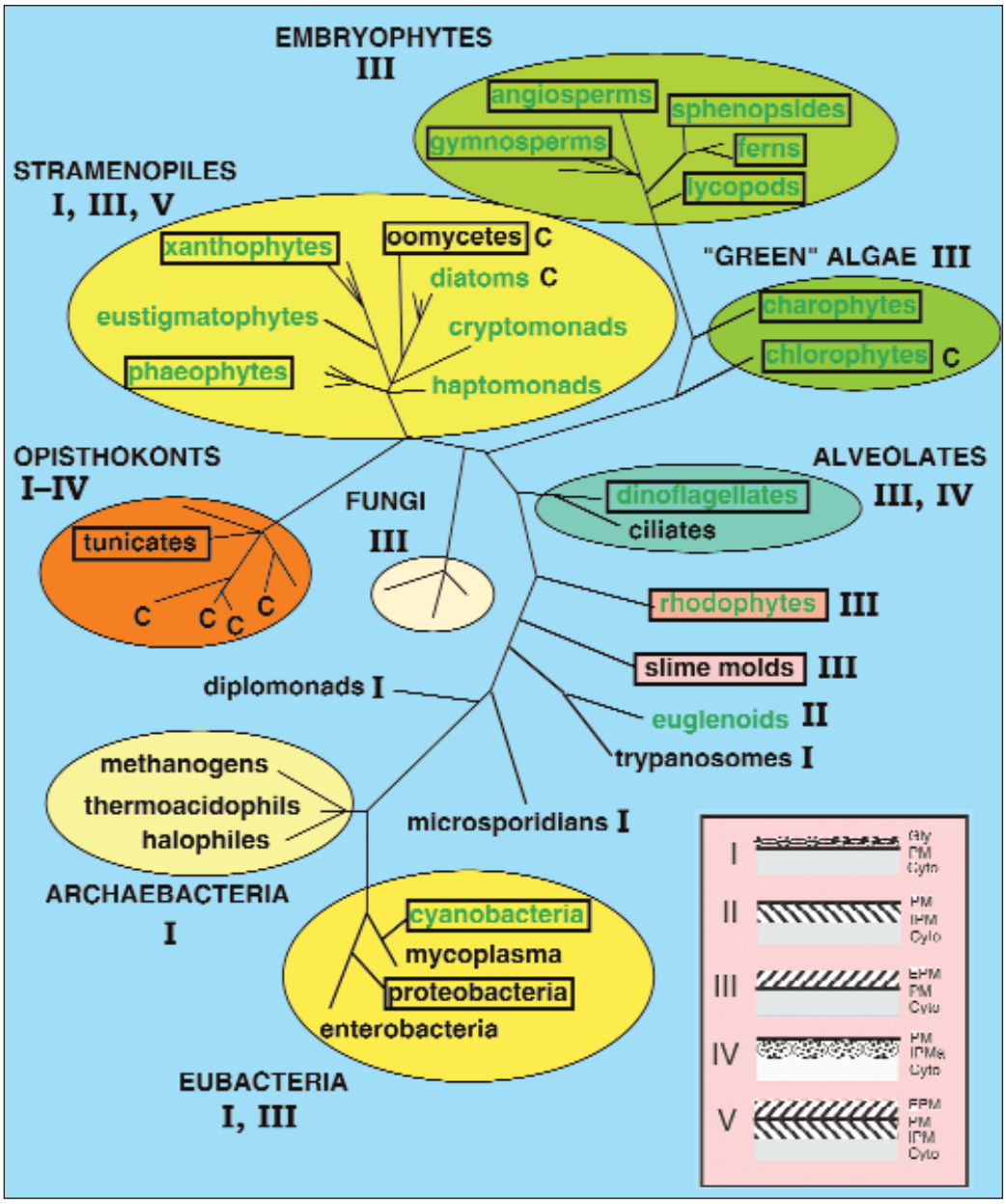

Figure 1. Distribution of five cell surface or cell wall types (I–V), and of the biosynthesis of cellulose and chitin, displayed on a highly simplified version of the Tree of Life. The types of cell surface or cell wall are based on the classification scheme of Becker (2000), shown at bottom right. Photoautotrophic groups are shown in lowercase green type, and heterotrophic lineages are in lowercase black type. "C" marks the clades capable of chitin synthesis; the names of those capable of cellulose synthesis are in rectangles. Abbreviations: Cyto, cytoplasm; EPM, materials external to the plasma membrane; Gly, glycocalyx; IPM, materials internal to the plasma membrane; IPMa, internal materials within cytoplasmic vesicles; PM, plasma membrane.

surrounding the cell walls of some cyanobacteria (e.g., *Phormidium autumnale, Oscillatoria princeps,* and *Nostoc punctiforme*) consists of a variety of pectic acids, mucopolysaccharides (containing ribose, galactose, rhamnose, and arabinose), and numerous cellulosic fibrils (Nobles et al. 2001). Likewise, some proteobacteria synthesize and extrude long cellulosic strands (e.g., *Agrobacterium* and *Rhizobium;* see Römling 2002).

