Introduction of algae

Algae are a unique group of organisms displaying a wide variety of reproductive patterns. Various division patterns can be found from simple division into two cells, similar to yeast (binary fission), to the formation of four and up to several thousand daughter cells in a single cell cycle in green algae dividing by multiple fission. In some algal species, both binary and multiple fission can be observed in the same organism, either under different growth conditions or at different phases of the life cycle. Furthermore, wide-ranging body organizational structures exist in algae, from unicellular organisms (microalgae) to multicellular ones resembling higher plants (macroalgae) with a very complex body shape built by morphologically distinct cells having various physiological roles. This section will deal only with the vegetative cell cycle of unicellular green algae, existing as single cells or gathered into coenobia (where daughter cells arising from a single mother cell stay connected together), colonies or filaments, but independent of each other. Although 60 years have passed since the first studies of the algal cell cycle, possible ways in which algae can still contribute to research into the biology of cell cycles are far from exhausted. The seemingly narrow range of these organisms provides such a broad variety of reproductive patterns that, in spite of extensive literature, they still represent a challenge for future researchers in cell cycle biology. The aim of this section is to summarize the significant progress made, from early historical findings up until the last few years, and to highlight the hidden potential of algae for the future. About 60 years ago, chlorococcal algae of the genus *Chlorella* were among the first microorganisms to be successfully grown in synchronous cultures and used for biochemical and physiological analyses of the cell

cycle. The first experiments were therefore carried out at the same time that Howard and Pelc first separated the cell cycle into four phases G1, S, G2 and M. From the early years, other green algae, *Desmodesmus* (*Scenedesmus*) and *Chlamydomonas* also formed prominent cell cycle models. Their multiple fission reproductive patterns are, as is described below, rather different from the patterns terminated by binary fission that are characteristic of most eukaryotic cells. The multiple fission cell cycle and mechanisms governing its regulation are the most important contributions that algal cell cycle studies have made to the general field of cell cycle research. The purpose of the cell cycle is to consistently reproduce all cellular structures in order to produce a new daughter cell.

Biosynthesis of the Cell Walls of the Algae

"Algae" constitute a diverse array of photosynthetic eukaryotes that are derived from multiple evolutionary origins and today occupy most of modern earth's photic zones. These organisms are of profound importance to many food chains as well as planet-wide gas exchange dynamics and mineralization processes. Algal cells, like virtually all forms of life, are covered by an extracellular matrix (ECM) that contributes significantly to cell-cell adhesion, cell expansion control, the sensing of environmental stressors, defense, reproduction and morphogenesis. Algal ECM also contributes prominently to human activities and has been harnessed for extraction of biochemicals that are used in the pharmaceutical, food and biofuel industries. The algal ECM exhibits many architectural designs that include multi-shaped scales, highly mineralized organic shells and crystalline glycoprotein coverings See Table 1. In most taxa of the late divergent green, brown and red algae, the ECM is typically characterized by cell walls that consist of

microfi brillar networks embedded in matrices of diverse polysaccharides and proteins. Approximately 450–500 million years ago, one group of green algae, the charophytes or “Charophycean Green Algae” (CGA or the Streptophyta), invaded terrestrial ecosystems and ultimately gave rise to modern land plants in arguably the most important event in the history of life on the planet. The cell walls of these early invaders and their extant relatives changed the biosphere profoundly and became a basis for modern human cultural evolution (e.g., agriculture, wood, paper and textiles). Currently, most of our knowledge of the structure and function of cell walls is centered on research dealing with those found in land plants and for good reason. Polymers of these cell walls constitute the largest source of annual renewable biomass on the planet. Likewise, the synthesis and maintenance of a cell wall is “front and center” in the physiology of a plant cell. A very large percentage of a land plant’s Photosynthetically-derived organic product and as much as 30 % of its genetic machinery are employed for the construction and maintenance of wall architecture. Algal cell walls and other ECM materials also contribute significantly to the biomass of specific c habitats and though no specifi c information is yet available, one might reasonably speculate that the cell wall of an alga requires similar investment of the cell’s genetic and photosynthetic machinery. Our current understanding of the developmental processes and regulatory controls required for the production of algal cell walls is not nearly as well resolved as that of land plants. In that land plant taxa that have been investigated thoroughly (e.g. Arabidopsis), a highly coordinated interaction of multiple subcellular systems functioning in response to multiple genetic prompts and environmental stresses that utilize complex signal cascades is required for competent synthesis, secretion and remodelling of the cell wall. For algae, recent research, especially that employing the advanced tools of

molecular biology biochemistry, cell biology, immunology and high throughput/rapid screening methodologies such as chemical genomics is beginning to yield new and valuable insight into the structure of algal cell walls and more importantly, their developmental dynamics.In this chapter, a review of some of these recent research efforts dealing with algal cell wall developmental dynamics in algae is provided.

