CHAPTER 6

Biochemistry and Molecular Biology of Cellulose Biosynthesis in Plants: Prospects for Genetic Engineering

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Contents

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Advances in Plant Biochemistry and Molecular Biology, Volume 1
ISSN 1755-0408, DOI: 10.1016/S1755-0408(07)01006-5

Abstract Cellulose is a major component of the plant cell wall, and understanding the mechanism of synthesis of this polysaccharide is a major challenge for plant biologists. Cellulose microfibrils are synthesized and assembled by membrane-localized protein complexes that are visualized as rosettes by freeze-fracture electron microscopy. Cellulose synthase is required for cellulose synthesis. So far only this enzyme has been localized to these cellulose-synthesizing complexes. Although it has not been possible to purify and fully characterize cellulose synthase activity from plants, it has been possible to obtain cellulose synthesis *in vitro* using membranes and detergent-solubilized membrane fractions. Cellulose synthase uses uridine 5′-diphosphate (UDP)-glucose as a substrate and polymerizes glucose residues into long β-1,4-linked glucan chains in a single-step reaction. Cellulose synthases are encoded by genes belonging to a superfamily, and each plant synthesizes a number of different cellulose synthases. Genetic analysis suggests that each cellulose-synthesizing complex contains at least three nonredundant cellulose synthases and mutation in any one of these cellulose synthases results in cellulose deficiency. More interestingly, different cellulose synthases perform cellulose synthesis in the primary cell wall and the secondary cell wall. Apart from the cellulose synthases, a number of other proteins have been suggested to play a role in cellulose synthesis, but so far their functions are not clearly understood. Genetic manipulation of cellulose synthesis in plants will therefore require not only a complete understanding of the different cellulose synthases but also other proteins that regulate the temporal and spatial synthesis and assembly of this very important polysaccharide.

Key Words: Cellulose, Cellulose biosynthesis, Cellulose synthase, Cellulose synthase-like, CesA, Csl, *Arabidopsis*, Cotton, *Acetobacter xylinum*, Genetic manipulations.

1. INTRODUCTION

Cellulose is an abundant biopolymer that is synthesized by all plants, most algae, a number of bacteria including cyanobacteria, the cellular slime mold, and the ascidians (a group of animals) (Brown, 1996). The major proportion of cellulose, produced in the biosphere by plants, adds strength to the plant cell wall and helps in determining the direction of cell and plant growth. The plant cell wall itself is a complex of polysaccharides, which include cellulose and noncellulosic polysaccharides (hemicelluloses and pectins), as well as lignins and proteins. All plant cells have a primary cell wall consisting of cellulose, hemicellulose, pectin, and proteins; however, some cells additionally have a secondary cell wall consisting mainly of cellulose and lignins, and it is in these cells that the proportion of

cellulose is increased considerably. The importance of cellulose as an essential component of plants and its uses in our daily lives cannot be overemphasized. Interestingly, cellulose also is the most important industrial polysaccharide, and considering its unique physical properties, it has been studied widely by chemists since its initial discovery by Anselme Payen almost 165 years ago (Klemm *et al.*, 2005).

Studies on the structure of cellulose have been crucial in developing concepts regarding the sites of cellulose synthesis and the mechanism by which it is synthesized (Preston, 1974). Although much more is known about the structure of cellulose (and these studies are still continuing) (Nishiyama *et al.*, 2003), the last decade and a half has witnessed a surge in our understanding of the biosynthesis of cellulose in plants. Many of these advances are related to the identification of genes for cellulose biosynthesis in plants (Arioli *et al.*, 1998; Pear *et al.*, 1996), analysis of mutants affected in cellulose biosynthesis (Robert *et al.*, 2004), the capability to analyze cellulose synthesis *in vitro* using cell-free extracts (Kudlicka and Brown, 1997; Lai-Kee-Him *et al.*, 2002), and visualization of enzymes involved in cellulose synthesis in living plant cells (Paredez *et al.*, 2006; Robert *et al.*, 2005). In this chapter, we will discuss the development of present-day concepts related to cellulose biosynthesis and the prospects of modifying this property in plants.

2. THE MANY FORMS OF CELLULOSE—A BRIEF INTRODUCTION TO THE STRUCTURE AND DIFFERENT CRYSTALLINE FORMS OF CELLULOSE

Unlike most known biopolymers, cellulose is a simple molecule that consists of an assembly of β-1,4-linked glucan chains. As a result, cellulose is defined less by its primary structure (β-1,4-linked glucose residues with cellobiose being the repeating unit in all chains) and more by its secondary and higher-order structure in which the chains interact via intramolecular and intermolecular hydrogen bonds, as well as van der Waals interactions, to give rise to different forms of cellulose (Fig. 6.1) (O'Sullivan, 1997). Cellulose exhibits polymorphism, and the different forms of cellulose are usually defined by their crystalline forms, although reference is also made to other forms of cellulose such as noncrystalline cellulose, amorphous cellulose, and more recently nematic-ordered cellulose (Kondo *et al.*, 2001). Whereas, the glucan chains are arranged in a specific manner with respect to each other in crystalline cellulose, no specific arrangement of the glucan chains occur in noncrystalline or amorphous cellulose. In contrast, nematic-ordered cellulose is highly ordered but not crystalline and is obtained by uniaxial stretching of water-swollen cellulose (Kondo *et al.*, 2004).

In general, cellulose produced by living organisms occurs as cellulose I and is assembled in a structure referred to as a microfibril (Fig. 6.2). The properties of the microfibril are determined by its size, shape, and crystallinity. The glucan chains in cellulose I are arranged in a parallel manner, and depending upon the arrangement of these chains, two crystalline forms of cellulose I—I_α and I_β—have been

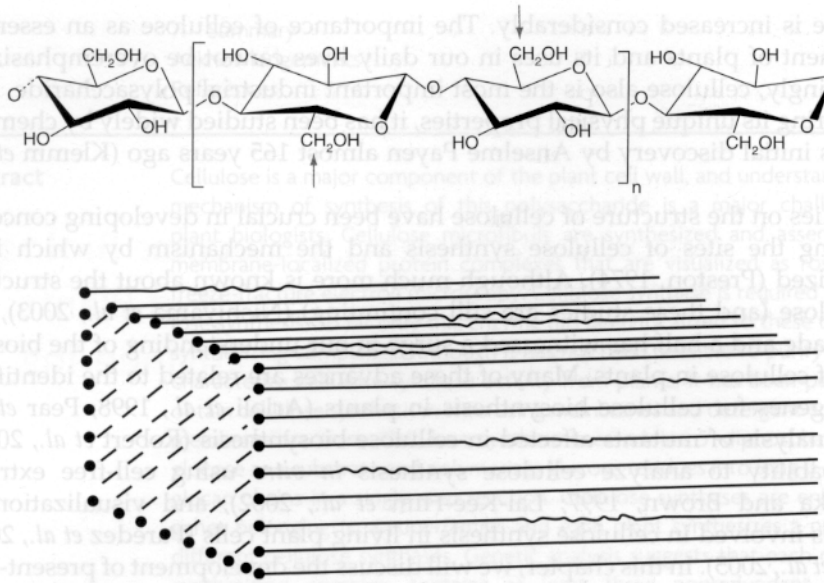

FIGURE 6.1 Top image is the structural formula for the β-1,4-linked glucan chain of cellulose. The bracketed region indicates the basic repeat unit, cellobiose, in the chain. The glucan chain has a twofold symmetry. The bottom image is a schematic representation of a crystalline cellulose I microfibril. (Reproduced from Brown, Jr. R. M., *J. Poly. Sci. Part A Poly. Chem.* **42,** 489–495.) (See Page 5 in Color Section.)

