

Cellulose Biosynthesis

Deborah P. Delmer¹ and Yehudit Amor

Department of Botany, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

INTRODUCTION

The Structure of Cellulose Confers Complexity upon the Synthetic Process

There is probably no major biochemical process in plants that is both so important and so poorly understood at the molecular level as cellulose synthesis. This is surprising, because the basic synthetic event is a simple polymerization of glucose residues from a substrate such as UDP-glucose to form the homopolymer β -1,4-D-glucan (for reviews on the structure and industrial uses of cellulose, see Kuga and Brown, 1991; French et al., 1993). However, this is a deceptively simple description, and the process is clearly very complex and requires many higher levels of organization. This complexity can be ascribed to a number of factors that relate to the forms and patterns in which cellulose is deposited in nature. First, the stereochemistry imposed by the β -1,4-glycosidic linkage creates a linear, extended glucan chain in which every other glucose residue is rotated $\sim 180^\circ$ with respect to its neighbor (Figure 1). This means that cellobiose, and not glucose, is the basic repeating unit of the molecule and contrasts with other glucan polymers such as starch (α -1,4-glucan) or callose (β -1,3-glucan; see Figure 1), in which the disaccharide is not the repeating unit and the chains are not perfectly extended but assume less ordered, helical configurations.

The extended nature of the β -1,4-glucan chain creates a situation in which chains can interact with each other in a very precise manner to form a rigid structure. Thus, cellulose in nature never occurs as a single chain but exists from the time of synthesis as a composite of many chains, called microfibrils (Figure 1). The chains associate very strongly via both intra- and interchain hydrogen bonding between glucose residues in a manner so precise that microfibrillar cellulose is largely crystalline. Although this notion has been controversial for many years, the general consensus now is that, in the cellulose I crystal (that is, the form found in nature), the chains are aligned parallel to each other. This at least eliminates complications of the sort involved in the synthesis of antiparallel chain structures such as DNA, and it suggests that the mechanism of polymerization of adjacent chains probably proceeds in a

similar manner throughout the microfibril. Chain length can vary among organisms, ranging from a low of ~ 2000 up to $\sim 20,000$ glucose residues, and virtually nothing is known about how chain length is determined. A further complication arises from the observation that, in nature, cellulose I exists in two different allomorphs, called I α and I β , that can be distinguished by ^{13}C -NMR (Atalla and VanderHart, 1989). Microfibril size can also vary among organisms, in general ranging from the so-called elementary fibril of ~ 36 chains to the very large fibrils of the cellulosic algae, which can contain more than 200 chains and are so highly organized that they can diffract as a single pure crystal (see Kuga and Brown, 1991). An additional level of organization is apparent from the observation that, as plant cells mature and deposit a thick secondary wall, microfibrils often are found further associated into macrofibrils or bundles (Figure 1).

Cellulose in the primary walls of dividing and elongating cells fulfills several functions, the most obvious being to provide strength. In most primary walls, cellulose exists as elementary fibrils that form a complex with xyloglucan (Hayashi, 1989). Tensile strength studies using herbicide-adapted plant cell cultures that virtually lack a cellulose-xyloglucan network provide an estimate that this network contributes $\sim 70\%$ of the total strength to normal primary walls (Shedletsky et al., 1992). In mature plants, the overall strength of the plant derives mainly from the very thick secondary cell walls characteristic of many mature cell types of the plant; because cellulose constitutes $\sim 40\%$ of the dry weight of these walls and is deposited in layers of alternating pitches, secondary wall cellulose can certainly be considered to be a major contributor to overall plant strength.

The process of cellulose deposition plays another critical role in plant development as well, in that the patterns in which cellulose is deposited determine, to a great extent, patterns of plant development (see Green and Selker, 1991). In growing cells, the pattern of cellulose deposition is generally transverse to the axis of elongation, thus forcing turgor-driven cell expansion along the elongation axis. Toward the end of elongation, the pattern shifts, and, depending upon the cell type involved, complex new patterns of cellulose deposition occur during the phase of secondary wall cellulose thickening. In sum, the process of cellulose synthesis not only involves chain polymerization but also includes mechanisms that determine how

¹ To whom correspondence should be addressed.

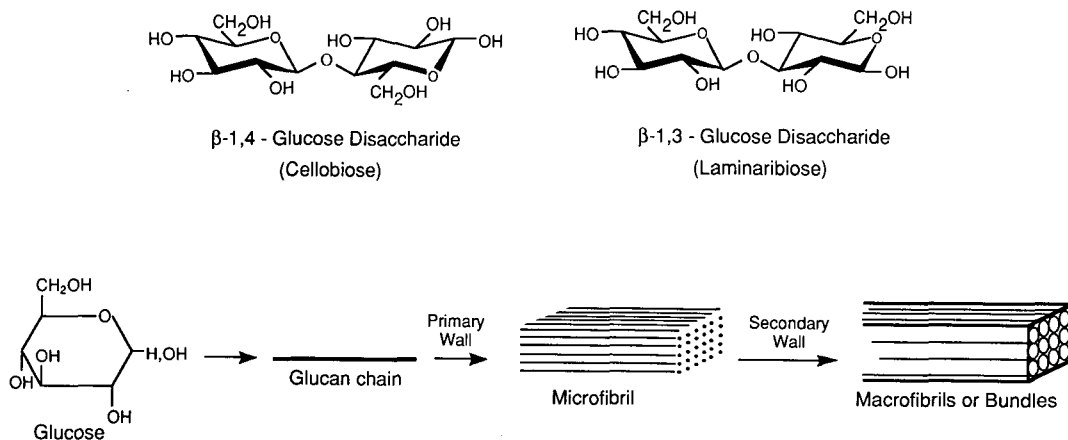


Figure 1. Steps in the Assembly of Native Cellulose.

The basic repeating unit of cellulose is the disaccharide cellobiose; each glucose residue is rotated $\sim 180^\circ$, which contrasts with the disaccharide unit (laminaribiose) found in the β -1,3-glucan callose. Glucose residues (in some activated form) are polymerized into individual chains; these further associate to form microfibrils. In the primary cell wall of plants, the so-called elementary fibril is a microfibril of ~ 36 chains, but microfibril size can vary among organisms. During synthesis of the plant secondary wall, microfibrils often associate further to form bundles.

and to what extent these chains associate to form micro- and macrofibrils. In the algae and higher plants, complex mechanisms are also required to regulate the orientation of deposition.

Model Systems for the Study of Cellulose Biosynthesis

No single system has emerged as ideal for the study of cellulose biosynthesis, but for those new to the field, it is helpful to describe briefly some that have proven particularly useful. Very few genera of bacteria synthesize cellulose, but the gram-negative bacterium *Acetobacter xylinum* secretes large quantities of cellulose as microfibrils from a row of synthetic sites along the longitudinal axis of the cell (for review, see Ross et al., 1991). The microfibrils from each synthetic site merge to form a large ribbon of cellulose; in the growth medium, these ribbons and associated cells tangle and form a floating pellicle that allows the nonmotile, strictly aerobic bacteria to grow in the higher oxygen tension at the surface. Upon contact with host plant cells, the tumor-forming bacterium *Agrobacterium tumefaciens* secretes cellulose fibrils from all sides of the cell, a process that aids in cell attachment and promotes virulence (see Matthysse et al., 1995a, 1995b). Both *A. xylinum* and *A. tumefaciens* can be grown in large quantities and can be transformed. Mutants of both can be selected or created that are impaired in cellulose biosynthesis, and genes and enzymes involved in this process have been identified in both bacteria. The cellulosic algae have proven quite useful for freeze-fracture studies in which putative synthase complexes were first visualized in the plasma membrane (Montezinos and Brown, 1976), but they have proven difficult to study at the biochemical and molecular levels. The slime mold *Dictyostelium discoideum* synthesizes cellulose at various stages in its life cycle (Blanton,

1993). A cellulose synthase activity has been demonstrated in this organism (Blanton and Northcote, 1990), and many sophisticated genetic approaches are available that make it very attractive for developmental studies of cellulose biosynthesis. The water mold *Saprolegnia* contains both β -1,4-glucan and β -1,3-glucan in its cell walls, and separable enzymes have been detected that synthesize these glucans in vitro (Bulone et al., 1990), but genetic studies are not advanced with this organism.

In higher plants, cotton fibers represent an interesting object of study (Basra and Malik, 1984; Ryser, 1985). These are single cells that elongate from the epidermal layer of the ovule, and they elongate synchronously within the boll. At the transition to secondary wall synthesis, the fibers transiently synthesize callose, followed by massive deposition of secondary wall cellulose (Maltby et al., 1979), in which the microfibrils are deposited in helical arrays in successive layers of alternating pitch. In contrast to other plant cells, cellulose constitutes more than 90% of the dry weight of the mature fiber cell. (Thus, when you don your jeans and lab coat, you are wrapping yourself in millions of these little cellulose factories!) Another interesting higher plant system is that of mesophyll cells of *Zinnia*, which can be induced in culture to undergo differentiation to form tracheary elements that show striking localized patterns of secondary wall cellulose deposition (Fukuda, 1991).

A WORKING MODEL OF THE MECHANISM OF CELLULOSE SYNTHESIS

To provide the reader with something to visualize as we proceed to discuss the process of cellulose synthesis in detail, we begin by presenting a hypothetical working model of how

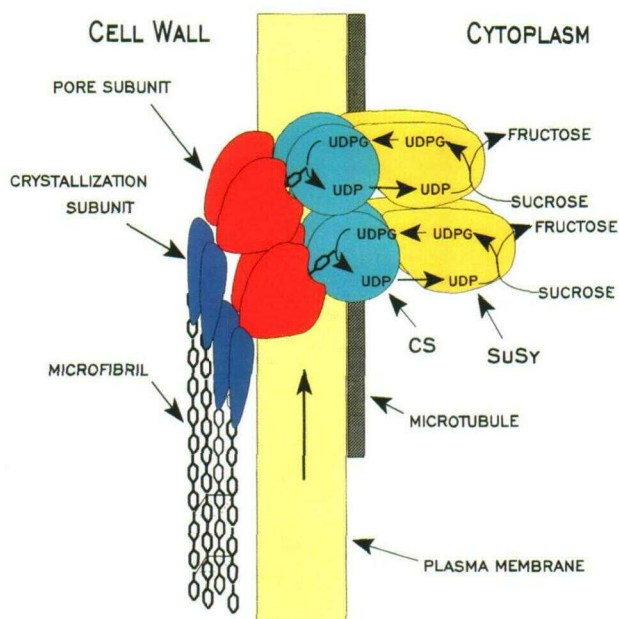


Figure 2. Hypothetical Model of a Cellulose Synthase Complex in the Plasma Membrane.