Table 1 Overview of extracellular coverings and their chemical constituents

Algal group	Components of extracellular coverings
Chlorophyta (green algae)	Scales, theca, cell wall
	Cellulose, mannans, xyloglucans, xylans, 1,3 ß-glucans, mixed linkage glucans, xylogalactorhamnans, rhamnoxyloglactogalacturonans, Kdo and 5-O-Kdo polymers, arabinogalactan proteins, extensin
Rhodophyta (red algae)	Cell wall
	Cellulose, xylans, mannans, sulfated mixed linkage glucans, sulfated galactans
Phaeophyta (brown algae)	Cell wall
	Cellulose, alginates, fucoidans
Haptophyta	Coccoliths
	Acidic polysaccharides, proteins, calcite
Dinophyta	Amphiesma
	Cellulose
Bacillariophyta	Frustule
	Mannose and 1,3 ß-glucans, multiple proteins, silica

These polymeric networks during expansion, development and in response to environmental stress. Production of the cell wall requires the temporally and geographically coordinated activities of the endomembrane system, cytoskeletal network, plasma membrane and the wall itself.

Cell Walls: An Architectural Paradigm with Much Fine Tuning

The cell wall of plants consists of a framework of fibrillar polysaccharides that is embedded in a matrix composed of neutral and charged polysaccharides, various proteins and in some cases, polyphenolics like lignin. This structural design is also found in cell walls

of late divergent taxa of the charophytes. Previous interpretations of the phylogeny of the charophytes suggested that the ancestors of modern day charophytes were capable of making the successful transition to land because specifi c features of their physiology and biochemistry allowed for a degree of pre adaptation to life on land. Further proposed that the ability of the charophytes to produce cell walls with specific polymer arrays was a critical aspect of this pre-adaptation for this terrestrial invasion. It is also important to note that the general architectural design of a fibril/matrix cell wall is also found within many taxa of the Chlorophycean green algae, the red algae (Rhodophyta), the brown algae (Phaeophyta) and Xanthophyceae (Fig. 1). Though the specifi c polymeric composition of both the fi brillar and matrix components may vary, it seems quite clear that the design of a matrixreinforced fibrillar composite (e.g., like reinforced concrete or fiberglass) was selected by a broad phylogenetic spectrum of algae as the most efficacious covering for survival in a wide range of habitats, each with specific challenges. The microarchitecture of the cell walls of algae and plants is not only complex, it is dynamic. The polymers therein are secreted/woven, incorporated and interconnected in precise networks that are closely regulated by genetic and environmental cues. Subsequent biochemical modulations alter these polymeric networks during expansion, development and in response to environmental stress. Production of the cell wall requires the temporally and geographically coordinated activities of the endomembrane system, cytoskeletal network, plasma membrane and the wall itself.

The Structural Framework of Microfibrils: Cellulose, Mannans and Xylans

The β-1,4-glucan polymer, cellulose, is the major fibrillar and load-bearing component of the cell walls of plants and many algal taxa (Popper et al. 2011). Cellulose is found in unbranched and insoluble "crystalline" strands called microfibrils that are products of inter-and intrapolymer hydrogen bonding of hydroxyl groups among adjacent β-1,4-glucan chains. Microfibrils are highly resistant to digestion and often have a tensile strength that is greater than some steels, i.e., advantageous features for a macromolecule that serves as the load-bearing framework of the cell wall. The infrastructure of the cellulose network in a cell wall and its functional role are also products of the complex and interconnected matrix of other polysaccharides and proteins that surrounds the microfibrils. The identification of the pathway and components of cellulose biosynthesis of photosynthetic eukaryotes is deeply grounded in algal-based studies. The biosynthetic machinery for making cellulose in these organisms is believed to be an evolutionary product of lateral gene transfer derived from an ancestral bacterial endosymbiont. Cellulose is most often synthesized in the plasma membrane and is subsequently deposited or incorporated into the cell wall. The enzyme complex that is responsible for cellulose synthesis is the cellulose synthesizing apparatus or CESA which has been classified in one super gene family containing nine cellulose synthase-like (Csl) families and one cellulose synthase (CESA) family (Yin et al. 2009). CESA synthesis occurs (i.e., synthesized or initially recognized) in the Golgi apparatus. It is transported to the plasma membrane in Golgi-derived vesicles where it is incorporated into terminal complexes (TCs) that fabricate cellulose microfibrils. TCs contribute both to the