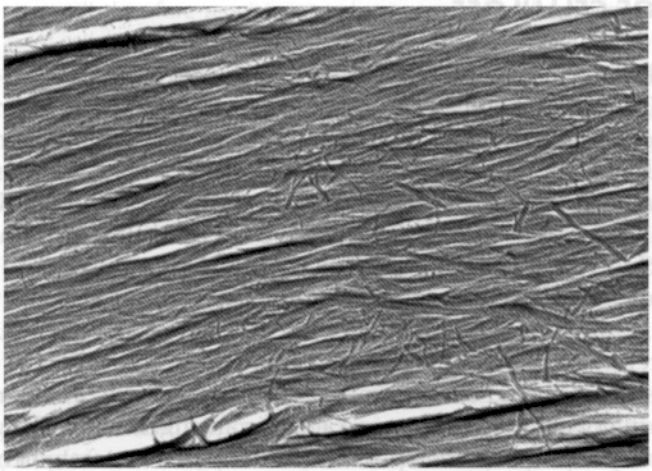

FIGURE 6.2 Freeze fracture image of cellulose microfibrils in the secondary wall of a developing cotton fiber. (*Unpublished image from R. Malcolm Brown, Jr. and Kazuo Okuda.*)

identified (Attala and Vanderhart, 1984). The more thermodynamically stable form of cellulose is cellulose II, and in this allomorph the glucan chains are arranged in an antiparallel manner. Cellulose II is produced in nature by certain organisms or under specific conditions but is generally obtained by an irreversible

process upon chemical treatment (mercerization or solubilization) of native cellulose I. Furthermore, cellulose III_I and cellulose III_{II} are obtained from cellulose I and cellulose II, respectively, in a reversible process, by treatment with liquid ammonia or some amines and the subsequent evaporation of excess ammonia, and cellulose IV_I and cellulose IV_{II} are obtained irreversibly by heating cellulose III_I and cellulose III_{II} respectively to 206 °C in glycerol (O'Sullivan, 1997). Implicit in the biosynthesis of cellulose is the role of the cellulose-synthesizing machinery that allows synthesis and organization of a metastable form of cellulose (cellulose I) that is found to be desirable in living organisms in comparison to the more stable cellulose II product. Whereas the assembly of the glucan chains (crystallization) endows cellulose with its characteristic properties, it is the synthesis of these β-1,4-linked glucan chains (polymerization) that is the focus of research for most biologists.

3. BIOCHEMISTRY OF CELLULOSE BIOSYNTHESIS IN PLANTS

3.1. UDP-glucose is the immediate precursor for cellulose synthesis

Although cellulose was characterized as an aggregation of glucose units by Anselme Payen in 1839, it was in 1895 that Tollens proposed that cellulose is a chain of glucose molecules (French, 2000). While the structure of cellulose was being determined and debated, studies on its biosynthesis did not truly begin until the identification of nucleotide sugars, and specifically UDP-glucose as a glucose donor in biosynthetic reactions (Leloir and Cabib, 1953). The transfer of glucose from UDP-glucose to cellulose was first described by Glaser in 1958 using particulate fraction from cell-free extracts of the bacterium *Acetobacter xylinum* (Glaser, 1958). However, when UDP-glucose was used as the sugar donor in experiments using digitonin-solubilized fractions from various plants, the polysaccharide product obtained *in vitro* was identified as callose (β-1,3-glucan) instead of cellulose (Feingold *et al.*, 1958). Using particulate extracts from plants, the synthesis of cellulose was reported by Barber and colleagues in 1964, and from their experiments these authors concluded that the sugar donor for synthesis of cellulose was guanosine 5′-diphosphate (GDP)-glucose and not UDP-glucose (Barber *et al.*, 1964). In these experiments, the particulate extracts from plants also allowed synthesis of an alkali-insoluble polysaccharide from GDP-mannose and from a mixture of GDP-glucose and GDP-mannose. Recently, a cellulose synthase-like protein (AtCslA9), identified as a β-glucomannan synthase, has been shown to possess β-mannan synthase, β-glucan synthase, and β-glucomannan synthase activities (Liepman *et al.*, 2005). This β-glucomannan synthase can catalyze the production of β-mannan when supplied with GDP-mannose, a β-glucan when supplied with GDP-glucose or β-glucomannan when supplied with a combination of GDP-glucose and GDP-mannose. It is now clear that in the earlier experiments where GDP-glucose was used as a sugar donor with plant extracts, techniques for characterizing the *in vitro* products did not allow a clear distinction to be made between the possible β-glucomannan product and cellulose

(Barber *et al.*, 1964; Chambers and Elbein, 1970). Moreover, it was felt at the time that synthesis of the major homopolymers of glucose in plants could be regulated by using different nucleotide sugars—UDP-glucose for callose synthesis, adenosine diphosphate (ADP)-glucose for starch synthesis, and GDP-glucose for cellulose synthesis (Barber *et al.*, 1964). We now know that in plants, although ADP-glucose is the precursor for starch synthesis, the precursor for synthesis of callose and cellulose is UDP-glucose. Support for the role of UDP-glucose as a precursor of cellulose in plants came from studies tracing the flow of carbon from glucose to cellulose in developing cotton fibers (Carpita and Delmer, 1981). Evidence for the role of UDP-glucose as the precursor for cellulose synthesis in plants did not come easily, and only a brief historical account is given here to highlight one of the many difficulties encountered in dissecting the mechanism of cellulose synthesis in plants. A detailed account of the early years and the progress that has been made since then is provided by Delmer in a number of excellent review articles (Delmer, 1983, 1999). Suffice it to say that as late as 1983, in one of her reviews Delmer summarized that "convincing *in vitro* synthesis of cellulose from UDP-glucose using plant extracts has never been conclusively demonstrated" (Delmer, 1983). In plants, UDP-glucose functions as a glucose donor in a number of glucosyl transfer reactions. From genome sequencing, it is now known that plants have the largest number of carbohydrate-modifying enzymes, and consequently UDP-glucose could participate as a glucose donor in many different reactions when unpurified plant extracts are used for *in vitro* cellulose synthesis (Coutinho *et al.*, 2003). Furthermore in plants, polysaccharides, such as xyloglucan, have a backbone similar to cellulose, and it is important to distinguish the synthesis of these polysaccharides from synthesis of cellulose. Although not much has changed since the early days in the manner in which *in vitro* cellulose synthesis reactions were performed, a few modifications in the reaction conditions and better product characterization (described later) has allowed conclusive demonstration of *in vitro* cellulose synthesis from UDP-glucose using extracts from a variety of plants (Colombani *et al.*, 2004; Kudlicka and Brown, 1997; Kudlicka *et al.*, 1995, 1996; Lai-Kee-Him *et al.*, 2002; Okuda *et al.*, 1993; Peng *et al.*, 2002).

3.2. *In vitro* synthesis of cellulose from plant extracts

3.2.1. The β-1,3-glucan synthase and lessons from *in vitro* β-1,3-glucan synthesis

To understand the biochemical machinery required for cellulose synthesis in plants, it is necessary to demonstrate *in vitro* synthesis of cellulose using plant extracts. Unfortunately, much to the dismay of most researchers studying cellulose biosynthesis, the major *in vitro* polysaccharide product synthesized from plant extracts using UDP-glucose as the precursor was and is still found to be callose, the β-1,3-glucan first reported from mung bean extracts by Feingold and colleagues in 1958 (Feingold *et al.*, 1958). Observing the synthesis of this polysaccharide in place of cellulose has been both frustrating and invigorating as it brings up a number of very interesting questions, many of which have not been fully answered.