A microfibril is synthesized via a multisubunit complex, with one unit of the complex responsible for the polymerization, secretion, and alignment of each glucan chain. For simplicity, the number of such units shown here in the complex and the number of chains formed are less than that expected in nature. This model should be considered purely speculative and is a composite derived from studies done with a variety of organisms, including bacteria, algae, and higher plants. See the text for evidence regarding the existence and function of the various subunits shown in the model. Interaction with microtubules would not be expected for a bacterial synthase, and the interaction with sucrose synthase (SuSy) has been implicated only in higher plants. The arrow in the plasma membrane shows the direction an algal or plant synthase complex is expected to move during microfibril synthesis. CS, catalytic subunit.

cellulose might be synthesized. Figure 2 shows a cellulose synthase complex at the plasma membrane. Because cellulose is synthesized as microfibrils, each complex should have one synthetic unit, including a catalytic subunit, per glucan chain. For simplicity, we show only four such units, but for an elementary microfibril there are most likely ~36 such units per complex. Each catalytic subunit accepts as a substrate an activated form of glucose, most likely UDP-glucose. This might come directly from the cytoplasm or, in higher plants, might be donated from a membrane-associated form of sucrose synthase. The growing chains may be secreted through the membrane via a pore, and an accessory protein may facilitate alignment of the chains to promote precise crystallization into cellulose I. We also discuss evidence for additional regulatory subunits, but for simplicity these are not included in the model shown in Figure 2. In bacteria, the complexes are stationary, and microscopic studies of the process suggest that the force of polymerization propels the entire cell backward

in the medium. In algae and plants, it is thought that the complex moves in the fluid mosaic membrane and that the pattern of movement determines the pattern of cellulose deposition and is somehow guided by microtubules that are either adjacent to, or directly connected with, the synthase complex.

With this model as background, we now proceed to examine the various steps in cellulose biosynthesis as we understand them today, to clarify what we know as distinct from what we only suspect or do not know at all, and in so doing, to point out which critical questions need to be addressed to understand the process fully. We have also indicated those areas in which molecular and genetic approaches may be most useful. Other reviews on cellulose synthesis in bacteria and higher plants are available (Delmer, 1990; Ross et al., 1991; Blanton and Haigler, 1995), as are some more general reviews on plant cell wall biosynthesis (Delmer and Stone, 1988; Bolwell, 1993; Iyama et al., 1993).

TRACING THE PATH OF CARBON INTO CELLULOSE

The only well-characterized cellulose synthase is that of *A. xylinum*. This synthase uses UDP-glucose directly as a substrate for polymerization *in vitro* (Ross et al., 1991), and a mutant blocked in ability to synthesize UDP-glucose from UDP-glucose pyrophosphorylase is cellulose deficient (Valla et al., 1989). Very recent and surprising results with *A. tumefaciens* suggest a substantially different pathway, in which glucose residues from UDP-glucose are transferred to lipids, which then serve as further intermediates from which oligosaccharides undergo a final polymerization via an endoglucanase functioning in a synthetic mode (Matthysse et al., 1995a). No stimulation of cellulose synthesis was observed by addition of cyclic diguanylic acid (c-di-GMP, an activator of *A. xylinum* cellulose synthase; see later discussion), although a previous report suggested that it plays a role in cellulose synthesis in *A. tumefaciens* (Amikam and Benziman, 1989). Five *A. tumefaciens* genes were identified as being involved in cellulose synthesis, just one of which bears homology with a synthase-related gene from *A. xylinum* (the *BcsA* gene; see later discussion; Matthysse et al., 1995b).

The nonbiological substrate β -cellobiosyl-fluoride has been shown recently to serve as a substrate for synthesis of cellulose I catalyzed by a purified cellulase in a nonaqueous environment, which favors synthesis rather than hydrolysis of the polymer (Lee et al., 1994). However, the possibility of a glucanase-catalyzed polymerization of cellulose *in vivo* is a truly novel idea. Such a reaction seems theoretically possible if catalyzed within the nonaqueous environment of the plasma membrane. The details of these new findings need to be confirmed further, but if correct, the pathway is definitely novel, and it would remain to be determined whether it is unique to *A. tumefaciens*. One attractive feature of this pathway would be the ability to transfer, via lipid intermediates, cellobiosyl, as opposed to glucosyl residues. In a pathway involving transfer of glucose only, it has been difficult to envision how large

complexes could add successive residues that are rotated 180°, a problem that could be avoided by transfer of an activated cellobiosyl group. However, an alternate solution to this problem is suggested in the imaginative model of Saxena et al. (1995), which proposes two separate UDP-glucose binding sites for transfer of successive glucose residues in opposite orientations.

Some years ago, a possible role for lipid intermediates in cellulose synthesis in plants was debated (see Delmer, 1983), but to date, no firm data exist to support this concept. Plant cells normally contain very high levels of UDP-glucose (in the millimolar range; Schlupmann et al., 1994). Using developing cotton fibers cultured *in vitro*, Carpita and Delmer (1981) traced the path of carbon from supplied radioactive glucose to cellulose. The results of such kinetic studies, coupled with computer simulation models, were consistent with but did not prove the interpretation that UDP-glucose is an intermediate in the pathway to cellulose.

UDP-glucose can be synthesized by UDP-glucose pyrophosphorylase by the reversible reaction $\text{glucose-1-P} + \text{UTP} \rightleftharpoons \text{UDP-glucose} + \text{PPi}$ (for review, see Kleczkowski, 1994). Most plant tissues contain very high levels of UDP-glucose pyrophosphorylase, so the capacity for UDP-glucose synthesis via this enzyme is theoretically high; in sink tissues, however, the enzyme probably functions in the direction of UDP-glucose degradation. A second possible route for UDP-glucose synthesis is the reversible reaction catalyzed by sucrose synthase (SuSy): $\text{sucrose} + \text{UDP} \rightleftharpoons \text{UDP-glucose} + \text{fructose}$. SuSy, which is found in high levels mainly in nonphotosynthetic tissues, is known to play a critical role in the degradation of sucrose, most notably during starch synthesis in sink tissues, where it clearly functions with the UDP-glucose pyrophosphorylase (in its degradative mode) to provide glucose-1-P for further production of the substrate for starch synthesis, ADP-glucose (Geigenberger and Stitt, 1993; see also Martin and Smith, 1995, this issue).

The maize shrunken (*sh1*) mutant, which is deficient in the ability to form starch, has a mutation in a gene coding for an endosperm-specific form of SuSy (Chourey and Nelson, 1976). The endosperm cells of the *sh1* mutant eventually deteriorate, apparently as a result of a defect(s) in cell wall formation, suggesting an important role for SuSy in some aspect of wall formation (Chourey et al., 1991). A specific inhibitor of cellulose synthesis, 2,6-dichlorobenzonitrile (DCB), inhibits the expression in maize protoplasts of a reporter gene directed by the promoter of the *sh1* gene (Maas et al., 1990). These authors interpreted this result as relating to the known regulation of the *sh1* gene by sugar levels and proposed that increased UDP-glucose levels may feed back to regulate SuSy gene expression. However, we have found that DCB does not significantly alter UDP-glucose levels in plants (D. P. Delmer, unpublished results), and DCB may thus affect expression by some other mechanism. SuSy also plays an important role in sucrose degradation to provide energy for phloem loading and to provide UDP-glucose for callose synthesis in sieve plates (Geigenberger et al., 1993; Martin et al., 1993; Nolte and Koch, 1993).

Recent studies with developing cotton fibers also suggest an important role for SuSy in providing UDP-glucose for cellulose synthesis (Amor et al., 1995). The most abundant of the membrane proteins that bind UDP-glucose is an 84-kD polypeptide that binds UDP-glucose in a Mg^{2+} -dependent manner (Delmer et al., 1991). Because this is close to the size of the catalytic subunit of bacterial cellulose synthase (see later discussion), we undertook to purify and characterize this polypeptide. Sequencing of several tryptic fragments from the purified polypeptide showed it to be a form of SuSy. Before this observation, all studies with SuSy had assumed that this enzyme was soluble in the cytoplasm. It is now known that >50% of total SuSy is very tightly associated with the membrane, suggesting a model in which some form of SuSy might be associated with the cellulose synthase complex and serve to channel carbon directly from sucrose via UDP-glucose to the complex (Amor et al., 1995; see also Figure 2).

The idea that SuSy is associated with the cellulose synthase complex is appealing for several reasons. First, it is much more efficient from an energy standpoint than a pathway involving UDP-glucose pyrophosphorylase, because cellulose would be synthesized "directly" from sucrose, with the UDP regenerated by the cellulose synthase for reuse by SuSy. Furthermore, glycosyltransferases such as bacterial cellulose synthase (Ross et al., 1991) and callose synthase (Morrow and Lucas, 1986) are inhibited by UDP; if the UDP is quickly reutilized by SuSy, then UDP levels could be kept low, thereby enhancing synthase activity. Finally, there would be little or no competition by other enzymes for use of the UDP-glucose produced because it would be channeled directly to the synthase catalytic subunit. Immunolocalization studies (Amor et al., 1995) show that during secondary wall synthesis in cotton fibers, SuSy is localized at the cell surface, often in arrays that parallel the helical pattern of cellulose deposition. This further supports the notion that SuSy may exist in a complex with cellulose synthase.