The ability of some Eubacteria to synthesize cellulose is intriguing in light of the endosymbiotic theory of the origin of eukaryotic cells and the appearance of cellulose in the walls of vastly unrelated photoautotrophic and heterotrophic eukaryotic species (figure 1). The endosymbiotic theory proposes that the first eukaryotic cells evolved as the result of a physiological confederation of metabolically diverse prokaryotes (Margulis 1981, Knoll 2003). The first chloroplasts are believed

to have evolved from photoautotrophic oxygen-liberating cyanobacterial-like prokaryotes that survived within heterotrophic bacterial host cells. Likewise, the first mitochondria are believed to have evolved from ancient heterotrophic aerobic or oxygen-tolerant prokaryotes similar to modern-day proteobacteria. These primary endosymbiotic events prefigured the most ancient heterotrophic (e.g., diplomonad, microsporidian) and photoautotrophic (e.g., rhodophyte, chlorophyte) eukaryotic lineages. Secondary endosymbiotic events—in which photoautotrophic eukaryotes became permanent residents within heterotrophic eukaryotic host cells—also occurred and probably gave rise to the euglenoids, cryptophytes, and other algal groups (McFadden 2001).

In the context of cell wall evolution, the lateral gene transfer between cyanobacterial-like endosymbionts and the nuclei of their host cells provides an explanation for the distribution of cellulose biosynthesis among some of the most ancient algal lineages. Likewise, secondary endosymbiotic events may explain why some more recently derived protist lineages possess the ability to synthesize cellulose. That lateral gene transfer failed to occur in some cases is indicated by organisms like the euglenoids, which are believed to have evolved by means of secondary endosymbiotic events. Many euglenoids are photoautotrophic, yet they are incapable of synthesizing cellulose. Although the algal endosymbiont in euglenoid cells may have had the capacity to produce cellulose, this ability was not transferred to euglenoids. However, all euglenoids synthesize the β-1,3 glucan polymer, called paramylon, which is chemically similar to callose (Bäumer et al. 2001). Additionally, recent research has shown that the machinery of cellulose synthesis can "default" to callose synthase when cell membranes are damaged (Delmer 1999).

Although cellulose synthesis in some algae can be ascribed to cyanobacterial-like endosymbionts (Nobles et al. 2001), many heterotrophic protists produce cellulose in various quantities, suggesting that this capacity was conveyed by proteobacterial-like ancestors to modern mitochondria, or that these heterotrophs once possessed chloroplasts and subsequently lost them. The latter possibility may explain the presence of cellulose in the walls of oomycetes (e.g., *Pythium aphanidermatum* and *Phytophtora cactorum;* see Helbert et al. 1997), which probably descended from a photosynthetic stramenopile ancestor (figure 1).

An additional level of complexity is added by the possibility that viruses have conveyed genetic information across eukaryotic lineages. For example, the CVK2 chlorovirus encodes enzymes for the synthesis of chitin synthase, a polysaccharide composed of N-acetyl-D-glucosamine, which is found in the walls of fungi and oomycetes and in the exoskeletons of many groups of invertebrates. When infected with the CVK2 virus, the unicellular chlorophyte *Chlorella* produces thin chitin fibrils on the surface of its cell wall (Kawasaki et al. 2002). Small quantities of chitin have also been detected in uninfected *Chlorella* species and in some diatoms, which are distantly related to the oomycetes (Kapaun and Reisser 1995, Shahgholi et al. 1996). It is therefore possible that chitin synthase genes have been transferred by viruses across a number of unrelated lineages (figure 1).

Gene sequences and synthesis

The lateral gene transfer hypothesis for cellulose synthesis is supported by recent studies that show remarkable molecular homologies among functionally nonredundant cellulose synthase genes (*CesA*) across diverse prokaryotic and eukaryotic species. Ultrastructural comparisons of the transmembrane complexes containing cellulose synthase proteins also support this hypothesis (Delmer 1999, Richmond and Somerville 2000, Nobles et al. 2001, Roberts et al. 2002, Römling 2002).

In terms of molecular homologies, recent studies show that all members of the *CesA* gene family isolated from land plants encode for integral membrane proteins with one or two transmembrane helices in the N-terminal protein region and three to six transmembrane helices in the C-terminal region (Richmond and Somerville 2000). Likewise, all members of this gene family share an N-terminal domain structure that includes a cytoplasmic loop consisting of four conserved regions (U1–U4), each of which contains a D residue or the QXXRW sequence (figure 2). The D-D-D-QXXRW motif is predicted to code for glycosyltransferase functionality. Three other features are shared among land plant cellulose synthases: (1) a strongly conserved region (CR-P) between the U1 and U2 conserved regions, (2) an N-terminal LIM-like zinc-binding domain, and (3) the so-called hypervariable region between U2 and U3 (Delmer 1999). The hypervariable region is now known to have strong sequence similarity among closely related species. It is therefore a highly conserved region within specific clades (Vergara and Carpita 2001).

Molecular comparisons indicate that the CR-P insertion and the D-D-D-QXXRW motif evolved before the appearance of the monophyletic land plants—indeed, before the appearance of eukaryotes—because both of these features have been identified in *CesA* proteins from the green alga *Mesotaenium caldariorum* (Roberts et al. 2002) and in putative *CesA* proteins from cyanobacteria (Nobles et al. 2001). Thus, the genomic roots of cellulose biosynthesis are bacterial. It is also clear that gene duplication and functional divergence occurred after ancient *CesA*-like genes became embedded within eukaryote genomes, because eukaryotic *CesA* proteins are functionally nonredundant and because they are arranged in structurally well-defined transmembrane structures, called terminal complexes (TCs), which are invariably involved with the assembly of cellulose. For example, among all land plants and some of their close algal relatives, TCs, as seen from the exterior surface of the plasma membrane, are solitary rosettes consisting of six granules (figure 3). Although the precise arrangement and number of the different cellulose synthases within an individual granule are currently unknown, research indicates that some *CesA* proteins (designated as *CesAi* proteins) initiate glucan chain formation by accepting glucose residues from a sugar nucleotide donor (most likely uridine 5'-diphosphate, or UDP), whereas other synthases further extend individual glucan chains (desig-