During normal development, cellulose is found in all plant cells, whereas callose generally is synthesized in response to wounding, physiological stress, or infection, and is a component of the cell plate in dividing cells apart from being present in specialized cells. As such, enzymes for synthesis of this polysaccharide are not expected to be active most of the time. The general explanation to account for the large amount of *in vitro* synthesis of callose as opposed to cellulose using plant extracts is that this occurs in response to the wounding or stress of the cells during cell breakage. Using antibodies against β-1,4-glucan synthase and β-1,3-glucan synthase, Nakashima *et al.* (2003) recently demonstrated that the activation of β-1,3-glucan synthase upon wounding may be dependent on the degradation of β-1,4-glucan synthases by specific proteases (Nakashima *et al.*, 2003). However, under appropriate conditions in the presence of UDP-glucose, plant extracts synthesize both callose and cellulose, and the optimal conditions required for synthesis of these two polysaccharides have been shown to be only slightly different. Whether the same enzyme catalyzes the synthesis of both callose and cellulose has been debated for a number of years, but so far no conclusive evidence is available in support of either the one enzyme-two polysaccharides or the one enzyme-one polysaccharide synthesis with respect to these two polysaccharides. Although it has been possible to separate the major cellulose-synthesizing and callose synthesizing activities by native gel electrophoresis, the polypeptide composition in these two fractions could not be completely analyzed (Kudlicka and Brown, 1997). Interestingly, relatively much more is known about the identity of the catalytic subunit of cellulose synthase as compared to the nature of the catalytic subunit of callose synthase. This is true, in spite of the fact that genes required for synthesis of β-1,3-glucans have been identified in yeast, and similar genes have been identified in a number of plants (Cui *et al.*, 2001; Doblin *et al.*, 2001; Hong *et al.*, 2001; Li *et al.*, 2003). Surprisingly, the proteins encoded by these genes do not show similarity to any known glycosyltransferase, much less the cellulose synthases. These proteins are classified as 1,3-β-D-glucan synthases and have been placed in family 48 of glycosyltransferases (http://afmb.cnrs-mrs.fr/CAZY/). In plants, genes encoding this protein form a gene family, and in *Arabidopsis* 10 members are identified in this gene family.

Since synthesis of β-1,3-glucans occurs much more readily when plant extracts are used *in vitro*, many more studies have reported on characterization of the conditions for β-1,3-glucan synthase activity and its purification from a variety of plants. As an example, optimal conditions for *in vitro* synthesis of β-1,3 glucan from *Arabidopsis* were defined by the presence in the reaction mixture of 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer, pH 6.8, 1 mM UDP-glucose, 8 mM Ca^{2+}, and 20 mM cellobiose (Lai-Kee-Him *et al.*, 2001). Similar conditions, in the presence or absence of Mg^{2+} in the reaction mix, have also been shown to be optimal for the synthesis of cellulose using plant extracts (Colombani *et al.*, 2004). Since both callose synthase and cellulose synthase are membrane proteins, the choice and concentration of detergents used during extraction of the proteins have been found to be very crucial in obtaining high specific activity of both callose synthase and cellulose synthase from plant extracts. Incorporating a variety of techniques, Dhugga and Ray (1994) purified

the β-1,3-glucan synthase activity 5,500-fold from pea homogenates and found two polypeptides that copurified with the enzyme activity (Dhugga and Ray, 1994). Unfortunately, the identity of these proteins could not be determined, although one of these polypeptides was shown to bind to UDP-glucose. In related sets of experiments, Kudlicka and Brown (1997) demonstrated separation of the callose synthase and cellulose synthase activities in digitonin-solubilized mung bean membranes using gel electrophoresis under nondenaturing conditions (Kudlicka and Brown, 1997). The polypeptide composition in the two fractions was analyzed by SDS-PAGE, and while three similar sized polypeptides were observed in both activities, polypeptides unique to each activity were also observed. However, the characterization of these polypeptides did not provide any further information regarding the similarities or differences between the two enzyme activities. As mentioned in this section, many of the studies for *in vitro* synthesis of callose were applicable to *in vitro* synthesis of cellulose using plant extracts. Interestingly, conclusive demonstrations of cellulose synthesis *in vitro* using plant extracts had to do more with utilizing a greater variety of techniques for product characterization than with development of novel assay methods.

3.2.2. Increasing cellulose synthase activity *in vitro* and utilizing more techniques for product characterization

Techniques to identify and characterize the cellulose product have played a crucial role in determining cellulose synthesis *in vitro*. Interestingly, many of the criteria used by Glaser in 1958 for *in vitro* cellulose production using bacterial extracts are still used for characterizing the cellulose product and determining the cellulose synthase activity, namely incorporation of ^{14}C-glucose from UDP-^{14}C-glucose into a hot alkali-insoluble fraction (Glaser, 1958). The product was further characterized by acid hydrolysis and/or enzymatic analysis using cellulases. Although less than 1% of the glucose from UDP-glucose was incorporated into the alkali-insoluble fraction in the *in vitro* reaction, the product was characterized as cellulose.

A major breakthrough in understanding cellulose biosynthesis in *A. xylinum* and increasing cellulose synthase activity in bacterial extracts came with the identification of cyclic di-guanosine monophosphate (c-di-GMP) as an allosteric activator of cellulose synthase (Ross *et al.*, 1986). This nucleotide is now recognized to be a regulator of many more bacterial functions than previously thought (D'Argenio and Miller, 2004). The addition of c-di-GMP in reaction mixtures using bacterial extracts led to a remarkable increase in incorporation of glucose from UDP-glucose into a cellulose product.

In another development, the *in vitro* product using bacterial extracts for the first time was visualized by electron microscopy, and this product was shown to bind to gold-labeled cellobiohydrolase providing evidence that this product is cellulose (Lin *et al.*, 1985). The *in vitro* product obtained using *A. xylinum* inner membrane was furthermore shown to be cellulose II (Bureau and Brown, 1987). The capability to synthesize large amounts of the *in vitro* product was crucial in performing X-ray diffraction, sugar analysis, linkage analysis and molecular weight analysis to demonstrate conclusively that the product was cellulose (Bureau and Brown, 1987).

Many of these techniques were later utilized by Okuda *et al.* (1993) using cotton fiber extracts to demonstrate the *in vitro* production of cellulose II (Okuda *et al.*, 1993). Additionally, the incorporation of glucose from UDP-glucose into an Updegraff reagent-resistant fraction was included to be a stricter criterion for the cellulose product. Although no activator comparable to c-di-GMP was identified for activation of the cellulose synthase from plant tissues, a number of nucleotides were found to increase the *in vitro* cellulose synthase activity (Li and Brown, 1993). Overall, the success in demonstrating cellulose synthesis *in vitro* is ascribed to the choice of plant tissue (cotton fibers), method of extraction, and the ability to synthesize large amounts of the *in vitro* product for characterization. Although cellulose was synthesized *in vitro* using plant extracts, the major product was still β-1,3 glucan, and this could be distinguished from cellulose using electron microscopy.