SYNTHESIS OF CELLULOSE IN VITRO

The first breakthrough in this area came when Aloni et al. (1982) achieved high rates of *in vitro* synthesis with membrane preparations of *A. xylinum* in the presence of GTP and a soluble protein factor. Later studies showed that the protein factor is a diguanylate cyclase that converts GTP to c-di-GMP (Ross et al., 1987, 1991). The finding that c-di-GMP is a specific activator of the bacterial synthase opened the way for detailed enzyme characterization and purification and for subsequent cloning of the genes (see later discussion). Cellulose synthesis by the *A. xylinum* enzyme occurs as a Mg^{2+} -dependent reaction that uses UDP-glucose as a substrate and, in the presence of micromolar levels of c-di-GMP, takes place at *in vitro* rates that can approach those observed *in vivo* (Aloni et al., 1982; Ross et al., 1987). No added primer is needed, even with the purified enzyme; however, one cannot exclude the possibility

that primer purifies with the enzyme. (In general, we can find no evidence for a primer requirement for synthesis of either cellulose or callose in any organism.)

There are now also firm reports of in vitro synthesis of β -1,4-glucan (not shown to be crystalline cellulose) from UDP-glucose using membrane preparations from *Dictyostelium* (Blanton and Northcote, 1990) and from the water mold *Saprolegnia* (Fevre and Rougier, 1981). In *Dictyostelium*, the in vitro activity approaches that observed in vivo; the activity is Mg^{2+} dependent and is stimulated by cellobiose but not c-di-GMP. Unfortunately, this enzyme has proven difficult to solubilize (Blanton and Northcote, 1990; Blanton and Haigler, 1995). In *Saprolegnia*, the β -1,4-glucan synthase activity is stimulated by Mg^{2+} , cellobiose, and c-di-GMP, but to a much lower extent than is the bacterial synthase (Girard et al., 1991). The β -1,3-glucan synthase of *Saprolegnia*, which can be separated from the β -1,4-glucan synthase (Bulone et al., 1990), is stimulated only by cellobiose and has a much higher K_m for UDP-glucose (Fevre and Rougier, 1981).

A major complication in all searches for in vitro cellulose synthase activity in plasma membranes derived from higher plants arises from the fact that such membranes have very high levels of callose synthase activity. Indeed, because callose has often been confused with cellulose in nonrigorous determinations of glucan structure, there have been a number of erroneous claims of cellulose synthesis that were subsequently disproven (see Delmer, 1987). Thus, it is difficult to discuss the synthesis of cellulose in plants without also discussing the properties of the callose synthase, which is now fairly well characterized. Both cellulose and callose synthase exist in the plasma membrane and appear to use the same substrate, yet their activities appear to be regulated in an opposing fashion in vivo. With the exception of specialized cells such as pollen tubes (see Schlupmann et al., 1993), callose is not normally a cell wall constituent; rather, it is deposited in response to perturbations such as mechanical and temperature stresses and pathogen invasion (for a comprehensive review, see Stone and Clarke, 1992). We have recently observed that when callose synthesis is induced by elicitors in vivo, there is a proportional drop in the rate of cellulose synthesis (E. Shedletzky and D.P. Delmer, unpublished observations), indicating that synthesis of the two polymers has opposing modes of regulation.

The discovery that callose synthase is activated by micromolar levels of Ca^{2+} provides at least partial explanation for the induction of callose synthesis in vivo, because elevation of cytoplasmic Ca^{2+} is known to occur in response to such perturbations (Kauss, 1991; Trewavas and Gilroy, 1991). However, there are conditions in which cytoplasmic Ca^{2+} rises, yet callose synthesis does not occur (see Kauss, 1991). The known additional requirement for a β -glucoside (for example, cellobiose) activator (Hayashi et al., 1987) may partially explain this finding. Instead of cellobiose, the native activator may be β -furfuryl- β -glucoside, the cytoplasmic level of which may vary in response to perturbation (Ohana et al., 1993). It is interesting that the requirement for a β -glucoside is shared also by the

eukaryotic cellulose synthase activities of *Dictyostelium* and *Saprolegnia*.

In studies with membranes derived from developing cotton fibers, R. M. Brown's group has demonstrated reproducible but low rates of 1,4- β -glucan synthesis simultaneous with much higher rates of callose synthesis in vitro (Li and Brown, 1993; Okuda et al., 1993). More recently, conditions were found in which the ratio of cellulose to callose was improved and the two types of products could be distinguished by electron microscopy; however, rates of cellulose synthesis were still low (Kudlicka et al., 1995). Although demonstration of some synthesis of cellulose in vitro is indeed important, the conditions used to demonstrate this cellulose synthase activity were similar to those traditionally used for callose synthase, including addition of Ca^{2+} . This should favor the activity of callose synthase and not that of cellulose synthase. Thus, at present it seems that we still are lacking an understanding of the critical factors necessary for obtaining substantial synthesis in vitro from UDP-glucose (for further discussion, see Delmer et al., 1993a).

With the recent discovery of a membrane-bound form of SuSy, we have begun to address the question of whether in vitro synthesis by disrupted and detergent-permeabilized cotton fibers might occur more readily if the substrate supplied were sucrose instead of UDP-glucose (Amor et al., 1995). Rates of β -1,4-glucan synthesis from sucrose can be obtained that are comparable with, and sometimes exceed, those for callose synthesis. In the presence of Ca^{2+} and cellobiose, callose can also be synthesized in this coupled reaction, and although other studies have obtained contrasting results (Li and Brown, 1993; Kudlicka et al., 1995), we have found that coupled activity for β -1,4-glucan synthesis is favored over that for callose in the absence of Ca^{2+} . Thus, it may be that the plant cellulose synthase normally accepts UDP-glucose readily only when it is transferred directly from SuSy in the complex, whereas callose synthase is more readily able to accept UDP-glucose in its free form. The coupled reaction from sucrose has been possible to demonstrate only with freshly detached cotton fibers permeabilized with digitonin, and the reaction is very labile and not demonstrable in membrane preparations. Such results suggest that preservation of some higher level of organization and/or regulatory factors is critical for obtaining good activity. The fact that either callose or cellulose can be synthesized from sucrose lends some support to the notion that both glucan synthases might be in the same complex (with SuSy), as suggested previously (Jacob and Northcote, 1985; Delmer, 1987).

OVERALL STRUCTURE OF THE CELLULOSE SYNTHASE COMPLEX

The Cellulose Synthase Catalytic Subunit

The only catalytic subunit of cellulose synthase that has been clearly identified is that of *A. xylinum*. In a preparation of

	* *		* * * * *
SPS 195	D S D T G G Q V K Y V V E L A		
SuSy 297	Y P D T G G Q V V Y I L D Q V		
AcsAB 177	W P P E K V R . V H I L D D G		
BcsA 177	W P P D K V N . V Y I L D D G		
CelA 155	Y P A D R F T . V W L L D D G		
ORF f692	W P K D K L N . I W I L D D G		
NodC 53	Y P G E . L R . V Y V V D D G		
HyalS 92	Y P L S E . . . I Y I V D D G		

Figure 3. Conserved Potential UDP-Glucose/UDP-N-Acetylglucosamine Binding Motif Found in Various Glycosyltransferases.

Sequences are derived from cDNA clones. Numbers refer to the position of the first amino acid residue of the motif within the derived sequence. SPS, spinach sucrose-P synthase (accession number P31928); SuSy, Arabidopsis SuSy (accession number X70990); AcsAB, fused *BcsA* and *BcsB* genes of the *A. xylinum* strain ATCC53582 (accession number X54676); *BcsA*, cellulose synthase catalytic subunit gene cloned from *A. xylinum* strain used by Wong et al. (1990; accession number M37202); CelA, *BcsA* gene homolog from *A. tumefaciens* (accession number L38609); ORF f692, open reading frame (ORF) from *E. coli* analogous to the *BcsA* gene (accession number U00039); NodC, β -1,4-N-acetylglucosamine synthase from *Rhizobium meliloti* (accession number X01649); HyalS, hyaluronan synthase from *Streptococcus pyogenes* (accession number L20853). The latter two gene products utilize UDP-N-acetylglucosamine instead of UDP-glucose as substrate. All accession numbers are for GenBank listings. Asterisks denote the most highly conserved residues.

cellulose synthase purified by product entrapment (see Lin and Brown, 1989), photo-labeling studies with azido-UDP-glucose led to labeling of an 83-kD polypeptide (Lin et al., 1990). N-terminal sequencing of this polypeptide allowed the gene to be cloned (Saxena et al., 1990). Using a cloning strategy based upon the approach of genetic complementation of a mutant defective in cellulose synthesis, Wong et al. (1990) independently cloned an operon of four *A. xylinum* genes (*BcsA* through *BcsD*) involved in cellulose synthesis, one of which (*BcsA*) is homologous to that cloned by Saxena et al. (1990).

There are no firm indications of the nature of the catalytic subunit of plant cellulose synthase. Li et al. (1993), using photo-affinity labeling with azido-UDP-glucose, identified a polypeptide of 37 kD that shows Mg^{2+} -dependent interaction with the substrate and was suggested as a likely candidate for the catalytic subunit. However, these results still remain correlative, because they were not done with purified cellulose synthase. In similar correlative studies with crude or partially purified preparations of callose synthase, polypeptides in the range of 52 to 57 kD (Frost et al., 1990; Delmer et al., 1991; Fredrikson et al., 1991; Li et al., 1993; Dhugga and Ray, 1994) or 28 to 35 kD (Fink et al., 1990; Meikle et al., 1991; Girard et al., 1992) have been suggested as likely candidates for the catalytic subunit of this enzyme. There is also progress in purification of callose synthase. For example, Dhugga and Ray (1994) achieved substantial enrichment for polypeptides of 55 and 70 kD in callose synthase preparations of fairly high purity, the former of which bound UDP-glucose and

may therefore be the catalytic subunit. Whether these studies are relevant to cellulose synthase will be unclear until it can be shown whether the two enzymes are related.