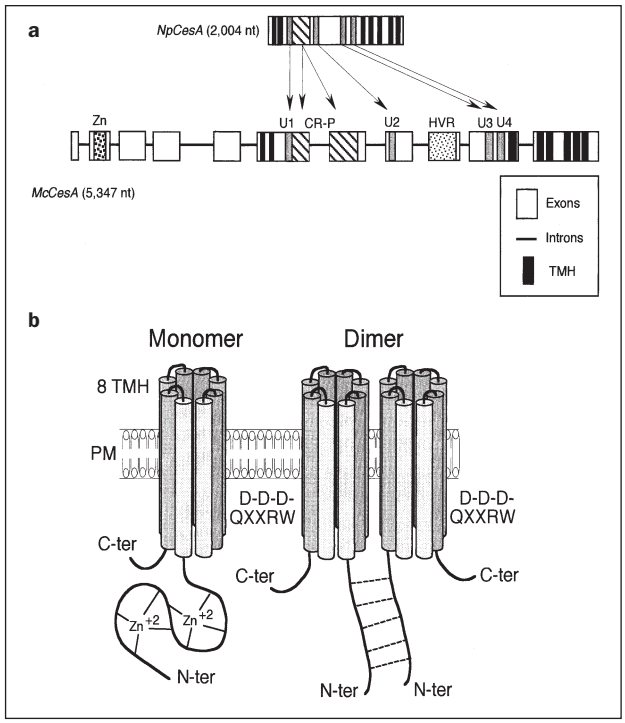

Figure 2. Cellulose synthase gene structure and hypothetical transmembrane protein folding. (a) Diagram aligning the cyanobacterium* Nostoc punctiforme*'s cellulose synthase gene (*NpCesA*) against that of the green alga* Mesotaenium caldariorum *(*McCesA*), showing the relative positions of the* McCesA *gene's N-terminal LIM-like zinc-binding domain region (Zn), eight transmembrane helix regions (TMH), four conserved cytoplasmic hoop regions (U1–U4), the strongly conserved region between U1 and U2 (CR-P), and the so-called hypervariable region (HVR) between U2 and U3. Adapted from Roberts and colleagues (2002). (b) Posited transmembrane-folding configurations across the plasma membrane (PM) for* CesA *monomers and dimers showing the putative locations of the active* CesA *site (the D-D-D-QXXRW protein region) for uridine 5'-diphosphate-glucose, or UDP-Glc, uptake and conversion into a cellulose glucan chain. Adapted from Delmer (1999) and Kurek and colleagues (2003).

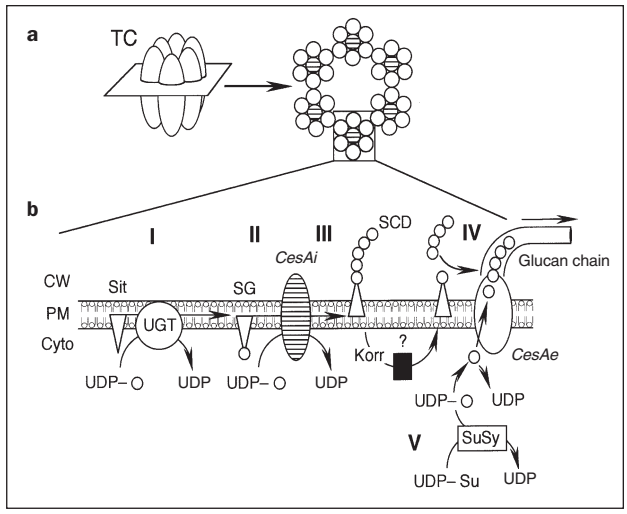

Figure 3. Land plant (embryophyte) cellulose synthase terminal complex (TC) and the as yet untested model of cellulose glucan chain synthesis created by Peng and colleagues (2002). (a) A typical TC consists of a rosette of six granules, each containing six functionally nonredundant cellulose synthases (the precise configuration is unknown). (b) Within each granule, a uridine 5'-diphosphate (UDP) glucosyl transferase, or UGT, conveys a glucose residue (circles) to a sitosterol molecule (Sit; step I) located on the cytoplasmic side (Cyto) of the plasma membrane (PM) to form the lipid-bound sugar sitosterol-β-glucoside (SG; step II). The SG molecule is subsequently extended by the addition of glucose from UDP-glucose, mediated by one initiating cellulose synthase (*CesAi*). The resulting lipid-linked oligosaccharide, sitosterol cellodextrin (SCD), is then "flipped" to the outer side of the cell membrane (PM), where the cellodextrin chain is cleaved by korrigan cellulase (Korr, which may or may not be a transmembrane constituent), subsequently bound to an elongating cellulose synthase (*CesAe*; step IV), and extended further by the addition of UDP-glucose provided by sucrose synthase (SuSy; step V) to form a single glucan chain extruded from one of 36 cellulose synthases in the TC. Adapted from Read and Bacic (2002).

nated as *CesAe* proteins). Several investigators have also suggested that dimers of cellulose synthase are required in order to provide a cooperative cellobiose-generating system (figure 2b; Carpita et al. 1996, Albersheim et al. 1997, Kurek et al. 2003).

