RESEARCH: BOTANY

A Recipe for Cellulose

Nicholas Carpita and Claudia Vergara

Cellulose is the world's most abundant biopolymer. These long paracrystalline cables, each containing several dozen glucose polymers, are spooled several times around plant cells to form the structural framework of the primary cell wall. Despite the efforts of many researchers since the mid-1960s, prolonged synthesis of cellulose has never been achieved in the test tube because the cellulose synthase machinery from flowering plants is so fragile. The enzyme polypeptide, too, has been elusive, even with use of affinity probes that mimic the substrate. In an age where tens of thousands of genes have been characterized, until very recently not a single gene encoding a higher plant polysaccharide synthase had been identified. This situation has now changed dramatically with a report by Arioli *et al.* on page 717 of this issue, in which a gene from the plant *Arabidopsis* is shown definitively to be responsible for cellulose synthesis (*1*).

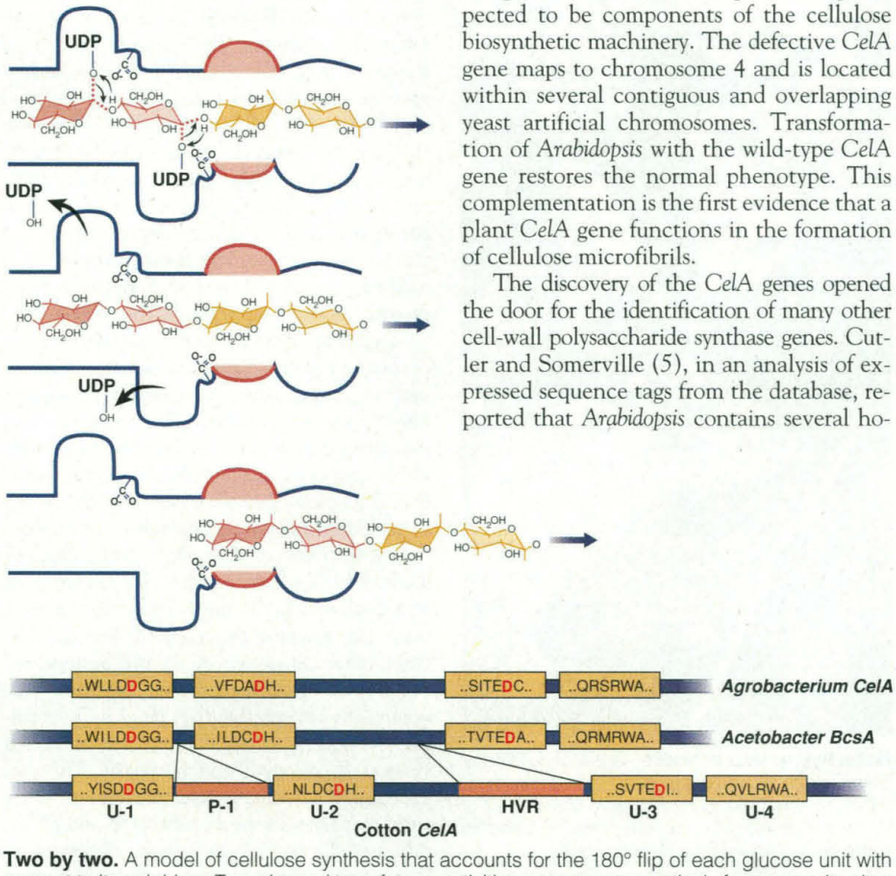
Early progress in the identification of synthase genes came from studies in bacteria. *Acetobacter xylinum* and *Agrobacterium tumefaciens* make cellulose that they extrude extracellularly and, unlike higher plant cellulose synthases, the bacterial synthases remain active when isolated. As a result, genes encoding polypeptides of the *Acetobacter xylinum* cellulose synthase were identified within an operon containing four genes (see figure) (*2*). Elation at this finding gave way to disappointment as the bacterial cellulose synthase clones, when used to probe plant libraries, failed to identify plant cellulose synthase homologs.