Another possible approach for identifying genes that code for UDP-glucose binding proteins is based upon the observation of Saxena et al. (1994) that a highly conserved sequence exists among the bacterial catalytic subunit and other enzymes that catalyze the polymerization of β -glycosyl residues from either UDP-glucose or UDP-N-acetylglucosamine. These include the NodC, hyaluronate synthase, and ExoO proteins. We have also examined motifs common to UDP-glucose binding proteins (D.P. Delmer and Y. Amor, unpublished data), and Figure 3 illustrates one such motif. Computer searches showed that a motif present in a UDP-glucose binding polypeptide isolated from sucrose-P synthase of spinach is also found in SuSy (Salvucci and Klein, 1993), in the BcsA product of two different strains of *A. xylinum*, and in the CelA product from *A. tumefaciens*. This motif is the same as the conserved motif identified by Saxena et al. (1994), which is also found in the NodC, hyaluronan synthase, and ExoO proteins. Our search also identified a similar sequence deduced from DNA sequencing experiments in *E. coli*, an organism not known to synthesize cellulose. Surprisingly, further analysis of this sequenced region by us (Figure 4) and by Sofia et al. (1994) identified three contiguous genes that show very good to weak homology with the *BcsA*, *BcsB*, and *BcsC* genes of *A. xylinum*. The function of these genes is completely unknown, but their existence is fascinating, and they clearly deserve further study.

The conservation of this UDP-glucose binding motif in a number of bacterial and plant proteins indicates that there may well be a similar region in higher plant cellulose and/or callose synthases. However, several groups, including our own, have been unable to identify the plant catalytic subunit gene using the complete *BcsA* gene as a probe. This suggests that the plant catalytic subunit is not highly homologous to the bacterial one. Furthermore, to our knowledge, the intensive sequencing now performed on random cDNA clones from a number of plant cDNA libraries has yet to yield a sequence showing strong homology with the *BcsA* gene. However, it may be that probes based upon specific and more highly conserved regions of the genes would be more successful. Hydrophobic cluster analyses of glycosyltransferase sequences (Saxena et al., 1995) also appear useful for identifying functionally important domains. At a minimum, such efforts might lead to the identification of genes coding for other important UDP-glucose binding proteins of plants.

Evidence for Additional Noncatalytic Subunits

It now seems clear that the *A. xylinum* synthase complex comprises at least one subunit in addition to the catalytic subunit. The second gene in the operon codes for a polypeptide of 86 kD, which migrates in SDS gels as a 93-kD polypeptide (Wong et al., 1990; Saxena et al., 1991). This polypeptide copurifies with the synthase complex (Lin and Brown, 1989; Mayer et

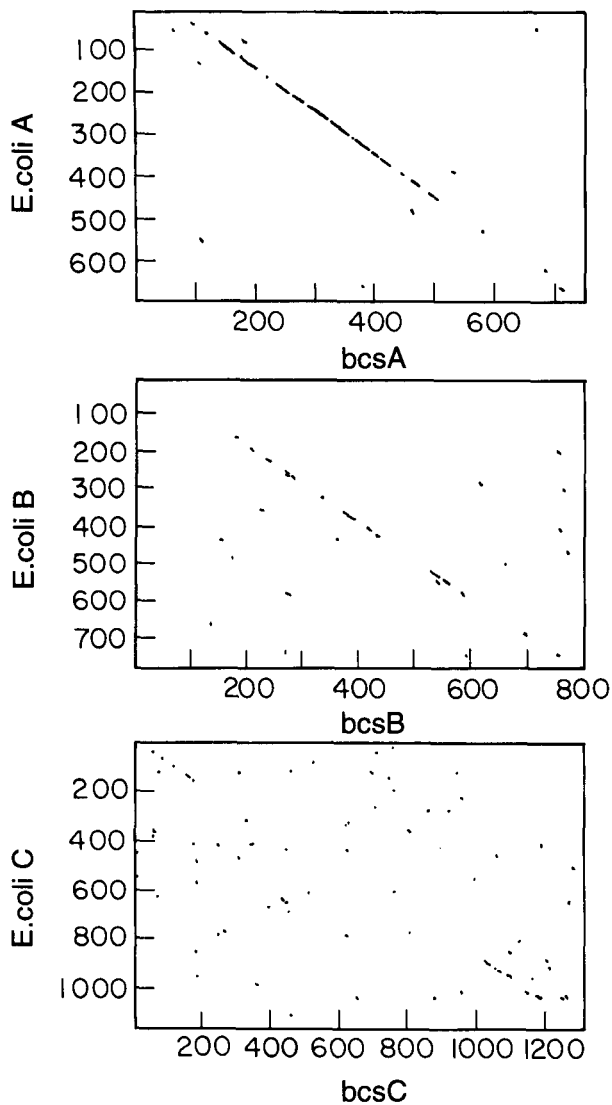


Figure 4. Sequence Comparisons among the *A. xylinum* BcsA, BcsB, and BcsC Proteins and Similar *E. coli* Protein Sequences.

One of two contiguous *E. coli* genes encodes a protein with high homology with the BcsA protein; the other shows moderate homology with the BcsB protein. A third *E. coli* gene codes for a protein showing weak homology with the BcsC protein. Amino acid sequences were compared using a Pustell Protein Matrix comparison on the MacVector Program (Scientific Imaging Systems, New Haven, CT). *E. coli* DNA sequences were from ORFs in the area of 76.0 to 81.5 min of the genome: *E. coli A* refers to ORF f692; *E. coli B* refers to ORF 779; *E. coli C* refers to ORF 1165. The GenBank accession number for this region of the chromosome is U00039 (also see Sofia et al., 1994).

al., 1991), and affinity-labeling studies suggest that it is most likely a regulatory subunit that binds c-di-GMP (Mayer et al., 1991). The roles of the polypeptides coded by the *BcsC* and *BcsD* genes are not entirely clear, but insertional mutagenesis of the *BcsC* gene resulted in loss of capacity to synthesize

cellulose (Wong et al., 1990; Saxena et al., 1994). The derived amino acid sequence of the *BcsC* gene product shows some homology with several bacterial pore-forming proteins (Saxena et al., 1994), suggesting the possibility that the *BcsC* gene may code for a pore protein that serves as the site of exit of the glucan chains (see Figure 2). Such pores have been visualized only once, by Zaar (1979). No putative pore protein copurifies with the synthase after solubilization with digitonin, nor does any polypeptide of the size predicted by the *BcsD* gene (17 kD). However, insertional mutagenesis of the *BcsD* gene creates a mutant that secretes only limited quantities of cellulose that, depending upon culture conditions, can have altered crystal structure (Saxena et al., 1994). From such a result, these authors suggest that the *BcsD* gene may be involved in determining the crystallization pattern of the microfibrils (see Figure 2).

In the absence of any purified cellulose synthase from higher plants, it is not possible to provide hard biochemical evidence to model the synthase complex. Amor et al. (1991) identified two polypeptides in membranes of cotton fibers that bind c-di-GMP with high affinity and specificity, but these have proven difficult to purify and characterize, and no further information is available at present concerning their possible function (Y. Amor and D.P. Delmer, unpublished data). Several reports indicate that polypeptides in the 30-kD range copurify with callose synthase activity. B.P. Wasserman's group has determined that several such peptides show homology with aquaporins (Wu and Wasserman, 1993; Qi et al., 1995), and these have been suggested as possible channels for export of glucan chains (Qi et al., 1995). Analysis of a recently cloned yeast gene presumed to code for a subunit of β -1,3-glucan synthase shows that the gene encodes a protein similar in structure to pore-like proteins involved in the export of β -1,2-glucan in bacteria (Cameron et al., 1994). In addition, Andrawis et al. (1993) have obtained evidence suggesting that purified cotton fiber annexin binds to, and influences the activity of, callose synthase. Recent crystallographic and biochemical studies with several animal annexins indicate that these proteins can function as Ca^{2+} channels (Huber et al., 1990; Berendes et al., 1993). Thus, it is possible that plant annexin may turn out to serve as a pore for callose secretion or, perhaps more likely, for allowing entry of Ca^{2+} , which would then cause localized activation of the callose synthase complex. Other studies identified a 65-kD membrane polypeptide that also interacts with cotton fiber callose synthase in a cation-dependent manner (Delmer et al., 1993b); the function of this protein is unknown, but recent cloning of the gene coding for this polypeptide indicates that it shares strong homology with another Ca^{2+} binding protein, calnexin (D.P. Delmer, unpublished data).

Morphological Studies on Cellulose Synthase Complexes

Preston (1964) predicted the existence of a cellulose synthase complex composed of many subunits, each of which would

catalyze the synthesis of an individual glucan chain; these chains would then self-associate during synthesis to form the microfibril (Figure 2). Montezinos and Brown (1976) were the first to actually visualize such a complex, which they observed at the ends of microfibrils in freeze–fracture studies of the plasma membrane of the cellulosic alga *Oocystis opiculata*. This terminal complex (TC) consists of a rectangular array of three linear rows of ~30 particles each. Similar complexes have been observed in many other cellulose-producing algae that are characterized by microfibrils of large size and high crystallinity (for discussion, see Okuda et al., 1994). In some other algae, such as *Micrasterias denticulata*, a highly ordered aggregate of six-particle, rosette-like structures has been observed at the ends of microfibrils (Giddings et al., 1980). In higher plants, similar rosettes are also observed singly in the plasma membrane, where they are concentrated at sites of high cellulose deposition, such as under the bands of cellulose formed in the walls of tracheary elements (Herth, 1985). Because different types of complexes (or portions of them) fracture to different faces of the plasma membrane, and because of limitations in resolution even with fine imaging, it is not yet known whether particles consist of identical subunits or several different types of subunit. Studies with antibodies are not yet feasible, because almost all complexes can be visualized only by freeze–fracture techniques.

Evidence that TCs and rosettes are involved in cellulose synthesis has been reviewed by Delmer (1987) and more recently by Blanton and Haigler (1995) and is, overall, fairly convincing. These complexes, particularly the rosettes of higher plants, are exceedingly labile, and great care and rapid freezing are necessary to preserve them (Herth and Weber, 1984). There is a fairly good correlation between complex size and shape and microfibril size and crystallinity. For higher plants, in which single rosettes are found, the size and hexameric appearance of the rosette fit a model in which each “subunit” of the hexamer synthesizes six glucan chains, leading to production of a 36-chain elementary fibril. This agrees with the prediction that the 35-glucan chain is the smallest aggregate that can form truly crystalline cellulose I (Chanzy et al., 1978). In the alga *Micrasterias*, in which rosettes are aggregated and microfibril size is much larger, the number of rosettes in each aggregated row corresponds well with the size of the microfibril produced (Giddings et al., 1980); a similar correlation between TC size and shape with microfibril size also holds for linear TCs (Hotchkiss, 1989).