Although much remains to be learned, a recent untested model of vascular plant cellulose synthesis provides some details of this process (Peng et al. 2002, Read and Bacic 2002). According to this model, UDP-glucosyl transferase, or UGT, conveys a glucose residue to a sitosterol molecule located on the cytoplasmic side of the plasma membrane (figure 3). This process forms the lipid-bound sugar sitosterol-β-glucoside, which is believed to subsequently extend by the addition of glucose from UDP-glucose mediated by *CesAi*. The resulting lipid-linked oligosaccharide (sitosterol cellodextrin, or SCD) is then "flipped" to the external surface of the cell membrane, where the cellodextrin is cleaved from the lipid by korrigan cellulase (which may not be a transmembrane component; see Szyjanowicz et al. 2004) and subsequently bound to *CesAe,* where it is extended further by the addition of UDP-glucose provided by sucrose synthase (figure 3). This model has not been subjected to independent testing, however, and so it must be considered tentative.

It is not known whether proteins other than those encoded by *CesA* genes are required for cellulose synthesis, and very few genes responsible for the synthesis of other cell wall

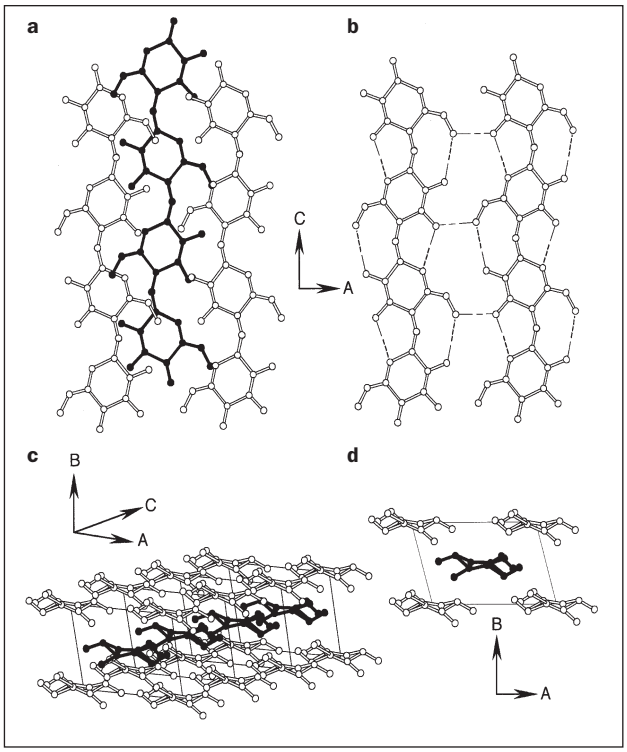

Figure 4. One of many allomorphic possibilities for the crystalline structure of crystalline cellulose I, viewed from different angles. (a) A–C projection (along the axes of three parallel chains in two planes). (b) A–C projection (on two parallel chains in one plane) showing hydrogen bonds (dashed lines). (c) Oblique A–B–C projection (on five parallel chains in three planes). (d) A–B projection (on five parallel chains in three planes; a cross-section of the structure is shown in panel c).

polysaccharides have been identified (but see Dhugga et al. 2004). However, the discovery of a large constellation of cellulose synthase–like (*Csl*) genes has opened the door to answering these questions and has already shed light on how gene duplication and divergence obtained the manifold polysaccharides found in plant cell walls. Specifically, reiterative database searches using *CesA* protein sequences from *Arabidopsis* and *Gossypium* reveal a gene superfamily consisting of six *Csl* subfamilies (Richmond and Somerville 2000). All of the *Csl* proteins appear to lack the cellulose synthase N-terminus LIM-like zinc-binding and RING finger domains, which are believed to play important roles in mediating protein-partnering or enzyme-targeted degradation. Some of these proteins appear to have fewer than the eight predicted *CesA* transmembrane domains, and some may be synthesized completely within the lumens of Golgi vesicles (Muñoz et al. 1996, Sterling et al. 2001). However, all *Csl* genes encode proteins with the D-D-D-QXXRW motif. Thus, even though no *Csl* gene has yet been linked directly to a specific protein, it is possible that the products of some *Csl* genes are involved in the synthesis of noncellulose cross-linking glycans or mix-linked glucans (Richmond and Somerville 2001).

Differences in the expression of *Csl* and *CesA* genes may also account for the differences observed for cell wall polysaccharide composition among various plant lineages (Matsunaga et al. 2004). In addition, various *Csl* and *CesA* genes may be developmentally programmed to switch on or switch off, thereby regulating and coordinating chemical differences in the cell walls of different parts of the same plant body.

Linear arrays and rosettes

The presence of cellulose in the surfaces or walls of many different kinds of organisms is not surprising. Cellulose is relatively "cheap" for organisms to manufacture, extremely hard to digest, and extraordinarily strong when placed in tension. Indeed, among diverse natural and artificial materials, cellulose is stronger in tension (in proportion to its density) than nylon, annealed aluminum, spiderwebs, tendons, or many high-tensile steels (Niklas 1992). This high density-specific tensile strength emerges from the participation in inter- and intrachain hydrogen bonding of all available hydroxyl groups among adjacently aligned β-1,4-glucan chains. This participation aggregates cellulose chains into insoluble crystalline strands reinforced by the dispersion forces among stacked heterocyclic rings (figure 4). In plant cell walls, the cable-like geometry of this unbranched covalent arrangement gives rise to extended structures called microfibrils, which are held together by structural proteins (e.g., extensin), a variety of pectins (e.g., homogalacturonan, rhamnogalacturonan, and arabinan), and hemicelluloses (e.g., xyloglucan, arabinoxylan, glucomannan, and xylan). In turn, microfibrils are aggregated into larger structures, called macrofibrils (figure 5).