The next breakthrough came as a result of two studies. First, when the deduced amino acid sequences of suspected bacterial synthases were subjected to hydrophobic cluster analysis, Saxena and colleagues (*3*) discovered that the synthase genes and those of other nucleotide-sugar–requiring enzymes contain short sequences of exceptionally high conservation, which are thought to be critical for uridine 5′-diphosphate (UDP)–glucose (UDP-Glc) binding and catalysis (see figure). Further, synthases able to make β-D-(1→4)–glycosyl linkages contain an additional domain and a total of four sequences of high similarity. In the second study, Pear *et al.* (*4*) found two plant homologs containing all four of the UDP-Glc–binding sequences of the *Acetobacter* cellulose synthase gene by a search of sequences in a cDNA library made from transcripts of cotton fibers taken at the onset of secondary wall cellulose formation. The plant *CelA* genes are highly expressed in fibers at the time of active secondary wall cellulose synthesis, encode polypeptides of about 110 kD, are predicted to have eight transmembrane domains, bind the substrate UDP-Glc, and contain two large domains unique to plants. After three decades, a prime candidate for a cellulose synthase gene had finally been identified.

Although the cotton *CelA* gene is a likely candidate for the catalytic cellulose synthase, the lack of in vitro synthesis of cellulose by the synthase has made direct proof of the function of the gene product difficult. In this issue, Arioli and his colleagues provide proof of function for a related homolog from *Arabidopsis* by complementation of a temperature-sensitive mutant. Several years ago, R. Williamson and his colleagues selected several *Arabidopsis* mutants in which the root tips swell at elevated temperatures. Actually, Williamson was using this strategy to identify microtubule mutants that had lost the ability to direct the orientation of cellulose microfibrils. These kinds of cytoskeletal mutants are predicted to result in isodiametric expansion of the root cells instead of elongation. Not all of the swelling mutants turned out to be cytoskeletal mutants—several of them were unable to make cellulose microfibrils. A long chromosome walk to the defective gene of one such mutant revealed that it was indeed a homolog of the cotton and other suspected *CelA* genes.

Curiously, the defective cellulose synthase seems to be able to make the individual β-D-(1→4)-glucan chains but is unable to organize them into a paracrystalline array. Arioli and his colleagues correlate this defect with a disorganization of the "particle rosettes," the plasma membrane complexes long suspected to be components of the cellulose biosynthetic machinery. The defective *CelA* gene maps to chromosome 4 and is located within several contiguous and overlapping yeast artificial chromosomes. Transformation of *Arabidopsis* with the wild-type *CelA* gene restores the normal phenotype. This complementation is the first evidence that a plant *CelA* gene functions in the formation of cellulose microfibrils.

The discovery of the *CelA* genes opened the door for the identification of many other cell-wall polysaccharide synthase genes. Cutler and Somerville (*5*), in an analysis of expressed sequence tags from the database, reported that *Arabidopsis* contains several ho-



Two by two. A model of cellulose synthesis that accounts for the 180° flip of each glucose unit with respect to its neighbor. Two glycosyl transferase activities operate cooperatively from opposite sites to add cellobiosyl units to the chain. Within three synthase genes (lower part) there are multiple domains for binding substrate (U-1 through U-4), UPD-Glc, and essential aspartyl residues (in red).

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mologs of cotton CelA, and that a number of other sequences, while not completely related to CelA, share significant identity to one or more of the suspected UDP-Glc–binding domains.

Why plants have so many different *CelA* and related genes is now a focus of investigation in a number of laboratories. The glucan chains in the cellulose microfibrils made in primary and secondary cell walls are different in their degree of polymerization, possibly as a result of different modes of catalysis and organization into paracrystalline arrays. Of the 40 or so cell types that plants make, almost all can be identified by unique features of their cell wall. The family of *CelA* genes may be required to encode the diverse cellulose synthases needed to build these specialized walls. For example, another *Arabidopsis* mutant was described in which the water-conducting cells form incomplete secondary walls (6), apparently as a result of defective cell-specific cellulose deposition. In addition, some of the related genes may well encode other glycosyltransferases, such as those that synthesize β-xylans, mannans, the backbone of xyloglucan, mixed-linkage β-glucans, or callose.