The cellulose II crystal structure is more thermodynamically stable than that of cellulose I and is the form obtained when cellulose crystallizes from solution. Thus, the metastable cellulose I appears to require the highly ordered synthetic process found in nature, and cellulose II is formed only in vivo in mutants or by in vitro synthesis (see Saxena et al., 1994). It now appears that the α form of cellulose I (triclinic, metastable) is the predominant type synthesized by linear TCs, whereas the monoclinic, more stable β form predominates in rosette-containing organisms (Atalla and VanderHart, 1989; Sugiyama et al., 1991). In sum, the type of synthase structure found

appears to be under genetic control and determines the size and crystalline structure of the microfibril formed (Haigler, 1985, 1991; Okuda et al., 1994; Blanton and Haigler, 1995). As a generalization, it seems that the large TCs produce a more highly ordered crystalline form of cellulose than that synthesized by rosettes.

ROLE OF THE CYTOSKELETON IN CELLULOSE SYNTHESIS

Except in special cases (see Reis et al., 1994), the cytoskeleton is somehow involved in directing the orientation of deposition of cellulose microfibrils in algae and plants (for review, see Giddings and Staehelin, 1991). Most studies have indicated that the cortical microtubule (MT) network is the major entity involved, because the alignment of this network is highly correlated with the alignment of the most recently deposited cellulose; furthermore, inhibitors that disrupt MT structure also lead to disorganized deposition of cellulose. Work with both developing cotton fibers and tracheary elements suggests that the actin cytoskeleton may also play a role in this process. Treating such cells with cytochalasins, which causes depolymerization of actin filaments, also disrupts MT and microfibril patterns (Kobayashi et al., 1987, 1988; Seagull, 1990). These results suggest that actin may play a primary role in setting the pattern of the cortical MT network, which in turn sets the pattern of cellulose deposition.

In animals, the small GTP binding proteins Rac and Rho are involved in the signal transduction pathway that regulates actin organization (Downward, 1992). We have recently identified two cotton genes whose products are homologous to animal Rac (Delmer et al., 1995). One of these two genes is particularly highly expressed in cotton fibers just at the time that the cytoskeleton undergoes the reorganization that leads to the helical patterns characteristic of the secondary cell wall. Rac proteins could potentially play other roles in cellulose and/or callose synthesis: in addition to its role in regulating actin organization, a Rac-related protein in yeast (CDC42) appears to be a specific activator of the yeast β -1,3-glucan synthase (Diaz et al., 1993; Mol et al., 1994).

There has been little progress in understanding how MTs might direct the movement of cellulose synthase complexes within the plasma membrane (see Giddings and Staehelin, 1991). One model suggests that they might interact directly with the complex; however, a more favored current model suggests that MTs serve as “fences” between which the complexes are constrained to move without any direct interaction. This model derives from several observations from freeze–fracture studies, in which rosettes appear to be abundant between MTs rather than in direct connection with them (Giddings and Staehelin, 1990). However, the concept of a direct connection between cellulose synthase and cytoskeleton, perhaps via accessory proteins, seems mechanistically more appealing. Reports have suggested a role for elongation factor (EF)-1 α

in interacting with and affecting the bundling of microtubules in a Ca^{2+} /calmodulin-dependent manner (Durso and Cyr, 1994) and in being involved in wall-membrane connections (Zhu et al., 1994). Thus, in addition to its involvement in the GTP-dependent elongation step of protein synthesis, EF-1 α clearly has other roles, and it is intriguing to speculate about whether this protein might interact with and promote the directed movement of cellulose synthase in plants.

Another question is what powers the movement of these complexes. The force of polymerization itself is considered sufficient to generate this movement; however, the major evidence for this is based upon the observation that nonflagellated *A. xylinum* cells are propelled through a liquid medium as they synthesize cellulose. However, the situation with bacteria is clearly different from that with plants, because this synthase is presumably stationary in the bacterial plasma membrane, and it remains to be proven that no additional energy inputs are required to generate cellulose synthase movement in algae and higher plants.

A number of recent studies indicate that cytoskeletal organization is under hormonal control (reviewed by Shibaoka, 1994). Particularly in the epidermal layer, which limits cell elongation, it now appears that gibberellic acid and auxin either maintain or reorient MTs in a transverse array and thus promote elongation. By contrast, the well-known swelling of plant stems in the presence of ethylene can be attributed to the effect of this hormone on MT orientation, which is to shift MTs away from the transverse. However, the mechanism by which hormones exert these effects is not at all clear, nor is it clear whether they affect actin function or that of MTs directly.

DISRUPTION OF CELLULOSE SYNTHESIS IN VIVO

A classic approach to dissecting metabolic pathways has been the use of either chemical inhibitors or mutants blocked in specific steps in the pathway. Two known herbicides, DCB and isoxaben, appear to disrupt plant growth by specific inhibition of cellulose synthesis (see Delmer et al., 1987; Heim et al., 1990b). Using photo-affinity labeling, Delmer et al. (1987) identified an 18-kD protein in cotton fibers that specifically binds an active analog of DCB; however, the protein is not abundant and, to date, has resisted complete purification and characterization. Other work from our laboratory (D.P. Delmer, unpublished data) indicates that DCB does not affect labeling of UDP-glucose *in vivo*; rather, it appears to exert its effect at some later step in the synthetic process. A number of reports show that DCB affects TC and rosette structure (see Mizuta and Brown, 1992), but its exact mode of action remains unclear. DCB has been shown to affect the expression of the SuSy gene (Maas et al., 1990); we have also found that the level of SuSy in cell lines adapted to growth on DCB is much lower than that in the parent lines (Y. Amor and D.P. Delmer, unpublished data), and this effect of DCB clearly deserves further study. The mode of action of isoxaben is also unclear; however,

several *Arabidopsis* mutants have been selected that are resistant to this herbicide, opening the possibility for the eventual cloning of the genes that confer herbicide resistance and that, by analogy, may also play a role in cellulose synthesis (Heim et al., 1989, 1990a). More recently, phthoxazolin has also been reported to inhibit cellulose synthesis, but no information is available concerning its mode of action (Omura et al., 1990).

One of the most promising new developments in the field of cellulose synthesis in higher plants has been the recent selection of several mutants that are impaired in the process. Potikha and Delmer (1995) have recently characterized an *Arabidopsis* mutant that is blocked in the ability to deposit secondary wall cellulose in the trichomes of leaf and stem. This mutant was identified because it lacks the birefringence under polarized light that is typical of ordered secondary wall cellulose. Direct analyses of cellulose content in isolated trichomes indicate that the cellulose level in mutant trichomes is only 17% of that in wild type; this residual level may be derived from the primary wall, which is formed normally in the mutant. Brittle culm mutants of barley are somewhat similar, in that the secondary cell walls of the culm have reduced cellulose content (Kokubo et al., 1991). Although the molecular lesions in these mutants remain to be discovered, it is clear now that it is possible to select for nonlethal mutants that are partially impaired in cellulose synthesis in specific cell types. The mutants thus raise the interesting possibility that there might be multiple forms of cellulose synthase that are under developmental regulation. For example, pollen tubes contain a callose synthase distinct from that of other tissues in that it is not regulated by Ca^{2+} (Schlupmann et al., 1993). However, it cannot be excluded that the mutations are in genes involved in cell-specific regulation of a common cellulose synthase.

Mutants apparently defective in cellulose synthesis may be included among the array of temperature-sensitive *Arabidopsis* mutants isolated as putative cytoskeletal mutants. Baskin et al. (1992) identified a number of such mutants by screening for mutants that show swelling of the root tip at the restrictive temperature. Indications are that some of these mutants are impaired in cellulose synthesis at high temperature (Betzner et al., 1995). These mutants, which map to distinct loci, are most likely impaired in primary wall cellulose synthesis; one is particularly interesting in that freeze-fracture studies indicate a disappearance of rosettes at the high temperature. If this result is confirmed, it would provide the strongest evidence yet for a role for rosettes in cellulose biosynthesis. The potential to clone this gene by chromosome walking exists, and further characterization of this mutant should provide valuable new insights into the process.

FUTURE DIRECTIONS

In conclusion, we summarize briefly some areas in which there is the most potential for future progress. First, regarding *in vitro*

cellulose synthesis, the evidence for a coupled reaction between SuSy and glucan synthases has led to the ability to obtain fairly high rates of cellulose synthesis *in vitro*; although the reaction is labile, it offers a new approach for characterization of the cellulose synthase activity. If such a complex between SuSy and glucan synthases does indeed exist, then biochemical approaches such as cross-linking or genetic approaches using the SuSy gene as "bait" in various types of interaction cloning systems (for example, Fields and Sternglanz, 1994; Stone et al., 1994) could lead to identification of other polypeptides in the synthase complex that might interact with the SuSy protein.

Second, the overall regulation of cellulose and callose synthesis as it relates to cell perturbation certainly deserves further study. Because of the known activation of callose synthase by cytosolic Ca^{2+} , it seems most likely that elevation of cytoplasmic Ca^{2+} will prove to be a negative regulator of cellulose synthesis. Because there is so much rapid progress in all aspects of signal transduction in plants, including that involving Ca^{2+} (Trewavas and Gilroy, 1991; Bowler and Chua, 1994), we anticipate that some of the knowledge gained from these advances can be applied to the regulation of both cellulose and callose synthesis. The limited data available that indicate opposing modes of regulation of the synthesis of these polymers also suggest another interesting area of study, namely, the relationship of cellulose and callose synthesis to the cell cycle. Because callose is synthesized at the cell plate (see Stone and Clarke, 1992), this raises the possibility that cellulose synthesis is inhibited at this stage of the cell cycle.