polymerization of the glucan chains and the assembly of these chains into microfibrils on the interior of the plasma membrane and ultimately moves to the outside via porin-based pores.

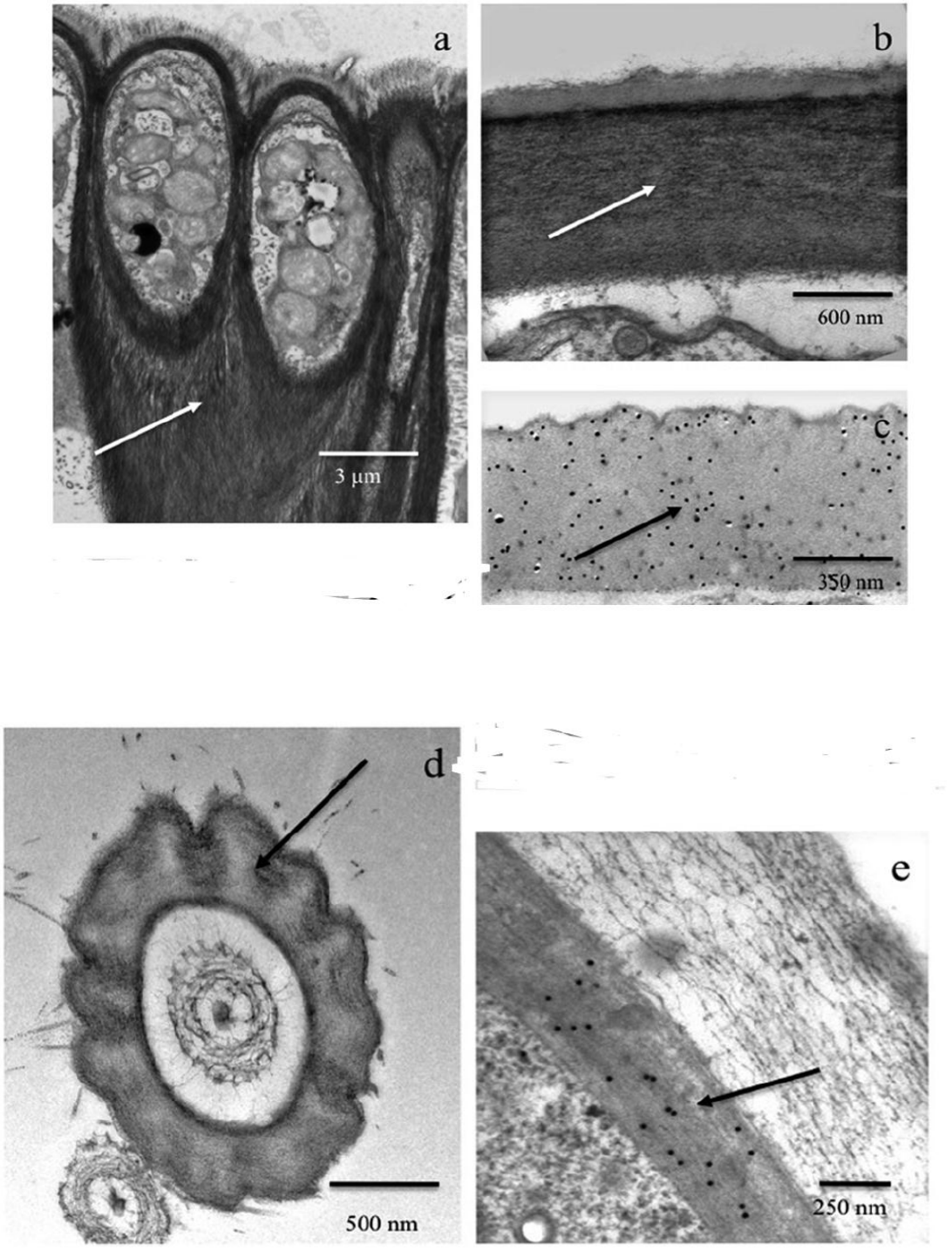
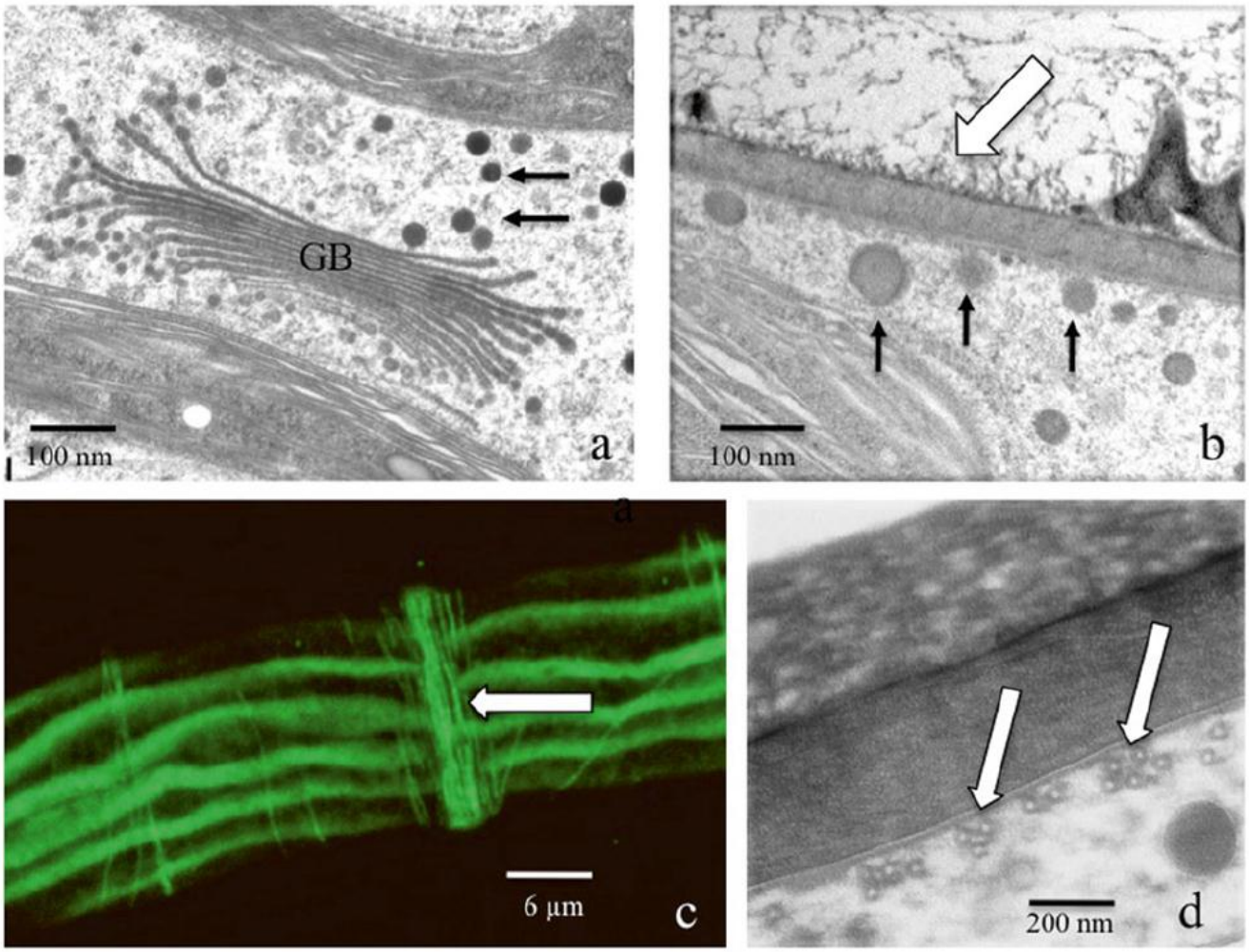