The mechanical behavior of microfibrils is governed in part by their dimensions (width, thickness, and length). Microfibrillar width and thickness are determined by the arrangement of TCs and the number of cellulose synthase units per TC, both of which tend to be highly conserved for individual species (figure 6; Tsekos 1999). For example, cellulose-producing eubacteria and all phaeophytes and rhodophytes (e.g., *Pelvetia* and *Ceramium,* respectively) typically have single linear TC arrays similar to those reported for the tunicate *Metandrocarpa uedai* (Kimura and Itoh 1996). Likewise, many xanthophyte and chlorophyte species have stacked linear TC arrays (e.g., *Vaucheria* and *Oocystis,* respectively). In contrast, all embryophytes and some charophyte algae (e.g., *Nitella*) have solitary TCs arranged in hexagonal rosettes (when viewed from the exterior plasma membrane surface). Among embryophytes, as noted above, each granule contains six cellulose synthase units, each of which produces one glucan chain. The typical embryophyte microfibril is thus described as consisting of 36 glucan chains, collectively measuring 3.5 micrometers (μm) by 3.5 μm in width and thickness (although this has yet to be determined unequivocally).

These and other examples can foster the belief that TC arrangement and cellulose synthase number per granule provide insights into phylogenetic relationships. In some cases, this may be true. However, suppositions based on this

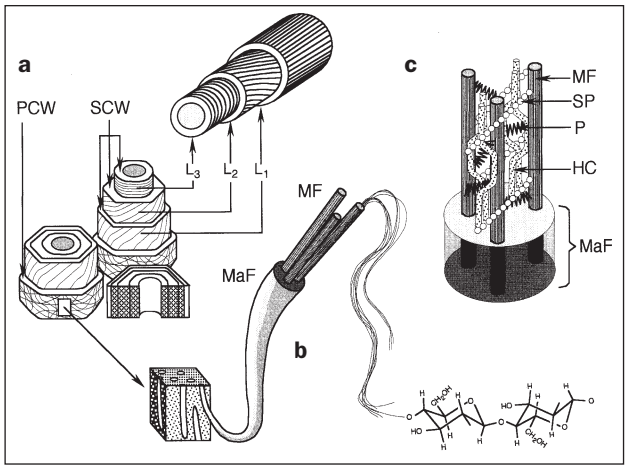

Figure 5. Architecture of the vascular plant cell wall. (a) Three adjoining cell walls with a primary cell wall (PCW) and a three-layered (L_1–L_3) secondary cell wall (SCW). The layers within the wall are produced sequentially, such that the PCW is external to the SCW and the oldest SCW layer (L_3) is in contact with the PCW. Extension of L_1–L_3 during cell growth in length results in the progressive passive realignment of cellulose microfibrils. (b) Cellulose microfibrils (MF), which are aggregated into macrofibrils (MaF), consist of 36 parallel glucan chains (with β-1,4 glucosic bonds; the molecular structure of the cellobiose monomeric unit is shown). (c) MFs in each MaF are linked by structural proteins (SP), pectins (P), and hemicelluloses (HC).

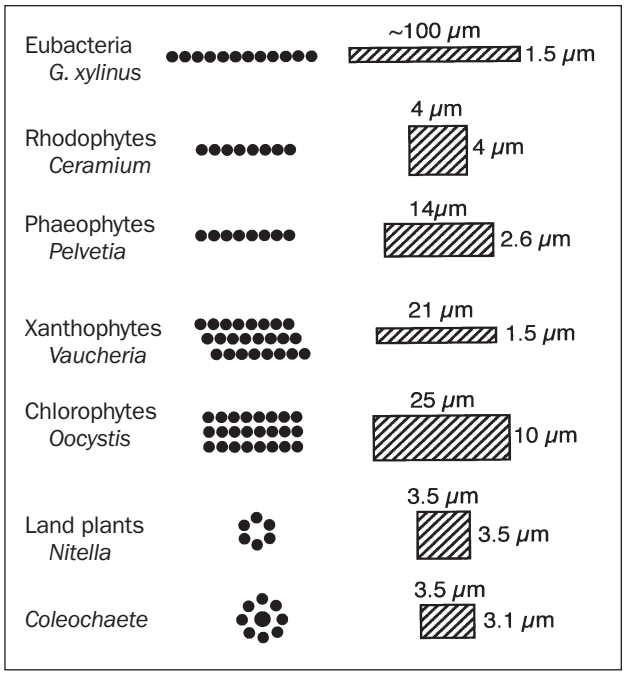

Figure 6. Diversity in the number and arrangement of cellulose synthase granules (black circles) in terminal complexes (TCs) (as seen from the plasma membrane exterior surface) and in the cross-sectional dimensions of cellulose microfibrils (cross-hatched areas). The TCs of some prokaryotes (e.g.,* Gluconacetobacter [*formerly* Acetobacter] xylinus*), rhodophytes (e.g.,* Ceramium*), and phaeophytes (e.g.,* Pelvetia*) are arranged in simple linear arrays (see figure 7a); those of some xanthophytes (e.g.,* Vaucheria*) and chlorophytes (e.g.,* Oocystis*) are arranged in stacked linear arrays. The TCs of all embryophytes and of the charophyte* Nitella *are arranged in hexagonal rosettes consisting of six granules (see figure 7b), while those of the charophyte* Coleochaete *are octagonal rosettes with nine granules. Adapted from Tsekos (1999).

approach can lead to highly problematic phylogenetic hypotheses (figure 6; Tsekos 1999), because even closely related species may differ in their TC arrangements and synthase numbers. The TC of the charophyte *Coleochaete,* for example, is an octagonal rosette of nine granules (Okuda and Brown 1992), which differs substantially from that of *Nitella* (figure 6).