Third, we predict that, in general, the cytoskeleton will be one of the most fruitful areas of research in plant biology in the near future. This should also have an impact on studies of the role of the cytoskeleton in cellulose synthesis. Combining the power of confocal microscopy with *in situ* immunolocalization studies, it is now possible to visualize cytoskeletal reorganization in living plant cells (Yuan et al., 1994). Given recent advances in microinjection techniques, it should be possible to dissect the signal transduction pathway from hormonal stimulation to cytoskeletal reorganization by microinjecting potential regulatory compounds that function downstream of the hormones and testing their effects in living cells. Recent advances in receptor research involving the hormones ethylene (Chang et al., 1993) and auxin (Jones and Prasad, 1992) undoubtedly will also contribute to our understanding of the regulation of cytoskeletal reorganization.

Finally, the power of genetics is beginning to show exciting potential for dissecting the process of cellulose synthesis. It is now clear that mutants impaired in various aspects of cellulose deposition can be selected by a variety of techniques. Clearly, the mutant approach is beginning to bear fruit in studies of β -glucan synthesis in fungi (Diaz et al., 1993; Cameron et al., 1994; Enderlin and Seltrennikoff, 1994; Roemer et al., 1994). The key to success in plants undoubtedly lies in the use of clever selection and screening techniques. Even in *A. xylinum*, many genes are involved in the process—not only those encoding synthase subunits, but also those whose products control synthesis and degradation of regulatory molecules

such as c-di-GMP (Ross et al., 1991). In plants, with the additional factors of cytoskeletal involvement as well as developmental regulation, one can expect that even more genes will be involved. Looking to the very long term, once critical genes are identified, the possibility exists for genetic modification of plants in ways that could alter the structure or crystallinity of cellulose produced and/or the timing and extent of its deposition in specific tissues of the plant.

REFERENCES

- Aloni, Y., Delmer, D.P., and Benziman, M. (1982). Achievement of high rates of *in vitro* synthesis of 1,4- β -D-glucan: Activation by cooperative interaction of the *Acetobacter xylinum* enzyme system with GTP, polyethylene glycol and a protein factor. *Proc. Natl. Acad. Sci. USA* **79**, 6448–6452.
- Amikam, D., and Benziman, M. (1989). Cyclic diguanylic acid and cellulose synthesis in *Agrobacterium tumefaciens*. *J. Bacteriol.* **177**, 6649–6655.
- Amor, Y., Mayer, R., Benziman, M., and Delmer, D. (1991). Evidence for a cyclic diguanylic acid-dependent cellulose synthase in plants. *Plant Cell* **3**, 989–995.
- Amor, Y., Haigler, C.H., Johnson, S., Wainwright, M., and Delmer, D.P. (1995). A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. *Proc. Natl. Acad. Sci. USA*, in press.
- Andrews, A., Solomon, M., and Delmer, D.P. (1993). Cotton fiber annexins: A potential role in the regulation of callose synthase. *Plant J.* **3**, 763–772.
- Atalla, R.H., and VanderHart, D.L. (1989). Studies on the structure of cellulose using raman spectroscopy and solid state ^{13}NMR . In *Cellulose and Wood: Chemistry and Technology*, C. Schuerch, ed (New York: Wiley Interscience), pp. 169–188.
- Baskin, T.I., Berzner, A.A., Hoggart, R., Cork, A., and Williamson, R.E. (1992). Root morphology mutants in *Arabidopsis thaliana*. *Aust. J. Plant Physiol.* **19**, 427–437.
- Basra, A.S., and Malik, C.P. (1984). Development of the cotton fiber. *Int. Rev. Cytol.* **89**, 65–113.
- Berendes, R., Burger, A., Voges, D., Demange, P., and Huber, R. (1993). Calcium influx through annexin V ion channels into large unilamellar vesicles measured with fura-2. *FEBS Lett.* **317**, 131–134.
- Betzner, A., Liangcai, P., Baskin, T., Herth, W., Cork, A., Birch, R., Rolfe, B., Redmond, J., and Williamson, R. (1995). Radial swelling mutants deficient in cellulose biosynthesis. *J. Cell. Biochem.* **21A** (suppl.), 440 (abstr. J5-013).
- Blanton, R.L. (1993). Prestalk cells in monolayer cultures exhibit two distinct modes of cellulose synthesis during stalk cell differentiation in *Dictyostelium*. *Development* **119**, 703–710.
- Blanton, R.L., and Haigler, C.H. (1995). Cellulose biosynthesis. In *Membranes: Specialized Functions in Plants*, M. Smallwood, P. Knox, and D. Bowles, eds (Greenwich, CT: JAI Press), in press.
- Blanton, R.L., and Northcote, D.H. (1990). A 1,4- β -D-glucan synthase system from *Dictyostelium discoideum*. *Planta* **180**, 324–332.
- Bolwell, G.P. (1993). Dynamic aspects of the plant extracellular matrix. *Int. Rev. Cytol.* **146**, 261–324.

- Bowler, C., and Chua, N.-H.** (1994). Emerging themes in plant signal transduction. *Plant Cell* **6**, 1529–1541.
- Bulone, V., Girard, V., and Fevre, M.** (1990). Separation and partial purification of 1,3- β -glucan and 1,4- β -glucan synthases from *Saprolegnia*. *Plant Physiol.* **94**, 1748–1755.
- Cameron, M., Forrest, F., Marrinam, J.M., Morin, N., Nilsen, J.B., Dahl, A.M., Mazur, P., Baginsky, K.W., Li, W., El Sherbeini, M., Clemas, J.A., Mandala, S.N., Frommer, B.A., and Kurtz, N.B.** (1994). *Saccharomyces cerevisiae* FKS1 (ETG1) gene encodes an integral membrane protein which is a subunit of 1,3- β -glucan synthase. *Proc. Natl. Acad. Sci. USA* **91**, 12907–12911.
- Carpita, N.C., and Delmer, D.P.** (1981). Concentration and metabolic turnover of UDP-glucose in developing cotton fibers. *J. Biol. Chem.* **256**, 308–315.
- Chang, K.C., Shing, K.F., Bleecker, A.B., and Meyerowitz, E.M.** (1993). Arabidopsis ethylene-response gene ETR-1: Similarity of products to two-component regulators. *Science* **262**, 539–544.
- Chanzy, H., Imada, K., and Vuong, R.** (1978). Electron diffraction from the primary wall of cotton fibers. *Protoplasma* **94**, 299–306.
- Chourey, P.S., and Nelson, O.E.** (1976). The enzymatic deficiency conditioned by the *shrunk-1* mutations in maize. *Biochem. Genet.* **14**, 1041–1055.
- Chourey, P.S., Chen, Y.C., and Miller, M.E.** (1991). Early cell degeneration in developing endosperm is unique to the *shrunk* mutation in maize. *Maydica* **36**, 141–146.
- Delmer, D.P.** (1983). Biosynthesis of cellulose. *Adv. Carbohydr. Chem.* **41**, 105–153.
- Delmer, D.P.** (1987). Cellulose biosynthesis. *Annu. Rev. Plant Physiol.* **38**, 259–290.
- Delmer, D.P.** (1990). The biochemistry of cellulose synthesis. In *The Cytoskeletal Basis of Plant Growth and Form*, C. Lloyd, ed (London: Academic Press), pp. 102–107.
- Delmer, D.P., and Stone, B.A.** (1988). Biosynthesis of plant cell walls. In *The Biochemistry of Plants*, Vol. 14, P.K. Stumpf and E.E. Conn, eds (San Diego: Academic Press), pp. 373–420.
- Delmer, D.P., Read, S.M., and Cooper, G.** (1987). Identification of a receptor protein in cotton fibers for the herbicide 2,6-dichlorobenzonitrile. *Plant Physiol.* **84**, 415–420.
- Delmer, D.P., Solomon, M., and Read, S.M.** (1991). Direct photolabeling with [³²P]UDP-glucose for identification of a subunit of cotton fiber callose synthase. *Plant Physiol.* **95**, 556–563.
- Delmer, D.P., Ohana, P., Gonen, L., and Benziman, M.** (1993a). In vitro synthesis of cellulose in plants: Still a long way to go. *Plant Physiol.* **103**, 307–308.
- Delmer, D.P., Volokita, M., Solomon, M., Fritz, U., Delphendahl, W., and Herth, W.** (1993b). A monoclonal antibody recognizes a 65 kDa higher plant membrane polypeptide which undergoes cation-dependent association with callose synthase in vitro and co-localizes with sites of high callose deposition in vivo. *Protoplasma* **176**, 33–42.
- Delmer, D.P., Pear, J., Andrawis, A., and Stalker, D.** (1995). Genes for small GTP-binding proteins analogous to mammalian Rac are highly expressed in developing cotton fibers. *Mol. Gen. Genet.*, in press.
- Dhugga, K.S., and Ray, P.M.** (1994). Purification of 1,3- β -glucan synthase activity from pea tissue: Two polypeptides of 55 and 70 kDa copurify with enzyme activity. *Eur. J. Biochem.* **220**, 943–953.
- Diaz, M., Sanchez, Y., Bennet, T., Sun, C.R., Godoy, C., Tamanoi, F., Duran, A., and Perez, P.** (1993). The *Schizosaccharomyces pombe* *cwg2+* gene codes for the β -subunit of a geranylgeranyltransferase type I required for β -glucan synthesis. *EMBO J.* **12**, 5245–5254.
- Downard, J.** (1992). Rac and rho in tune. *Nature* **359**, 273–274.
- Durso, N.A., and Cyr, R.J.** (1994). A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1 α . *Plant Cell* **6**, 893–905.
- Enderlin, C.S., and Selitrennikoff, C.P.** (1994). Cloning and characterization of a *Neurospora crassa* gene required for (1,3) β -glucan synthase activity and cell wall formation. *Proc. Natl. Acad. Sci. USA* **91**, 9500–9504.
- Fevre, M., and Rougier, M.** (1981). β -1,3 and β -1,4-glucan synthesis by membrane fractions from the fungus *Saprolegnia monoica*. *J. Gen. Microbiol.* **103**, 297–306.
- Fields, S., and Sternglanz, R.** (1994). The two-hybrid system: An assay for protein-protein interactions. *Trends Genet.* **10**, 286–292.
- Fink, J., Jeblick, W., and Kauss, H.** (1990). Partial purification and immunological characterization of 1,3- β -glucan synthase from suspension cells of *Glycine max*. *Planta* **181**, 343–348.
- Fredrikson, K., Kjellbom, P., and Larsson, C.** (1991). Isolation and polypeptide composition of 1,3- β -glucan synthase from plasma membranes of *Brassica oleracea*. *Physiol. Plant.* **81**, 289–294.
- French, A.D., Bertoniere, N.R., Battista, O.A., Cucolo, J., and Gray, D.G.** (1993). Cellulose. In *Encyclopedia of Chemical Technology*, 4th ed., Vol. 5, K. Othmer, ed (New York: John Wiley and Sons), pp. 476–496.
- Frost, D.J., Read, S.M., Drake, R.R., Haley, B.E., and Wasserman, B.P.** (1990). Identification of the UDP-glucose binding polypeptide of callose synthase from *Beta vulgaris* L. by photoaffinity labeling with 5-azido-UDP-glucose. *J. Biol. Chem.* **265**, 2162–2167.
- Fukuda, H.** (1991). Tracheary element formation as a model system of cell differentiation. *Int. Rev. Cytol.* **136**, 289–332.
- Geigenberger, P., and Stitt, M.** (1993). Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. *Planta* **189**, 329–339.
- Geigenberger, P., Langenberger, S., Wilke, I., Heinike, D., Heldt, H.W., and Stitt, M.** (1993). Sucrose is metabolized by sucrose synthase and glycolysis within the phloem complex of *Ricinus communis* L. seedlings. *Planta* **190**, 446–453.
- Giddings, T.H., and Staehelin, L.A.** (1990). Microtubule-mediated control of microfibril deposition: A re-examination of the hypothesis. In *The Cytoskeletal Basis of Plant Growth and Development*, C.W. Lloyd, ed (London: Academic Press), pp. 85–99.
- Giddings, T.H., Brower, D.L., and Staehelin, L.A.** (1980). Visualization of particle complexes in the plasma membrane of *Micrasterias denticulata* associated with the formation of cellulose fibrils in primary and secondary cell walls. *J. Cell Biol.* **84**, 327–339.
- Girard, V., Fevre, M., Mayer, R., and Benziman, M.** (1991). Cyclic diguanylic acid stimulates 1,4- β -glucan synthase from *Saprolegnia*. *FEMS Microbiol. Lett.* **82**, 293–296.
- Girard, V., Bulone, V., and Fevre, M.** (1992). Separation and partial peptide characterization of β -1,3-glucan synthase from *Saprolegnia*. *Plant Sci.* **82**, 145–153.
- Green, P.B., and Selker, J.M.L.** (1991). Mutual alignments of cell walls, cellulose and cytoskeletons: Their role in meristems. In *The Cytoskeletal Basis of Plant Growth and Development*, C.W. Lloyd, ed (London: Academic Press), pp. 303–322.