In later studies, using a variety of detergents, Kudlicka *et al.* (1995) was able to demonstrate not only an increase in the amount of cellulose synthesized *in vitro*, but also the production of cellulose I using plant extracts (Kudlicka *et al.*, 1995). Lai-Kee-Him *et al.* (2002) used detergent solubilized microsomal fractions from suspension-cultured cells of blackberry (*Rubus fruticosus*) for *in vitro* cellulose synthesis (Lai-Kee-Him *et al.*, 2002). These investigators found that the detergents Brij 58 and taurocholate were effective in solubilizing membrane proteins that allowed synthesis of both cellulose and callose given UDP-glucose as the substrate. Roughly 20% of the *in vitro* product was cellulose with taurocholate as the detergent, and no Mg^{2+} was required. The cellulose product was characterized by methylation analysis, electron microscopy, electron and X-ray synchrotron diffractions, and resistance to Updegraff reagent. Cellulose microfibrils were obtained *in vitro*, and they had the same dimensions as microfibrils isolated from primary cell walls. However, the cellulose diffracted as cellulose IV_I, a disorganized form of cellulose I that is formed when the fibrillar material contains crystalline domains that are too narrow or too disorganized to be considered real cellulose I crystals (Lai-Kee-Him *et al.*, 2002).

In related studies, but using immunoaffinity purified cellulose synthase from mung bean hypocotyls, Laosinchai (2002) also demonstrated the *in vitro* synthesis of cellulose microfibrils (Laosinchai, 2002).

3.3. Purification and characterization of cellulose synthase activity

Cellulose synthase is the enzyme that performs cellulose biosynthesis. Purification of this enzyme is a major objective for understanding its properties and in determining its structure and mode of regulation. Cellulose synthase is a membrane protein and like most membrane proteins its purification has eluded investigators interested in isolating it. However, significant progress has been made in purifying the cellulose synthase activity from *A. xylinum* using the product entrapment technique utilized for purification of the chitin synthase activity in yeast (Lin and Brown, 1989). In *A. xylinum*, using a combination of detergent solubilization and product entrapment methods, two major polypeptide bands were identified in the purified fraction. One of these polypeptides was shown to selectively bind UDP-glucose, and this polypeptide was identified as the

cellulose synthase catalytic subunit (Lin *et al.*, 1990). The other polypeptide was shown to bind the activator c-di-GMP (Mayer *et al.*, 1991). Sequence information obtained from these polypeptides was useful in identifying the corresponding genes from *A. xylinum* (Saxena *et al.*, 1990, 1991). However, similar progress has not been made with purifying the cellulose synthase activity in plants. Laosinchai (2002) used immunoaffinity techniques to purify cellulose synthase activity from mung bean fractions that synthesized cellulose microfibrils *in vitro* (Laosinchai, 2002). Unfortunately, sufficient amounts of the protein could not be isolated for further characterization of this activity. The cellulose synthase activity purified from *A. xylinum* utilizes UDP-glucose as the substrate and is activated by c-di-GMP. The cellulose synthase activity in plants is also shown to use UDP-glucose as the substrate, but it is not activated by c-di-GMP. Instead, the plant activity is influenced positively in the presence of cellobiose (Li and Brown, 1993). Although no requirement for a primer has been observed for cellulose synthesis *in vitro* using bacterial or plant extracts, a proposal for the requirement of a sterol-glucoside primer has been made for cellulose synthesis in plants (Peng *et al.*, 2002). This proposal is based on the observation that cotton fiber membranes synthesized sitosterol-cellodextrins (SCDs) from sitosterol-β-glucoside (SG) and UDP-glucose under conditions that favor cellulose synthesis (Peng *et al.*, 2002). As a result, this model invokes a number of other components besides cellulose synthase and UDP-glucose, in a multistep reaction scheme, as opposed to the single-step polymerization reaction that requires only cellulose synthase and UDP-glucose. Since most of the experiments demonstrating *in vitro* cellulose synthesis do not suggest the requirement for a primer and no new evidence has been provided in support of the multistep reaction scheme, the current view is that polymerization of glucose residues from UDP-glucose occurs in a single-step reaction catalyzed by the cellulose synthase.

Interestingly, many of the features of cellulose synthases from different organisms are predicted from the derived amino sequences following identification of the genes for cellulose synthases in these organisms.

4. MOLECULAR BIOLOGY OF CELLULOSE BIOSYNTHESIS IN PLANTS

4.1. Identification of genes encoding cellulose synthases in plants

Cellulose synthase genes were first identified in *A. xylinum* and subsequently in other bacterial species (Matthysse *et al.*, 1995b; Saxena *et al.*, 1990; Wong *et al.*, 1990) before they were identified in plants (Arioli *et al.*, 1998; Pear *et al.*, 1996). *A. xylinum* produces abundant amounts of cellulose, and it has been a model organism for studies on cellulose biosynthesis, so it is not surprising that cellulose biosynthesis genes were first identified in this organism. Interestingly, the genes from this organism were not found to be useful in isolating cellulose synthase genes from other organisms by nucleic acid hybridization techniques. However, Saxena *et al.* (1995) compared the derived amino acid sequence of the bacterial

cellulose synthase with other proteins and found them useful in identifying conserved amino acid residues in β-glycosyltransferases, more specifically the conserved residues and sequence motif identified as D, D, D, QXXRW in processive β-glycosyltransferases (Saxena *et al.*, 1995). Based on the deduced amino acid sequences of bacterial cellulose synthases and other β-glycosyltransferases, genes for plant cellulose synthases were first identified by random sequencing of a cotton fiber cDNA library (Pear *et al.*, 1996). Two cDNA clones (*GhCesA1* and *GhCesA2*) were identified from the cotton fiber cDNA library, and the derived amino acid sequence of GhCesA1 gave the first glimpse of the primary structure of a plant cellulose synthase (Pear *et al.*, 1996). In addition to the transmembrane regions and the conserved residues found in bacterial cellulose synthase, the cellulose synthase from plants was found to have additional features—the presence of two regions (originally referred to as CR-P and HVR) within the globular domain that contained the conserved residues and a zinc-finger domain at the N-terminus.

Around the same time that cDNA clones encoding cellulose synthases were identified in cotton by random sequencing (Pear *et al.*, 1996), a number of cDNA clones encoding amino acid sequences containing the D, D, D, QXXRW conserved residues and sequence motif were identified by sequence analysis of expressed sequence tag (EST) sequences of *Arabidopsis* and rice that were available in the public databases (Cutler and Somerville, 1997; Saxena and Brown, 1997). However, the proteins encoded by these cDNA clones did not show the additional features identified in the cotton cellulose synthases; instead these proteins resembled more the primary structure of the bacterial cellulose synthase and were referred to as cellulose synthase-like proteins with a role possibly in the synthesis of β-linked polysaccharides other than cellulose (Cutler and Somerville, 1997). Soon thereafter, a superfamily of genes encoding cellulose synthases (CesA) and cellulose synthase-like (Csl) proteins were identified in a large number of plants (Richmond and Somerville, 2000). The presence of a large number of genes belonging to the cellulose synthase superfamily in each plant was surprising at first, but the role of many of these CesA genes in cellulose biosynthesis became obvious following analyses of a number of *Arabidopsis* mutants affected in cellulose biosynthesis. Interestingly, two cellulose synthase genes were earlier identified in *A. xylinum* (Saxena and Brown, 1995). Although both genes encode a functional cellulose synthase as determined by *in vitro* cellulose synthase activities in mutants, only one gene was found to be essential for normal *in vivo* cellulose synthesis in *A. xylinum* (Saxena and Brown, 1995).