Although TC arrangement and cellulose synthase number per TC dictate microfibrillar width and thickness (and possibly cellulose allomorphism; see Koyama et al. 1997), little is known about the factors influencing microfibrillar length, which is also an important determinant of cell wall strength. Across many different natural and artificial sources, the degree of cellulose crystallinity is correlated with glucan chain length (Delmer 1999). The siphonous green alga *Boergesenia forbesii* holds the current record in this regard (i.e., 23,000 units per chain). However, microfibrillar length often exceeds the glucan chain length predicted on the basis of cellulose crystallinity, indicating that chain initiation and termination probably occur multiple times during the fabrication of individual microfibrils. Although various hypotheses have been proposed to explain glucan chain termination (e.g., chain cleavage to relax localized tension resulting from "out-of-step" synthases in adjoining granules), none satisfactorily explains how entire TCs are initiated or terminated.

Much remains to be learned about how TCs are globally coordinated to fabricate the cellulosic infrastructure of an entire cell wall. The most prevalent hypothesis is that TCs are guided by cortical microtubules (figure 7). If this is true, cytoskeletal architecture controls microfibrillar deposition and cell wall texture such that a change in the architecture ought to produce different depositional patterns and wall textures. This hypothesis is consistent with the parallel alignment of microfibrils and microtubules that is frequently reported for recently synthesized parts of plant cell walls (Wymer and Lloyd 1996, Taiz and Zeiger 2002). It also sheds light on anisotropic cell growth, because most researchers believe that cells enlarge by a process involving enzymatic stress relaxation and mechanical slippage (creep) of cellulose microfibrils, which make up the bulk of the load-bearing polysaccharide network in cell walls (Cosgrove 1996).

As noted above, cellulose is one of the strongest materials when placed in tension. Microfibrils thus are likely to func-

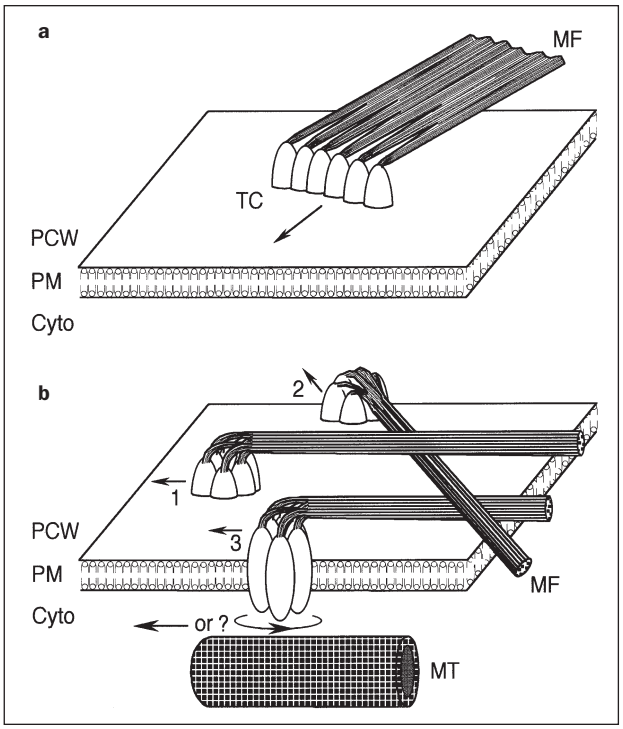

Figure 7. Surface views of transmembrane terminal complexes (TCs) arranged in linear arrays and rosettes. Direction of TC movement across the plasma membrane (PM) is indicated by arrows. (a) A single linear TC array producing a broad sheetlike cellulose microfibril (see figure 6). (b) Rosette TCs producing three microfibrils that crisscross in the primary cell wall (PCW) as a consequence of different rates of moment. Microfibrils (MFs) are frequently aligned with cortical microtubules (MTs) in the cytoplasm (Cyto). It is unclear whether MT orientation directly dictates that of the MF, or whether the initial orientation of MF extrusion by a TC dictates the MF's subsequent orientation.

tion as "tensile cables," anchored together by other cell wall constituents (figure 5c). This mechanochemical configuration permits fully turgid cells, especially thin-walled ones, to retain their shape; that is, thin-walled cells are hydrostatic mechanical devices (Niklas 1989, 1992). However, for cells to expand and permanently grow in size, the cell wall must be "relaxed" by allowing microfibrils to slip past one another. Likewise, changes in the orientation of microfibrils (affected by cytoskeletal reorientation of microtubules) are required to alter the direction of subsequent preferential cell expansion and growth. The deposition of transverse microfibrils in recently produced cell wall layers would predispose cells to expand longitudinally, whereas longitudinal microfibrils would favor transverse expansion.

Although the mechanics of cell wall expansion and isotropic growth are comparatively well understood, recent work indicates that microtubule and microfibril organization is only part of a much more complex story (Seagull 1991, Wiedemeier et al. 2002, Smith 2003). For example, the cells of the temperature-sensitive *mor1-1* mutant of *Arabidopsis thaliana* lose their orderly cortical microtubule arrangements at their restrictive culture temperatures. Although they also lose their capacity for anisotropic growth (which suggests a random arrangement of microtubules), these cells nevertheless retain parallel microtubule arrangements. Using the same mutant, Himmelspach and colleagues (2003) investigated whether well-ordered, preexisting microfibrils or cortical microtubules are essential for the resumption of normal (longitudinally aligned) microfibrils. Their protocol involved the transient disruption of microfibril organization with a brief treatment of the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile, and the subsequent examination of the alignment of newly formed microfibrils as cellulose synthesis was recovered at the mutant's nonpermissive culture temperature. Despite the presence of disordered microtubules (and the initially random cell wall texture of the microfibrils), new microfibrils formed in transverse and longitudinal patterns.