As the *CelA* genes were sequenced and the multiple binding sites for the substrate UDP-Glc were deduced, researchers began to rethink the biochemical mechanism of cellulose synthesis. The catalysis of this polymerization must overcome a steric problem because the β(1→4) linkage requires each glucose unit to be flipped nearly 180° with respect to its neighbors (see figure). To make such a linkage by addition of one sugar unit at a time, either the synthase or the growing chain must rotate 180°, or the sugar must be added in a constrained position and flip or flop alternately into the proper orientation by some other factor associated with the synthase. Because a cellulose microfibril is composed of several dozen chains, each arising from a synthase, such a drastic reorientation of enzyme or substrate would be unlikely. Saxena *et al.* (3) revived an old idea that the unit of addition is cellobiose, but with a new twist. With multiple UDP-Glc binding sites, Saxena and colleagues reasoned that two UDP-Glc–binding domains positioned 180° from each other make two simultaneous glycosyl transfers that add cellobiose units to the growing chain, circumventing the need for reorientation of synthase or chain. As it had not been established whether the glucosyl units were added to the reducing or nonreducing end of the chain, they also reasoned that retention of the UDP moiety at the reducing end kept the growing end of the chain activated. A similar two-site model was proposed by Carpita *et al.* (7), but in this model the two glucosyl units are simultaneously polymerized at the nonreducing end. Another version of this model of cellulose synthesis at the nonreducing end was presented recently, with the additional proposal that the glycosyl units form covalent intermediates with serine or threonine in the active site (8). However, Koyama *et al.* (9) showed by direct staining of the reducing ends and by microdiffraction electron crystallographic analysis that the glucose units are indeed added to the nonreducing end. They also invoke the two-site model for addition of glucosyl units, but the C-4 hydroxyl of the sugar to be added is activated not by a hydroxyl amino acid, but by aspartic acid—the very amino acid that hydrophobic cluster analysis of the original *CelA* genes had predicted to be essential in three of the four highly conserved domains (see figure).

What other polypeptides are involved in the synthesis of cellulose? The identification of a Zn-binding domain in the NH_2-terminal region of CelA (*10*) not only indicates that the polypeptide interacts with another protein, but also provides a molecular means to identify a ligand. Although there is much yet to be learned about polysaccharide synthesis in plants, the long decades of disappointment are over, and the discovery of the first synthase genes marks a new age of progress in learning how plants make their cell walls.

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SIGNAL TRANSDUCTION

Lipid-Regulated Kinases: Some Common Themes at Last

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Kinases are enzymes that add phosphates to small molecules or other enzymes, creating active signaling molecules or turning enzymes on or off. Cells use this device over and over again to change their internal biochemistry in response to signals from the outside. One class of these kinases [phosphoinositide 3-kinases (PI 3-kinases)] phosphorylate lipids to form the second messenger phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3] in response to a very wide range of extracellular stimuli. PtdIns(3,4,5)P_3 acts on pathways that control cell proliferation, cell survival, and metabolic changes—often through two different protein kinases, p70 ribosomal protein S6 kinases ($p70^{s6k}$) and protein kinase B (PKB). Until now the way PtdIns(3,4,5)P_3 regulates these two kinases has appeared to be very different, with each new advance adding to the impression that they had very little in common. However, two reports in this issue of *Science* on pages 707 and 710 (*1*, *2*) and two recent reports in *Current Biology* (*3*, *4*) add considerably to the picture of how these kinases are regulated, and in so doing reveal surprising similarities in their control mechanisms. The same regulatory themes likely apply to many protein kinases, significantly simplifying the task of understanding their regulation.

The ability of PKB, also known as Akt, to phosphorylate its substrates is increased after the cell is exposed to growth factors and insulin, and this increase occurs via a pathway that includes PI 3-kinase. In addition to its kinase domain, PKB contains an amino-terminal pleckstrin homology (PH) domain, to which the PtdIns(3,4,5)P_3 signaling molecule binds (*5*). In addition, PKB itself is phosphorylated when PtdIns(3,4,5)P_3 is present by two different protein kinases, 3-phosphoinositide-dependent protein kinase 1 (PDK1), which

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