- Haigler, C.H.** (1985). The functions and biogenesis of native cellulose. In *Cellulose Chemistry and Its Applications*, T.P. Nevell and S.H. Zeronian, eds (Chichester, UK: Ellis Horwood), pp. 30–83.
- Haigler, C.H.** (1991). Relationship between polymerization and crystallization in microfibril biogenesis. In *Biosynthesis and Biodegradation of Cellulose*, C.H. Haigler and P.J. Weimer, eds (New York: Marcel Dekker), pp. 99–124.
- Hayashi, T.** (1989). Xyloglucans in the primary cell wall. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 139–168.
- Hayashi, T., Read, S.M., Bussell, J., Thelen, M., Lin, F.C., Brown, R.M., and Delmer, D.P.** (1987). UDP-glucose: (1,3)- β -Glucan synthases from mung bean and cotton: Differential effects of Ca^{2+} and Mg^{2+} on enzyme properties and on macromolecular structure of the glucan product. *Plant Physiol.* **83**, 1054–1062.
- Heim, D.R., Roberts, J.L., Pike, P.D., and Larrinua, I.M.** (1989). Mutation of a locus of *Arabidopsis thaliana* confers resistance to the herbicide isoxaben. *Plant Physiol.* **90**, 146–150.
- Heim, D.R., Roberts, J.L., Pike, P.D., and Larrinua, I.M.** (1990a). A second locus of *Arabidopsis thaliana* that confers resistance to the herbicide isoxaben. *Plant Physiol.* **92**, 858–861.
- Heim, D.R., Skomp, J.R., Tschabold, E.E., and Larrinua, I.M.** (1990b). Isoxaben inhibits the synthesis of acid insoluble cell wall materials in *Arabidopsis thaliana*. *Plant Physiol.* **93**, 695–700.
- Herth, W.** (1985). Plasma membrane rosettes involved in localized wall thickening during xylem vessel formation of *Lepidium sativum*. *Planta* **164**, 12–21.
- Herth, W., and Weber, G.** (1984). Occurrence of the putative cellulose-synthesizing "rosettes" in the plasma membrane of *Glycine max* suspension culture cells. *Naturwissenschaften* **71**, 153–154.
- Hotchkiss, A.** (1989). Cellulose biosynthesis: The terminal complex hypothesis and its relationship to other contemporary research topics. In *Plant Cell Wall Polymers: Biogenesis and Biodegradation*, N.G. Lewis and M.G. Paice, eds (Washington, DC: American Chemical Society), pp. 232–247.
- Huber, R., Romisch, J., and Paques, E.P.** (1990). The crystal and molecular structure of human annexin V, an anticoagulant protein that binds to calcium and membranes. *EMBO J.* **9**, 3867–3874.
- Iyama, K., Lam, T.B.T., Meikle, P.J., Ng, K., Rhodes, D.I., and Stone, B.A.** (1993). Cell wall biosynthesis and its regulation. In *Forage Cell Wall Structure and Digestibility*, H.G. Jung, D.R. Buxton, R.D. Hatfield, and J. Ralph, eds (Madison, WI: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America), pp. 621–683.
- Jacob, S.R., and Northcote, D.H.** (1985). In vitro glucan synthesis by membranes of celery petioles: The role of the membrane in determining the type of linkage formed. *J. Cell Sci.* **2** (suppl.), 1–11.
- Jones, A.M., and Prasad, P.V.** (1992). Auxin-binding proteins and their possible roles in auxin-mediated plant cell growth. *Bioessays* **14**, 43–48.
- Kauss, H.** (1991). The role of the plasma membrane in host-pathogen interactions. In *The Plant Plasma Membrane*, C. Larsson and I.M. Moller, eds (Berlin: Springer-Verlag), pp. 320–350.
- Kleczkowski, L.A.** (1994). Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants. *Phytochemistry* **37**, 1507–1515.
- Kobayashi, H., Fukuda, H., and Shibaoka, H.** (1987). Reorganization of actin filaments associated with the differentiation of tracheary elements in *Zinnia mesophyll* cells. *Protoplasma* **138**, 69–71.
- Kobayashi, H., Fukuda, H., and Shibaoka, H.** (1988). Interrelation between the spatial disposition of actin filaments and microtubules during the differentiation of tracheary elements in cultured *Zinnia* cells. *Protoplasma* **143**, 29–37.
- Kokubo, A., Sakurai, N., Kuraishi, S., and Takeda, K.** (1991). Culm brittleness of barley (*Hordeum vulgare* L.) mutants is caused by smaller number of cellulose molecules in cell wall. *Plant Physiol.* **97**, 509–514.
- Kudlicka, K., Brown, R.M., Jr., Li, L., Lee, J.H., Shen, H., and Kuga, S.** (1995). β -Glucan synthesis in the cotton fiber. IV. In vitro assembly of the cellulose I allomorph. *Plant Physiol.* **107**, 111–123.
- Kuga, S., and Brown, R.M., Jr.** (1991). Physical structure of cellulose microfibrils: Implications for biogenesis. In *Biosynthesis and Biodegradation of Cellulose*, C.H. Haigler and P.J. Weimer, eds (New York: Marcel Dekker), pp. 125–142.
- Lee, J.H., Brown, R.M., Jr., Kuga, S., Shoda, S., and Kobayashi, S.** (1994). Assembly of synthetic cellulose I. *Proc. Natl. Acad. Sci. USA* **91**, 7425–7429.
- Li, L., and Brown, R.M., Jr.** (1993). β -Glucan synthesis in the cotton fibers. II. Regulation and kinetic properties of beta-glucan synthases. *Plant Physiol.* **101**, 1143–1148.
- Li, L., Drake, R.J., Clement, S., and Brown, R.M., Jr.** (1993). β -Glucan synthesis in the cotton fibers. III. Identification of UDP-glucose-binding subunits of beta-glucan synthases by photoaffinity labeling with [β - ^{32}P]5'-N 3 -UDP-glucose. *Plant Physiol.* **101**, 1149–1156.
- Lin, F.C., and Brown, R.M., Jr.** (1989). Purification of cellulose synthase from *Acetobacter xylinum*. In *Cellulose and Wood—Chemistry and Technology*, C. Scheurch, ed (New York: Wiley Interscience), pp. 473–492.
- Lin, F.C., Brown, R.M., Jr., Drake, R.R., Jr., and Haley, B.E.** (1990). Identification of the uridine-5'-diphosphoglucose (UDP-Glc) binding subunit of cellulose synthase in *Acetobacter xylinum* using the photoaffinity probe 5-azido-UDP-Glc. *J. Biol. Chem.* **265**, 4782–4784.
- Maas, C., Schall, S., and Werr, W.** (1990). A feedback control element near the transcription start site of the maize *shrunk1-1* gene determines promoter activity. *EMBO J.* **9**, 3447–3452.
- Maltby, D., Carpita, N.C., Montezinos, D., Kulow, C., and Delmer, D.P.** (1979). β -1,3-Glucan in developing cotton fibers: Structure, localization, and relationship of synthesis to that of secondary wall cellulose. *Plant Physiol.* **63**, 1158–1164.
- Martin, C., and Smith, A.M.** (1995). Starch biosynthesis. *Plant Cell* **7**, 971–985.
- Martin, T., Frommer, W.B., Salanoubat, M., and Willmitzer, L.** (1993). Expression of an Arabidopsis sucrose synthase gene indicates a role in metabolization of sucrose both during phloem loading and in sink organs. *Plant J.* **4**, 367–378.
- Matthysse, A.G., Thomas, D.O.L., and White, A.R.** (1995a). Mechanism of cellulose synthesis in *Agrobacterium tumefaciens*. *J. Bacteriol.* **177**, 1076–1081.
- Matthysse, A.G., White, S., and Lightfoot, R.** (1995b). Genes required for cellulose synthesis in *Agrobacterium tumefaciens*. *J. Bacteriol.* **177**, 1069–1075.
- Mayer, R., Ross, P., Weinhouse, H., Amikam, D., Volman, G., Ohana, P., Calhoun, R.D., Wong, H.C., Emerick, A.W., and Benziman, M.** (1991). Polypeptide composition of bacterial cyclic diguanylic acid-dependent cellulose synthase and the occurrence of immunologically crossreacting proteins in higher plants. *Proc. Natl. Acad. Sci. USA* **88**, 5472–5476.