These and other experiments indicate that preexisting microtubule or microfibril templates may not be required for the resumption of prior microfibrillar organization. But this does not preclude the possibility that microtubules influence the direction of cellulose extrusion, which in turn dictates subsequent microfibrillar orientation. Given that recently formed portions of microfibrils are anchored in the cell wall, it is possible that TCs are dynamically propelled in the same direction in which they initially extrude microfibrils (Staehelin and Giddings 1982). If so, an initial parallel alignment of microtubules and microtubules may be a transient phenomenon (figure 7).

Tying up loose ends

Any emphasis on cellulose synthesis and the fabrication of cellulose microfibrils can give the impression that the cell walls of land plants are predominantly cellulosic and chemically uniform. This impression is quickly dispelled, however, by recent research into the chemical diversification of primary cell walls during the radiation of embryophytes and their divergence from their nearest living algal relatives, the charophytes. For example, the cell walls of charophytes and embryophytes contain uronic acids and mannose (figure 8). However, those of charophytes, such as *Nitella* and *Chara,* lack detectable quantities of the amino acid hydroxyproline, which is a major component in the glycoproteins and extensins of embryophyte cell walls (Gotteli and Cleland 1968). Xyloglucan is also absent from the cell walls of charophytes, yet this polysaccharide is present in the cell walls of bryophyte (moss, liverwort, hornwort) gametophytes and of angiosperm and pteridophyte (horsetail, fern, lycopod) sporophytes (Popper and Fry 2003). Accordingly, hydroxyproline-rich and xyloglucan-rich walls probably evolved after the divergence of charophytes and embryophytes from their last common ancestor (figure 8).

Recent immunological studies of primary cell walls further indicate that chemical fine-tuning attended the evolution of the water-conducting cell walls of bryophytes. Using a battery

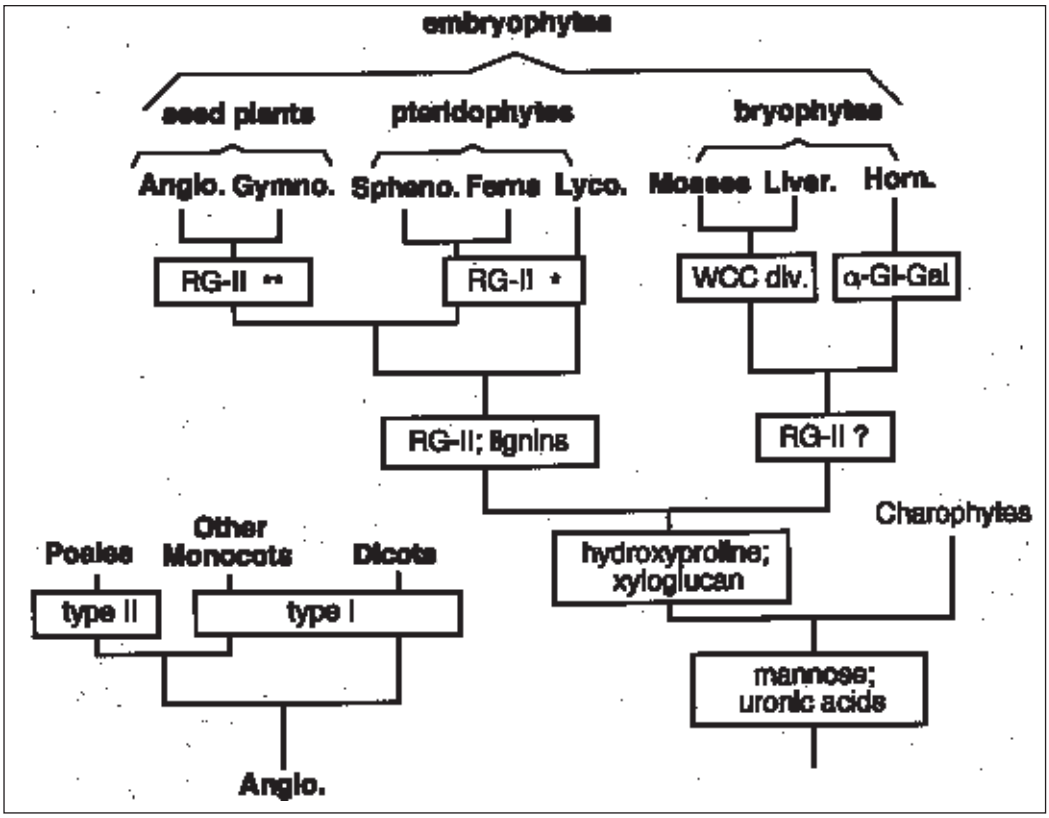

Figure 8. Some phylogenetic differences in the chemical composition of the primary cell walls of extant embryophytes and charophytes. All of the taxa shown here possess walls containing uronic acids and mannose, in addition to cellulose and other materials. The primary walls of all nonvascular embryophytes (bryophytes, including mosses, liverworts, and hornworts), pteridophytes (sphenopsids [horsetails], ferns, and lycopods), and seed plants (angiosperms and gymnosperms) also contain hydroxyproline and xyloglucan. The secondary cell walls of pteridophytes and seed plants may be lignified; the walls of pteridophytes and most angiosperms (and possibly gymnosperms) contain the borate cross-linked pectic polysaccharide rhamnogalacturon II (RG-II), but in different concentrations (high [**] or low [*]) that correlate loosely with average plant height. Although low concentrations of RG-II may occur in specific cell types of some bryophytes, chemical diversification in the composition of water-conducting cell types is evident among the mosses and liverworts (water-conducting cell diversification, or WCC div), and unique wall constituents have been detected in hornworts (e.g., α-D-glucuronosyl-(1,3)-L-galactose; α-Gl-Gal). The panel at bottom left shows differences between the cell wall composition of the Poales (type II cell wall designation; Carpita 1996) and of other monocots and dicots (type I designation; Carpita 1996). Abbreviations: Angio, angiosperms; Gymno, gymnosperms; Horn, hornworts; Liver, liverworts; Lyco, lycopods; Spheno, sphenopsids (horsetails).