4.2. Mutant analysis allowed identification of genes for cellulose synthases and other proteins required for cellulose biosynthesis

4.2.1. Identification and functional characterization of cellulose synthases in plants by analysis of mutants and gene expression studies

Although a majority of the *CesA* and *Csl* genes have been identified from genome and EST sequences, at least six of the *CesA* genes in *Arabidopsis* were identified by mutant analysis. In a number of cellulose-deficient *Arabidopsis* mutants, the mutations were mapped to genes that encoded for cellulose

synthases (Arioli *et al.*, 1998; Fagard *et al.*, 2000; Scheible *et al.*, 2001; Taylor *et al.*, 1999). Interestingly, although all the mutants exhibited different phenotypes, they all showed a deficiency in the amount of cellulose produced. The first mutant, where the mutation was identified in a gene that encoded for a cellulose synthase, was a temperature-sensitive root-swelling mutant (*rsw1*) (Arioli *et al.*, 1998). At the nonpermissive temperature, the mutant produced a larger proportion of noncrystalline cellulose in place of crystalline cellulose, and the rosette terminal complexes (TCs) normally associated with cellulose microfibrils were not observed by freeze-fracture electron microscopy. The mutation in the cellulose synthase gene (*rsw1* gene; *AtCesA1*) led to the substitution of valine for alanine at position 549 of the cellulose synthase protein and this change resulted in all the different phenotypes associated with the *rsw1* mutant (Williamson *et al.*, 2001). No biochemical changes have been characterized in the mutant protein, but it appears that at the nonpermissive temperature, the cellulose synthase is not assembled into a rosette structure. Although the mutation results in the reduction of crystalline cellulose at the nonpermissive temperature, noncrystalline cellulose still is produced suggesting that the *rsw1*-encoded cellulose synthase is able to synthesize the β-1,4-glucan chains, but does not allow for their assembly to take place, or alternatively these chains are synthesized by cellulose synthases encoded by other genes, where the assembly of these cellulose synthases is affected by the *rsw1* mutation. Changes in cell shapes and sizes suggested that the Rsw1 cellulose synthase contributed to cellulose in the primary wall. Interestingly, a number of questions still remain to be answered in terms of how the *rsw1* mutation affects cellulose biosynthesis.

A number of *irregular xylem* mutants (*irx* mutants) have been isolated by screening cross-sections of stems of *Arabidopsis* plants (Turner and Somerville, 1997). The mutations resulted in collapse of mature xylem cells in the inflorescence stems, and in many of these mutants there was a significant decrease in the amount of cellulose in the secondary cell wall of cells in the xylem. Genes mutated in some of the *irx* mutants were identified to encode for cellulose synthases. The null mutation in the *irx3* mutant results in a stop codon that truncates the cellulose synthase (Irx3; AtCesA7) by 168 amino acids (Taylor *et al.*, 1999) In two *irx1* mutants (*irx1-1* and *irx1-2*), the mutations were mapped to a different cellulose synthase gene that altered the amino acids at positions 683 ($D^{683}N$) in Irx1-1 and 679 ($S^{679}L$) in Irx1-2 (Taylor *et al.*, 2000). Both these amino acid positions reside within the conserved region of the Irx1 cellulose synthase (AtCesA8). RNA analysis indicated that *irx1* and *irx3* are highly expressed in stems but not in leaves, suggesting that both genes are involved in cellulose synthesis during secondary cell wall formation. Examination of the phenotypes of the xylem elements by electron microscopy showed that the same cell type is affected in the *irx1* and *irx3* mutants, indicating that products of both the *irx1* and *irx3* genes are required within the same cell for normal cellulose synthesis during secondary cell wall formation (Taylor *et al.*, 2000). These results allowed development of the concept regarding the nonredundant nature of cellulose synthases and the requirement of more than a single cellulose synthase in each cell for normal cellulose synthesis. Using biochemical and immunological methods, Taylor *et al.* (2000) furthermore

demonstrated that the Irx1 and Irx3 cellulose synthases associate with each other, and suggested that this association is required for cellulose synthesis (Taylor *et al.*, 2000). Even as different models to explain the requirement of two different cellulose synthases for cellulose synthesis were being proposed, another gene (*irx5*) encoding for a different cellulose synthase (Irx5; AtCesA4) was identified in a further screen of *irx* mutants and it was found that the *irx1*, *irx3*, and *irx5* genes were coexpressed in the same cells (Perrin, 2001; Taylor *et al.*, 2003). Using detergent-solubilized extracts, the proteins encoded by these three genes were shown to interact with each other, and it was suggested that all three gene products probably are required for the formation of the cellulose-synthesizing complexes (rosette TCs) in plants. Interestingly, the presence of all three cellulose synthases (AtCesA8, AtCesA7, and AtCesA4), but not their activity, is required for correct assembly and targeting of the cellulose-synthesizing complex during secondary wall cellulose synthesis (Taylor *et al.*, 2004). Overall, the *irx* mutants have been crucial in not only identifying the cellulose synthase genes that are required for cellulose synthesis during secondary wall formation, but also in formulating the concept that the assembly of the cellulose-synthesizing complexes (rosette TCs) in plants requires more than a single isoform of cellulose synthase. Fig. 6.3 shows immunogold labeling of the rosette TCs from *Vigna angularis* using an antibody to a cellulose synthase.

The protein regulator of cytokinesis 1 (*PRC1*) gene in *Arabidopsis* encodes AtCesA6, and like the *rsw1* mutant of AtCesA1, mutation in this gene exhibits decreased cell elongation, especially in roots and dark-grown hypocotyls, because

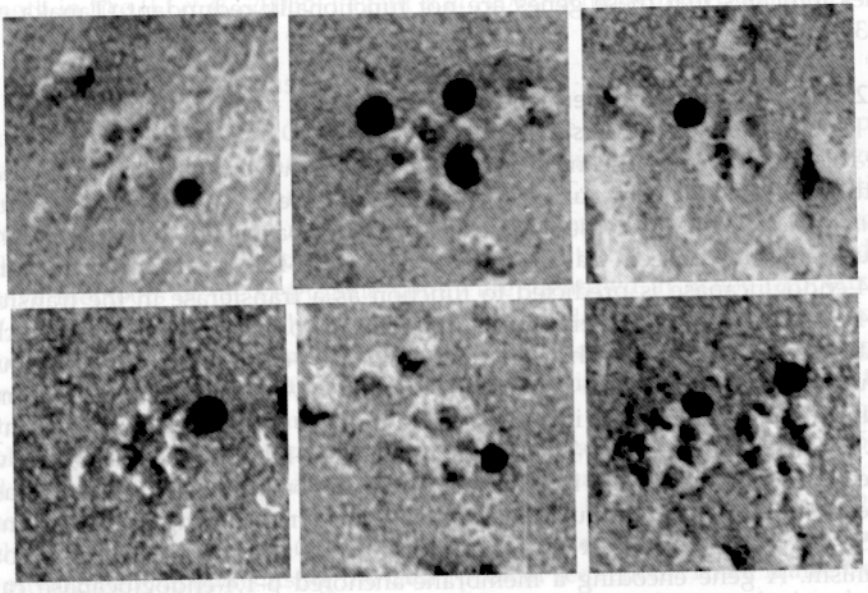

FIGURE 6.3 Rosette terminal complexes from *V. angularis* that were immunogold labeled with an antibody to cellulose synthase. (Reproduced from Kimura, S., Laosinchai, W., Itoh, T., Cui, X., Linder, R., and Brown, R. M., Jr. (1999). *Plant Cell* **11**, 2075–2085.)