- Meikle, P.J., Ng, K.F., Johnson, E., Hoogenraad, J.J., and Stone, B.A. (1991). The β -glucan synthase from *Lolium multiflorum*: Detergent solubilization, purification using monoclonal antibodies, and photoaffinity labeling with a novel photoreactive pyrimidine analog of uridine 5'-diphosphoglucose. *J. Biol. Chem.* **266**, 22569–22581.
- Mizuta, S., and Brown, R.M., Jr. (1992). Effects of 2,6-dichlorobenzonitrile and Tinopal LPW on the structure of the cellulose synthesizing complexes of *Vaucheria hamata*. *Protoplasma* **166**, 200–207.
- Mol, P.C., Park, H.-M., Mullins, J.T., and Cabib, E. (1994). A GTP-binding protein regulates the activity of (1 \rightarrow 3)- β -glucan synthase, an enzyme directly involved in yeast cell wall morphogenesis. *J. Biol. Chem.* **269**, 31267–31274.
- Montezinos, D., and Brown, R.M., Jr. (1976). Cellulose microfibrils: Visualization of biosynthetic and orienting complexes in association with the plasma membrane. *Proc. Natl. Acad. Sci. USA* **84**, 6985–6989.
- Morrow, D.L., and Lucas, W.J. (1986). (1-3)- β -Glucan synthase from sugar beet. I. Isolation and solubilization. *Plant Physiol.* **81**, 171–176.
- Nolte, K.D., and Koch, D.E. (1993). Companion-cell specific localization of sucrose synthase in zones of phloem loading and unloading. *Plant Physiol.* **101**, 899–905.
- Ohana, P., Benziman, M., and Delmer, D.P. (1993). Stimulation of callose synthesis *in vivo* correlates with changes in intracellular distribution of the callose synthase activator β -furfuryl- β -glucoside. *Plant Physiol.* **101**, 187–191.
- Okuda, K., Li, L., Kudlicka, K., Kuga, S., and Brown, R.M., Jr. (1993). β -Glucan synthesis in the cotton fiber. I. Identification of β -1,4- and β -1,3-glucans synthesized *in vitro*. *Plant Physiol.* **101**, 1131–1142.
- Okuda, K., Tsekos, I., and Brown, R.M., Jr. (1994). Cellulose microfibril assembly in *Erythrocladia subintegra* Rosenv.: An ideal system for understanding the relationship between synthesizing complexes (TCs) and microfibril crystallization. *Protoplasma* **180**, 49–58.
- Omura, S., Tanaka, Y., Kanaya, I., Shinose, M., and Takahashi, Y. (1990). Phthoxazolin, a specific inhibitor of cellulose biosynthesis, produced by a strain of *Streptomyces* sp. *J. Antibiot.* **43**, 1034–1036.
- Potikha, T., and Delmer, D.P. (1995). A mutant of *Arabidopsis thaliana* displays altered patterns of cellulose deposition. *Plant J.* **7**, 453–460.
- Preston, R.D. (1964). Structural and mechanical aspects of plant cell walls with particular evidence to synthesis and growth. In *The Formation of Wood in Forest Trees*, M.H. Zimmermann, ed (New York: Academic Press), pp. 169–201.
- Qi, X., Tai, C.-Y., and Wasserman, B.P. (1995). Plasma membrane intrinsic proteins of *Beta vulgaris* L. *Plant Physiol.* **108**, 387–392.
- Reis, D., Vian, B., and Roland, J.-C. (1994). Cellulose-glucuronoxylans and plant cell wall structure. *Micron* **25**, 171–187.
- Roemer, T., Paravicini, G., Payton, M.A., and Bussey, H. (1994). Characterization of the yeast (1-6)- β -glucan biosynthetic components, Kre6p and Skn1p, and genetic interactions between the PKC1 pathway and extracellular matrix assembly. *J. Cell Biol.* **127**, 567–579.
- Ross, P., Weinhouse, H., Aloni, Y., Michaeli, D., Ohana, P., Mayer, R., Braun, S., deVroom, E., van der Marel, G.A., van Boom, J.H., and Benziman, M. (1987). Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature* **325**, 279–281.
- Ross, P., Mayer, R., and Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* **55**, 35–58.
- Ryser, U. (1985). Cell wall biosynthesis in differentiating cotton fibres. *Eur. J. Cell Biol.* **39**, 236–265.
- Salvucci, M.E., and Klein, R.R. (1993). Identification of the uridine-binding domain of sucrose-phosphate synthase. *Plant Physiol.* **102**, 529–536.
- Saxena, I.M., Lin, F.C., and Brown, R.M., Jr. (1990). Cloning and sequencing of the cellulose synthase catalytic subunit gene of *Acetobacter xylinum*. *Plant Mol. Biol.* **15**, 673–683.
- Saxena, I.M., Lin, F.C., and Brown, R.M., Jr. (1991). Identification of a new gene in an operon for cellulose biosynthesis in *Acetobacter xylinum*. *Plant Mol. Biol.* **16**, 947–954.
- Saxena, I.M., Kudlicka, K., Okuda, K., and Brown, R.M., Jr. (1994). Characterization of genes in the cellulose-synthesizing operon (acs Operon) of *Acetobacter xylinum*: Implication for cellulose crystallization. *J. Bacteriol.* **176**, 5735–5752.
- Saxena, I.M., Brown, R.M., Jr., Fevre, M., Geremia, R.A., and Henrissat, B. (1995). Multidomain architecture of β -glycosyl transferases: Implications for mechanism of action. *J. Bacteriol.* **177**, 1419–1424.
- Schlupmann, H., Bacic, A., and Read, S.M. (1993). A novel callose synthase from pollen tubes of *Nicotiana*. *Planta* **191**, 470–481.
- Schlupmann, H., Bacic, A., and Read, S.M. (1994). Uridine diphosphate glucose metabolism and callose synthesis in cultured pollen tubes of *Nicotiana glauca* Link et Otto. *Plant Physiol.* **105**, 659–670.
- Seagull, R.W. (1990). The effects of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibers. *Protoplasma* **159**, 44–59.
- Shedletzky, E., Shmuel, M., Trainin, T., Kalman, S., and Delmer, D.P. (1992). Cell wall structure in cells adapted to growth on the cellulose-synthesis inhibitor 2,6-dichlorobenzonitrile: A comparison between two dicotyledonous plants and a graminaceous monocot. *Plant Physiol.* **100**, 120–130.
- Shibaoka, H. (1994). Plant hormone-induced changes in the orientation of cortical microtubules: Alterations in the cross-linking between microtubules and the plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 527–544.
- Sofia, H.J., Burland, V., Daniels, D.L., Plunkett, G., III, and Blattner, F.R. (1994). Analysis of the *Escherichia coli* genome. V. DNA sequence of the region from 76.0 to 81.5 min. *Nucleic Acids Res.* **22**, 2576–2586.
- Stone, B.A., and Clarke, A.E. (1992). *The Biology of (1-3)- β -Glucans*. (La Trobe, Australia: La Trobe University Press).
- Stone, J.M., Collinge, M.A., Smith, R.D., Horn, M.A., and Walker, J.C. (1994). Interaction of a protein phosphatase with an Arabidopsis serine-threonine receptor kinase. *Science* **266**, 793–795.
- Sugiyama, J., Persson, J., and Chanzy, H. (1991). Combined infrared and electron diffraction study of the polymorphism of native celluloses. *Macromolecules* **24**, 2461–2466.
- Trewavas, A., and Gilroy, S. (1991). Signal transduction in plant cells. *Trends Genet.* **7**, 356–361.
- Valla, S., Coucheron, D.H., Fjaervik, E., Kjosbakken, J., Weinhouse, H., Ross, P., Amidam, D., and Benziman, M. (1989). Cloning of a gene involved in cellulose biosynthesis in *Acetobacter xylinum*: Complementation of cellulose-negative mutants by the UDPG pyrophosphorylase structural gene. *Mol. Gen. Genet.* **217**, 26–30.
- Wong, H.C., Fear, A.L., Calhoun, R.D., Eichinger, G.H., Mayer, R., Amikam, D., Benziman, M., Gelfand, D.H., Meade, J.H., Emerick, A.W., Bruner, R., Ben-Basat, B.A., and Tai, R. (1990). Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. *Proc. Natl. Acad. Sci. USA* **87**, 8130–8134.

Wu, A., and Wasserman, B.P. (1993). Limited proteolysis of (1,3)- β -glucan (callose) synthase from *Beta vulgaris* L.: Topology of protease-sensitive sites and polypeptides identification using Pronase E. *Plant J.* **4**, 683–695.

Yuan, M., Shaw, P.J., Warn, R.M., and Lloyd, C.W. (1994). Dynamic reorientation of cortical microtubules, from transverse to longitudinal, in living plant cells. *Proc. Natl. Acad. Sci. USA* **91**, 6050–6053.

Zaar, K. (1979). Visualization of pores (export sites) correlated with cellulose production in the envelope of the gram-negative bacterium *Acetobacter xylinum*. *J. Cell Biol.* **80**, 773–777.

Zhu, J.-K., Damsz, B., Kononowicz, A.K., Bressan, R.A., and Hasegawa, P.M. (1994). A higher plant extracellular vitronectin-like adhesion protein is related to the translational elongation factor-1 α . *Plant Cell* **6**, 393–404.