Fig. 1 Overview of the diversity of cell walls in the algae. (**a**) The thick fibrillar walls (*arrow*) of the epidermal cells of the "button" of the brown alga, *Himenthalia*. (**b**) The thickened wall (*arrow*) of an internodal cell of the CGA, *Chara corallina*. (**c**) Anti-mannan (LM21) labelling of the cell wall (*arrow*) of the green alga, *Codium fragile*. (**d**) The thickened cellulosic wall (*arrow*) of a hair cell of the green alga, *Chaetosphaeridium*. (**e**) Anti-MLG labelling of the cell wall (*arrow*) of the desmid, *Pleurotaenium trabecula*. All images are TEM

Cellulose biosynthesis requires nucleotide sugars and is often associated with sucrose synthase, endo-β 1-4 glucanses and annexins. Freeze fracture/etch-transmission electron microscopy (TEM)-based imaging has demonstrated that in land plants and most of the late divergent taxa of the charophytes, the TCs are arranged in hexameric rosettes of 25–30 nm. Each of the six components of the rosette contains six CESA units, each of which produces one β1-4 glucan chain. The 36 glucan chains produced by a single rosette, associate via H-bonds to form a microfibril of approximately 2–4 nm in width and thickness. However, new imaging technology has shown that microfibrils may be considerably smaller, consisting of 24 or 18 chains formed by rosettes containing three CESA components. The verification of the role of TCs in cellulose biosynthesis was provided through labelling of TCs with a monoclonal antibody (mAb) specific for CESA using the green alga, Micrasterias.

In many algal groups, microfibril size may also be much greater than that of land plants. For example, in some red algae, microfibrils may measure from 25 to 68 nm. This size variation and the deposition/layering of cellulose microfibrils in the wall architecture are directly related to variations in the architecture of the TC. Chlorophycean green algae possess linear TCs that contain three rows of particles while in brown algae, single linear TCs with up to 100 nm subunits yield distinct ribbon-like microfibrils. The red alga, Erythrocladia subintegra (= Sahlingia subintegra), 1 produces single linear terminal complexes that measure 180 by 35 nm arranged in fourrows of 30–140 particles, while in the genera, *Radicilingua* and *Laurencia* , two rows of three particles each have been observed. These smaller complexes though could represent microfibril terminating synthesizing complexes. In the Xanthophytes, distinct stacked or diagonally-arranged linear TCs have been described. During different phases of the cell or developmental cycles of plants and

algae, cellulose microfibrils are often deposited in specific layers or lamellae. Cellulose microfibrils have great strength under tension but are relatively weak against shear and compressive forces. Therefore, the production of cellulose in multiple layers allows the cell to resist forces both internal and external from various directions. The orientation of cellulose microfibrils in the layers of the cell wall also correlates closely with the orientation of cortical microtubules found just underneath the plasma membrane. In land plants, this microfibril-microtubule orientation has been demonstrated using live-cell imaging. Here, microtubules guide the trajectories of GFP-labelled TCs in the plasma membrane that, in turn, orient the deposition of microfibrils. The exact molecular mechanisms behind the function of the microtubules are not yet fully resolved. They may play a role in controlling the velocity of the cellulose synthesis machinery in the plasma membrane, determine the density of TCs in the plasma membrane or target the insertion of CESA. However, in older epidermal cells of Arabidopsis roots, it has been shown that cellulose deposition and microtubule orientation may sometimes be independent of each other. The interaction of cortical microtubule networks and cellulose has also been explored in algae. In the desmid, *Penium margaritaceum* , a band of cortical microtubules found in the main pre-division expansion zone is arranged in the same orientation as that of the innermost layer of cellulose (Fig. 2). If the microtubule network is altered with the pharmacological agent, oryzalin, the cellulose-based inner wall layer thins considerably causing the cell to swell (Domozych et al. 2014b). Actin networks also influence cellulose synthesis including regulating the rate of CESA vesicle delivery to the plasma membrane in land plants, most likely through its role in cytoplasmic streaming. The actin machinery may also control the amount of time CESA is situated in the plasma membrane. Finally, actin



basedtransport may be responsible for recycling of CESA via clathrin-mediated endocytosis to the Trans Golgi network/early endosome. In red algae, limited data suggests that cellulose synthase enzyme complexes are also synthesized in the Golgi apparatus. Microfibrils are synthesized at the plasma membrane and are deposited in variably oriented layers. No direct correlation between cortical microtubule arrangement and microfibril deposition in the cell wall has been described but it is very likely that actin plays a key role in cellulose deposition. In some brown algae, TC activity and microfibril production are controlled most likely by actin and not by microtubules.