of cellulose deficiency in the primary wall (Fagard *et al.*, 2000). In addition to similar mutant phenotypes, both *AtCesA1* and *AtCesA6* also show similar expression profiles in various organs and growth conditions suggesting coordinated expression of at least two distinct cellulose synthases (*AtCesA1* and *AtCesA6*) in most cells (Fagard *et al.*, 2000). However, differences were observed in the embryonic expression of these two *CesA* genes (Beeckman *et al.*, 2002). Mutations in the *ixr1* and *ixr2* genes confer resistance to the cellulose synthesis inhibitor isoxaben and these two genes encode AtCesA3 and AtCesA6, respectively (Desprez *et al.*, 2002; Scheible *et al.*, 2001). The cellulose synthases identified by analysis of the *rsw1*, *ixr1*, and *PRC1/ixr2* mutants involve members of the CesA family (AtCesA1, AtCesA3, and AtCesA6) required for primary wall cellulose synthesis. Although no physical interactions have been determined for these cellulose synthases, studies of inhibition of cellulose synthesis by isoxaben suggest that AtCesA3 and AtCesA6 together form an active protein complex in which the involvement of AtCesA1 may be required (Desprez *et al.*, 2002).

Brittle culm mutants have been identified in barley, maize, and rice. The cellulose content in the cell walls of cells in the brittle culm mutants of barley was found to be lower than the wild-type plants, but no significant differences were found in the amount of the noncellulosic components of the cell wall (Kokubo *et al.*, 1989, 1991). Brittle culm mutants in rice were useful in identifying three *CesA* genes (*OsCesA4*, *OsCesA7*, and *OsCesA9*) (Tanaka *et al.*, 2003). The three genes are expressed in seedlings, culms, premature panicles, and roots, but not in mature leaves. The expression profiles are almost identical for these three genes, and decrease in the cellulose content in the culms of null mutants of the three genes indicates that these genes are not functionally redundant (Tanaka *et al.*, 2003).

4.2.2. Identification of other genes/proteins which may be required for cellulose biosynthesis in plants

The role of β-1,4-endoglucanase during cellulose synthesis was first proposed by Matthysse *et al.* (1995a,b) during analysis of cellulose-minus mutants in *Agrobacterium tumefaciens* (Matthysse *et al.*, 1995a,b). In this bacterium, cellulose synthesis is suggested to proceed via the formation of lipid-linked intermediates, and a β-1,4-endoglucanase is predicted to function as a transferase in the transfer of β-1,4-linked glucan oligomers from a lipid carrier to the growing cellulose chain (Matthysse *et al.*, 1995a). The gene encoding β-1,4-endoglucanase is organized with the cellulose synthase gene in an operon in *A. tumefaciens*, and a similar organization of these genes is observed in a number of other bacteria (Matthysse *et al.*, 1995b; Römling, 2002). The organization of a β-1,4-endoglucanase gene with the cellulose synthase gene in the same operon in bacteria has been taken as an indication that β-1,4-endoglucanase probably has a role during cellulose synthesis. So far, there is no direct demonstration for this role in bacteria or any other organism. A gene encoding a membrane-anchored β-1,4-endoglucanase called KORRIGAN also has been identified in a dwarf mutant of *Arabidopsis* (Nicol *et al.*, 1998). In plants, the KORRIGAN protein is believed to function during primary or secondary wall cellulose synthesis (Lane *et al.*, 2001;

Mølhøj *et al.*, 2002; Nicol *et al.*, 1998; Sato *et al.*, 2001; Szyjanowicz *et al.*, 2004; Zuo *et al.*, 2000). Its exact function during cellulose synthesis remains to be determined, although various roles have been assigned to it such as terminating or editing the glucan chains emerging from the cellulose synthase complex before their crystallization into a cellulose microfibril. Alternately it could cleave sterol from the sterol-glucoside primer that is suggested to initiate glucan chain formation (Peng *et al.*, 2002). However, recent evidence does not support this role (Scheible and Pauly, 2004). A membrane-bound sucrose synthase, which converts sucrose to UDP-glucose, may be physically linked to the cellulose synthase complex for channeling UDP-glucose to the cellulose synthase in plants, and suppression of this gene has been shown to effect cotton fiber initiation and elongation (Amor *et al.*, 1995; Ruan *et al.*, 2003).

Proteins that may indirectly influence cellulose biosynthesis include those that are required for *N*-glycan synthesis and processing (Lukowitz *et al.*, 2001). One of these proteins is glucosidase I, which trims off the terminal β-1,2-linked glucosyl residue from N-linked glycans and is involved in the quality control of newly synthesized proteins that transit through the endoplasmic reticulum (ER) (Boisson *et al.*, 2001; Gillmor *et al.*, 2002). Another protein could be glucosidase II that removes the two internal β-1,3-linked glucosyl residues subsequent to the action of glucosidase I in the quality control pathway (Burn *et al.*, 2002b). Other proteins that influence cellulose production include KOBITO, a membrane-anchored protein of unknown function that is suggested to be a part of the cellulose synthase complex, and COBRA, a putative glycosylphosphatidylinositol (GPI)-anchored protein, which upon being inactivated, dramatically reduces culm strength in rice (Li *et al.*, 2003b; Pagant *et al.*, 2002; Schindelman *et al.*, 2001).

4.3. The cellulose synthase genes

As of June 2006, *CesA* and *Csl* gene sequences have been identified in 252 plant species (http://cellwall.stanford.edu/). In *Arabidopsis*, 10 *CesA* and 30 *Csl* genes have been identified. Similar numbers of *CesA* and *Csl* genes have been identified in other plants as well. In rice, at least 12 *CesA* genes have been identified by analysis of cDNA, ESTs, and genome sequencing (http://cellwall.stanford.edu/). Twelve members of the *CesA* gene family are identified in maize (Appenzeller *et al.*, 2004). In most cases, the *CesA* genes are found to be dispersed on different chromosomes and have similar numbers of exons and introns. The *CesA* genes identified in maize from cDNA analysis and mapping studies were found to be distributed to different chromosomes, similar to the *Arabidopsis CesA* genes (Holland *et al.*, 2000). In *Arabidopsis*, the genes range in size from 3.5 to 5.5 kbp and contain 9–13 introns and the CesA transcripts range in size from 3.0 to 3.5 kb, encoding proteins that are 985–1,088 amino acids in length (Richmond, 2000). Orthologs of the *Arabidopsis CesA* genes have been identified in a number of plants by phylogenetic analysis using the CesA protein sequences. Three maize CesAs, ZmCesA10–12 cluster with the *Arabidopsis* CesAs that are shown to be involved in secondary wall cellulose synthesis. *ZmCesA10*, *ZmCesA11*, and *ZmCesA12* group with *AtCesA4* (Irx5), *AtCesA8* (Irx1), and *AtCesA7* (Irx3), respectively and are

probable orthologs of these genes. Based on expression patterns, these three genes appear to be coordinately expressed (Appenzeller *et al.*, 2004). Likewise, *OsCesA7*, *OsCesA4*, and *OsCesA9* are the orthologous genes in rice, as are barley *HvCesA4*, *HvCesA5/7*, and *HvCesA8* genes, respectively (Burton *et al.*, 2004; Tanaka *et al.*, 2003).