of monoclonal antibodies, Ligrone and colleagues (2002) examined the distribution of polysaccharide and glycoprotein carbohydrate epitopes in the cell walls of liverwort and moss gametophytes. Their analyses show that bryophyte cell wall components are as complex and cell-type specific as those of vascular plants, and that water-conducting cell types probably have manifold independent evolutionary origins. Such chemical fine-tuning continues to shed light on evolutionary hypotheses. For example, a unique cell wall disaccharide, α-D-glucuronosyl-(1,3)-L-galactose, has been reported for the hornwort *Anthoceros,* which is consistent with the supposition that the hornworts are an evolutionarily isolated group of nonvascular embryophytes.

The structure and concentration of other primary cell wall constituents offers insights into vascular plant evolution (figure 8). For example, the borate cross-linked pectic polysaccharide rhamnogalacturonan II (RG-II) has 12 different sugars and 20 different glycosidic linkages. This structure is highly conserved across all of the vascular plant species currently examined, indicating that it probably originated in the last common ancestor of the tracheophytes (Matsunaga et al. 2004). However, comparisons among phyletically diverse

vascular plants (e.g., lycopod, fern, and angiosperm species) reveal higher concentrations of RG-II in the walls of seed plants than in the walls of pteridophytes, whereas bryophyte cell walls have little or no RG-II. In addition, with the exception of grasses (Poales), the concentration of RG-II in angiosperm primary cell walls is significantly higher than in the walls of pteridophytes (Matsunaga et al. 2004).

Although the bulk chemical composition of tissues can significantly obscure the chemistry of individual cell types, it appears that the variation of RG-II concentration *in toto* correlates, albeit loosely, with the tensile strength of the primary cell wall (figure 8; Ryden et al. 2003). This correlation is interesting because numerous studies show that flowering plants require boron for their normal growth and development, particularly the development of their vascular cell types, and because the amount of boron required correlates with the amount of pectin and RG-II in cell walls (O'Neill et al. 2003, Matsunaga et al. 2004). Therefore, it is reasonable to suggest that subtle changes in the cell wall concentration of RG-II have attended the evolution of the flowering plants.

Research into plant cuticles is also shedding light on land plant evolution. The cuticle is an integral part of the epidermal cell walls (and, in some species, subepidermal cell walls) of vascular plants. It reduces the loss of water, the entry of pathogenic organisms and various organic compounds, and the deleterious effects of excessive sunlight. It can also serve as a "tensile skin" that provides mechanical support to aerial plant parts (Wiedemann and Neinhuis 1998, Matas et al. 2004). Therefore, the cuticle was undoubtedly a critical adaptation for plant survival on land. It is not clear, however, whether cuticularized primary cell walls evolved after the divergence of nonvascular and vascular land plants, or before the divergence of embryophytes and charophytes from their last common ancestor. A few pyrolysis gas chromatography and mass spectroscopy studies report cuticles in bryophytes, whereas a cuticle-like material appears to surround the air pores on the dorsal surface of the thalloid liverwort *Marchantia paleacea*. Structural similarities between the surface layers of some charophytes and bryophytes have also been reported (Cook and Graham 1998). Although the chemical nature of these nonvascular plant cuticle–like materials is currently unknown, the capacity to impregnate external primary cell walls with a resistant matrix of biopolymers similar to those found in vascular plant cuticles may well be a pleisomorphic feature binding the charophytes and embryophytes.

Conclusions

Every major clade of unicellular organism has evolved some form of specialized external or internal material in direct contact with the plasma membrane, presumably in response to sustained selection for the maintenance or regulation of cell shape, osmoregulation, protection against pathogens, or diffusion barriers limiting the passage of macromolecules. One of the most prevalent cell wall materials, especially among eukaryotic photoautotrophs, is cellulose. Comparative ultrastructural and molecular studies support the hypothesis that the ability to synthesize cellulose across otherwise very different eukaryotic lineages was the result of ancient lateral gene transfers between prokaryotic "host" cells and eubacterial-like endosymbionts capable of cellulose synthesis. Secondary endosymbiotic events also occurred in the course of life's history. Subsequent modification and elaboration of cell wall chemistry and architecture undoubtedly attended the evolutionary divergence and radiation of individual eukaryotic lineages as a result of genomic changes resulting in the ability to synthesize novel cell wall polysaccharides and proteins. Embryophytes and charophytes, which share a last common ancestor, possess cell walls that differ chemically in many ways. Likewise, the primary cell walls of the nonvascular and vascular plants differ chemically in ways that suggest adaptive fine-tuning to different physiological or environmental conditions rather than neutral mutation. Future research will undoubtedly shed light on the genetics of cell wall synthesis and provide insights into whether gene duplication and subsequent functional divergence of the *Csl* and *CesA* proteins are responsible for phyletic differences in the composition of cell wall polysaccharides. Likewise, future research will provide a better understanding of how multicellular plants alter the chemical compositions of individual cells and tissue types during growth and development.

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