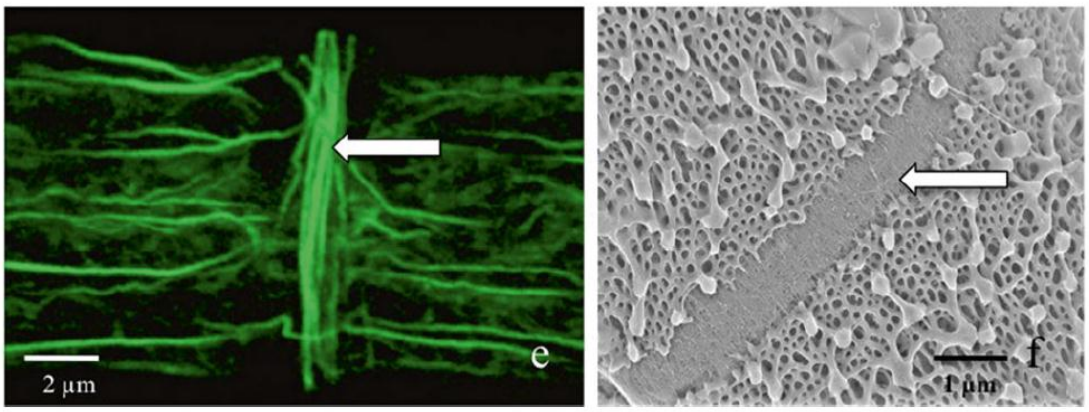


Fig. 2 The coordinated activities of multiple subcellular systems during wall formation in *Penium margaritaceum*. (**a**) The Golgi Body (GB) is the site of synthesis of HG. The HG is carried to the expansion zone via small vesicles (*arrows*). (**b**) The vesicles (*small arrows*) move to the expansion band (*large arrow*) and release the HG to the apoplast. (**c**, **d**) The expansion band is highlighted by a cortical band of microtubules (*arrows*). **c** = anti-tubulin. Confocal laser scanning microscopy (CLSM); **d** = TEM. (**e**) CLSM imaging of rhodamine phalloidin labeling of a transient cortical actin band (*arrow*) that underlies the expansion band. (**f**) FESEM Imaging of the expansion band highlighting the zone where the HG outer layer has yet to form

Cellulose may also be part of other algal extracellular coverings that are not considered as typical cell walls. Thecate dinofl agellates (Pyrrhophyta, Dinophyceae) produce an extracellular covering called the amphiesma. This highly complex structure consists of an outer continuous membrane i.e., the plasma membrane, an outer plate, a single membrane bound compartment that contains a cellulosebased theca and an inner pellicle. The theca is processed through the Golgi apparatus and an associated network of Golgi-derived vesicles. The amphiesma is often shed during development via a process called ecdysis. While much remains to be resolved about the amphiesma and the cellulosic theca, it has also been shown that cellulase activity was critical for coordination of cell cycle activity when amphiesma development occurs. Many algal taxa produce microfibrillar wall materials that are not cellulosic. In coenocytic Chlorophycean green algae, microfibrils consist of β-1,3 xylans, β-1,4 mannans and heteropolymeric fibrillar polysaccharides (Fig. 1). In red algae, microfibrillar β1-4 mannans, β1-3 or β1-4 linked xylans have been described. To date, little detailed information is available about their

biosynthesis. Few detailed studies are available today concerning the mechanisms and regulatory controls of cellulose synthesis in algae. However, new efforts and technologies offer much promise. For example, genomic sequencing of key taxa of several algal groups is rapidly progressing and the development of transformed cell lines expressing fluorescent protein- CESA complexes for cell wall studies of algae have recently made significant progress. New developments in high resolution light, electron and atomic force microscopy coupled with new cellulose specific probes should soon provide great insight into cellulose production in algae. Finally, "chemical genetics"-based screening of large arrays specific bio-active molecules including cellulose synthesis-affecting agents have provided new tools for investigating the specifics of the cell all biosynthetic machinery that may also be applied to algae. All of these innovations will soon resolve many questions about cellulose in algae and contribute significantly to current and future efforts to harness the algal cell wall polysaccharides for biofuel production.