Orthologs of the *Arabidopsis CesA* genes required for secondary wall cellulose synthesis have also been identified by expression analysis of normal wood undergoing xylogenesis in hybrid aspen (Djerbi *et al.*, 2004). Four *CesAs*, *PttCesA1*, *PttCesA3–1*, *PttCesA3–2*, and *PttCesA9* were shown to exhibit xylem-specific expression, with the derived amino acid sequences and expression profiles of *PttCesA3–1* and *PttCesA3–2* being very similar, suggesting that they represent redundant copies of a *CesA* with the same function. Phylogenetic analysis indicates that the xylem-specific CesAs from hybrid poplar cluster with similar CesAs from other poplars and *Arabidopsis*. PttCesA1 is most similar to AtCesA4, PttCesA3–1, and PttCesA3–2 are closest to AtCesA8, and PttCesA9 is closest to AtCesA7 (Djerbi *et al.*, 2004). Although it has been possible to identify orthologs of CesAs required for secondary wall cellulose synthesis in various plants, the relationship between the CesAs involved in primary wall cellulose synthesis from different plants is not as clear. From phylogenetic analysis, it appears that the genes for primary wall cellulose synthesis have duplicated relatively independently in dicots and monocots (Appenzeller *et al.*, 2004).

4.4. The cellulose synthase protein

The cellulose synthase genes identified in *A. xylinum* encode either the catalytic subunit consisting of 754 amino acids and 9 potential transmembrane regions or a longer protein of approximately 1,550 amino acids consisting of the cellulose synthase catalytic domain and an activator (c-di-GMP)-binding domain with 9 potential transmembrane regions (Saxena *et al.*, 1990, 1991, 1994; Wong *et al.*, 1990). The catalytic region in these proteins was predicted to have the conserved residues and sequence motif identified as D, D, D, QXXRW (Saxena *et al.*, 1995). *CesA* genes in plants encode a large, multipass transmembrane protein with a globular region containing the D, D, D, QXXRW motif. The CesA proteins in plants have a plant-specific conserved region (CR-P) and a hypervariable region (HVR-2) within the cytosolic globular region that contains the conserved residues. A conserved, extended N-terminal region is shown to have two zinc-finger domains resembling LIM/RING domains followed by a HVR-1 region (Kawagoe and Delmer, 1997). The RING domains are predicted to mediate protein–protein interactions. Using the yeast two-hybrid system, it has been shown that the zinc-finger domain of GhCesA1 is able to interact with itself to form homodimers or heterodimers with the zinc-finger domain of GhCesA2 in a redox-dependent manner (Kurek *et al.*, 2002). This dimerization of CesAs is supposed to represent the first stage in the assembly of the rosette TC (Saxena and Brown, 2005).

5. MECHANISM OF CELLULOSE SYNTHESIS

5.1. Role of primer and/or intermediates during cellulose synthesis?

In straightforward terms, cellulose biosynthesis requires the enzyme cellulose synthase for catalyzing the polymerization of glucose residues from UDP-glucose into a β-1,4-linked glucan chain. This simple mechanism envisions direct polymerization without the need for any intermediates or a primer. Cellulose biosynthesis has been demonstrated *in vitro* using membrane and detergent-solubilized extracts from *A. xylinum* and a number of plants in the presence of only UDP-glucose (Kudlicka and Brown, 1997; Lai-Kee-Him *et al.*, 2002; Lin and Brown, 1989; Okuda *et al.*, 1993). The synthesis of cellulose *in vitro* with the minimal added components in the reaction mixture strongly supports the direct polymerization of glucose without any requirement for a primer. However, in the absence of purified cellulose synthases it is not possible to completely exclude the role of other proteins or components contributed by the membrane fraction or detergent extracts during cellulose synthesis. In 2002, Peng *et al.* proposed a model for cellulose biosynthesis in which they suggested that SG serves as a primer for synthesis of SCDs by CesA proteins (Peng *et al.*, 2002). According to their model, a membrane-associated endoglucanase Kor (encoded by the *Korrigan* gene) cleaves SCDs giving rise to SG and cellodextrins (CDs). In the next step, the CDs undergo β-1,4-glucan chain elongation catalyzed by CesA proteins. The glucose moiety of SG is found to be attached via its reducing end to sitosterol and chain elongation in the first step is predicted to proceed from the nonreducing end. Based on this model, plants deficient in sitosterol are expected to show a severe phenotype due to impairment in cellulose synthesis (Peng *et al.*, 2002). A number of mutants deficient in sitosterol content have been identified in *Arabidopsis*. However, *dwf1/dim* mutants of *Arabidopsis* that have a severe reduction in sitosterol content have been rescued to the wild type by brassinosteroid (BR) treatment suggesting that sitosterol may not have a major role in cellulose biosynthesis (Clouse, 2002). In the absence of any direct evidence for the role of sitosterol in cellulose biosynthesis, doubts have been raised regarding the proposed involvement of SG as a primer (Somerville *et al.*, 2004).

5.2. Addition of glucose residues to the growing glucan chain end

The glucose residues in the β-1,4-linked glucan chains in cellulose are arranged such that each residue is inverted with respect to its neighbor, giving rise to a twofold screw axis and a rather flat chain. If this arrangement of sugar residues is established during synthesis, it would entail either the rotation of the glucan chain or the cellulose synthase for addition of successive glucose residues to the growing end. A model suggesting that the active site of the enzyme can position two UDP-glucose molecules in an orientation such that the two glucose residues are positioned inverted to each other in the catalytic pocket was proposed by Saxena *et al.* (1995), and it was suggested that the glucose residues could be added sequentially or simultaneously to the growing end (Saxena *et al.*, 1995).

The growing end was later shown to be the nonreducing end of the β-1,4-linked glucan chain during cellulose synthesis (Koyama *et al.*, 1997). Alternatively, the twofold symmetry in the glucan chain can be obtained from a single catalytic center, based on the reasoning that there is a fairly large degree of freedom of rotation about the β-glycosidic bond. According to this proposal, the glucose residue added in one orientation relaxes into the native orientation after polymerization (Delmer, 1999). Other proposals have suggested that two catalytic centers may be present in two subunits and be organized following dimerization or two different catalytic domains within the same catalytic site participate in the dual addition (Albersheim *et al.*, 1997; Charnock *et al.*, 2001). Cellulose synthase and other processive β-glycosyltransferases have so far resisted crystal structure determination although structure of a nonprocessive β-glycosyltransferase (SpsA from *Bacillus subtilis*) has been determined (Charnock and Davies, 1999). The SpsA protein lacks the conserved QXXRW motif found in the processive enzymes, and studies with site-directed mutants of cellulose synthase have indicated a role of this motif during the synthesis of cellulose (Saxena *et al.*, 2001). The structure of the globular region of the *A. xylinum* cellulose synthase containing all the conserved aspartic acid residues and the QXXRW motif was predicted using the genetic algorithm, and it was estimated that the central elongated cavity can accommodate two UDP-glucose residues (Saxena *et al.*, 2001). The alternating orientation of the *N*-acetylglucosamine (GlcNAc) residues within the chitin chain also led to the proposal that chitin synthases possess two active sites, and this possibility was tested using UDP-derived monomeric and dimeric inhibitors of chitin synthase activity *in vitro* (Yeager and Finney, 2004). Using these inhibitors, it was found that uridine-derived dimeric inhibitors exhibited a 10-fold greater inhibition of chitin synthase activity as compared to the monomeric control, consistent with the presence of two active sites in chitin synthases (Yeager and Finney, 2004).

6. PROSPECTS FOR GENETIC ENGINEERING OF CELLULOSE BIOSYNTHESIS IN PLANTS

6.1. Manipulation of cellulose biosynthesis in plants

Genetic modifications for improvement of specific traits or the addition of new traits to economically important plants is a major objective worldwide. Not only is cellulose a constituent of all plants, a number of plants (such as cotton and forest trees) are grown specifically for their cellulose content. In general, the objective of genetic manipulation of the cellulose synthesizing capacity in these plants is to either increase the amount of cellulose or modify the physical properties of the cellulose during synthesis. For example, the secondary cell wall in cotton fibers determines the fiber properties. Considering that the secondary cell wall in cotton fibers is approximately 95% cellulose, the properties of the cotton fiber are dependent not only on the amount of cellulose deposited, but also on other features such as the structure and orientation of the cellulose microfibrils and the degree of

polymerization of the glucan chains. Additionally, manipulation of cellulose synthesis in a number of crop plants may be important for improving specific agronomic traits. As an example, stalk lodging in maize results in significant yield losses, and an increase in the cellulose content in the cells in the stalk may allow improvements in stalk strength and harvest index (Appenzeller *et al.*, 2004). Apart from its importance in the growth and development of plants, cellulose is also an abundant renewal energy resource that is present in the biomass obtained from agricultural residues of major crops. Corn stover is the most abundant agriculture residue in the United States and it can be used for various applications including bioethanol production (Kadam and Mcmillan, 2003). Increasing the content of cellulose and reducing the lignin content of corn plants is therefore considered to be beneficial for ethanol production.

Cellulose biosynthesis in plants can be modified by manipulation of the cellulose synthase (*CesA*) genes or other genes that influence cellulose production. *CesA* genes have been identified in most plants, and as a result they are prime targets for directly modifying cellulose synthesis by genetic manipulation. *CesA* genes are part of a gene family, and as a result a number of features of these genes will have to be analyzed before they can be manipulated usefully. Some of these features may include understanding of the expression of the different *CesA* genes, the redundant nature of each gene in a specific cell type, and the phenotype that is generated when each gene is mutated or overexpressed (Holland *et al.*, 2000). In corn, the majority of the cellulose in the stalk is in the vascular bundles. Based on their expression patterns, 3 of the 12 *CesA* genes in corn appear to be involved in cellulose synthesis during secondary wall formation and their promoter sequences have been identified (Appenzeller *et al.*, 2004). These promoters can now be used for expression of *CesA* genes in specific cell types for increasing their cellulose content.

Direct modification of cellulose content by manipulation of the cellulose synthase genes has been performed in only a few cases so far. To improve fiber quality of cotton fibers, the *A. xylinum acsA* and *acsB* genes were transferred to cotton (Li *et al.*, 2004). The fiber strength and length of fibers were found to be greater in the transformed plants, as well as the cellulose content was found to be higher in the transformed plants as compared to the control plants. In potato, cellulose content was modified in the tuber using sense and antisense expression of the full length *StCesA3* and class-specific regions (CSR) of the four potato *CesA* cDNAs (Oomen *et al.*, 2004). The antisense and sense *StCesA3* transformants demonstrated that the cellulose content could be decreased to 43% and increased to 200% of the wild type, respectively, by modifying the RNA expression levels (Oomen *et al.*, 2004). Interestingly, the increase in cellulose content by increasing expression of a single *CesA* gene was found to be remarkable considering that multiple copies of different CesAs are believed to be required for assembly of cellulose-synthesizing complexes. The utility of antisense transgenic lines in generating a range of phenotypes is suggested to be particularly useful, especially where null mutations are potentially lethal (Oomen *et al.*, 2004). In *Arabidopsis*, the transgenic approach using antisense expression exhibited a slightly different phenotype as compared to a mutation in the corresponding gene

(Burn *et al.*, 2002a). The modulation of *CesA* RNA expression levels and concomitantly cellulose content has also been demonstrated in tobacco plants using virus-induced silencing of a cellulose synthase gene (Burton *et al.*, 2000). Apart from the *CesA* genes, genes with an indirect role in cellulose biosynthesis, such as the sucrose synthase, have been manipulated in the cotton fiber using suppression constructs. A 70% or more suppression of the sucrose synthase activity in the ovule led to a fiberless phenotype suggesting that this enzyme has a rate-limiting role in the initiation and elongation of fibers (Ruan *et al.*, 2003). In other instances, while some researchers have shown an increase in cellulose accumulation following manipulation of genes for reduced lignin synthesis in aspen trees (Hu *et al.*, 1999; Li *et al.*, 2003a), other researchers did not find any evidence in support of enhanced cellulose synthesis upon severe downregulation of lignin biosynthetic genes (Anterola and Lewis, 2002). It is believed that the synthesis of cellulose is interconnected with the synthesis of other components of the plant cell wall, and manipulation of a number of genes would therefore affect cellulose production. However, not much is known as to how the different pathways are interconnected, but a systems view of these interactions is beginning to emerge (Somerville *et al.*, 2004).

6.2. Influence of cellulose alterations in plants

Cellulose in the plant cell wall influences a number of traits, and although not much is known in terms of the effects on the plant upon increase of cellulose content in the cell wall, a number of studies have linked mutations in the genes encoding cellulose synthases and other proteins that may be required for cellulose synthesis to changes in other properties. For example, the *Arabidopsis* cellulose synthase (*AtCesA3*) mutant, *cev1*, is found to be resistant to fungal pathogens and is constitutively activated for defense pathways in a manner similar to that for the pathogen-induced pmr4 mutant (Cano-Delgado *et al.*, 2003; Ellis *et al.*, 2002; Nishimura *et al.*, 2003). Moreover, there is an accumulation of transcripts that are induced by jasmonic acid (JA) and ethylene in this mutant (Ellis and Turner, 2001; Ellis *et al.*, 2002). Increased ethylene production and/or sensitivity was observed for *cesA3*^{eli1}, *cesA6*^{prc1}, *kor1*, *elp1/pom1*, and in wild-type plants treated with 2,6-dichlorobenzonitrile (DCB) or isoxaben (Cano-Delgado *et al.*, 2003; Desnos *et al.*, 1996; Ellis and Turner, 2001; Ellis *et al.*, 2002; Zhong *et al.*, 2002). Only a brief list of changes have been mentioned here, but as is clear from these results that changes in cellulose synthesis/content in the cell wall are sensed by cells directly or indirectly through as yet unknown mechanisms.

7. SUMMARY

Cellulose is a component of all plant cells, and modification of the cellulose content or properties can have dramatic effects on the form and function(s) of specific parts or the entire plant. Cellulose synthase is the enzyme required for biosynthesis of cellulose, and a number of genes encoding this protein form part

of a gene family in plants. Although plants are well endowed with genes for cellulose synthases, and expression of most of the *CesA* genes have been observed in most tissues, mutations in some of them can have very different effects. At the same time increased expression of some of the *CesA* genes may result in increased synthesis of cellulose in specific cells and tissues. More importantly, the direction in which the cellulose microfibrils are assembled in the primary cell wall helps determine the direction of cell elongation. In cells with a secondary cell wall, the orientation of the cellulose microfibrils influences the properties of the cell. Although the general view is that microtubules play a role in determining the direction of cellulose synthesis, not much is known as to how this occurs. For effective manipulation of cellulose synthesis in plant cells, it is necessary that we not only understand the machinery responsible for cellulose biosynthesis, but also as to how it is assembled, localized, and regulated.

ACKNOWLEDGEMENTS

The authors acknowledge support from the Division of Energy Biosciences, Department of Energy (Grant DE-FG03-94ER20145), and the Welch Foundation (Grant F-1217).

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