The polysaccharides of cell walls are widely thought to be formed by polymerization of monosaccharides. We hypothesize that the backbones of the six polysaccharides that are present in the primary walls of all higher plants may instead be formed by polymerization of disaccharide intermediates.

It is readily apparent that the backbone of rhamnogalacturonan I is composed of many repeats of a diglycosyl unit, namely rhamnosyl-galactosyluronic acid (Lau et al., 1985). But what of the homogalacturonan backbone of rhamnogalacturonan II and the backbone of homogalacturonan itself? They appear to be formed from the single glycosyl residue galactosyluronic acid repeated many times. However, if one looks at the three-dimensional structures, the backbones are constructed of diglycosyl repeating units (Rees and Welsh, 1977; Morris, 1986). This is equally true of the cellulose backbone of xyloglucan (Bauer et al., 1973; Levy et al., 1991), the \( \beta-1,4 \)-glucan chains of cellulose itself (Gibeaut and Carpita, 1994; Delmer and Amor, 1995), and the \( \beta-1,4 \)-xylan backbone of arabinobioxyan (Carpita, 1983). Researchers have long recognized that cellulose and the backbone of xyloglucan are composed of disaccharide repeats. Thus, the backbones of the six polysaccharides present in all of the primary cell walls studied to date are composed of diglycosyl repeating units. Diglycosyl residue repeats are also characteristic of the extracellular matrix glycosaminoglycans of animals. This is true even though the backbones of many other polysaccharides (starch, lipopolysaccharides, and capsular polysaccharides) do not have backbones of diglycosyl units.

The backbones of xyloglucan and cellulose are linear chains of \( \beta-\text{d-glucosyl} \) residues, and the backbone of arabinobioxyan is a linear chain of \( \beta-\text{d-xylosyl} \) residues. These residues both take the \( ^4\text{C}_1 \) chair form when connected glycosidically in 4-linked chains (Fig. 1A). In the \( ^4\text{C}_1 \) chair form, O-1 and O-4 of \( \beta-\text{d-glucosyl} \) and \( \beta-\text{d-xylosyl} \) residues are in the equatorial configuration (Fig. 1A). Furthermore, the backbones of the corresponding polysaccharides tend to assume the energetically favored ribbon-like form that allows the glucan chains of cellulose to hydrogen-bond to one another to form crystalline fibers. Xyloglucan and arabinobioxyan take this form when they hydrogen-bond to cellulose. Even xyloglucan in solution is thought to have a closely related structure (Levy et al., 1991). In the ribbon-like form, every glucosyl or xylosyl residue is flip-flopped in relation to its two immediate neighbors.

The backbones of rhamnogalacturonan II and homogalacturonan are linear chains of \( \alpha-\text{d-galactosyluronic} \) acid residues. The O-1 and O-4 of the \( \alpha-\text{d-galactosyluronic} \) acid residues of homogalacturonan are both in the axial configuration (Fig. 1B). Thus, when a 4-linked chain of these residues is interconnected by glycosidic bonds in the \( \alpha \) configuration, as in homogalacturonan, a puckered ribbon-like chain is believed to form in which every galactosyluronic acid residue is flip-flopped in relation to its neighbors, similar to the glucosyl residues of cellulose and the xylosyl residues of the arabinobioxyan backbone.

The flip-flopping of \( \alpha-\text{d-galactosyluronic} \), \( \beta-\text{d-glucosyl} \), or \( \beta-\text{d-xylosyl} \) residues results in the corresponding face being "up" and then "down" in neighboring glycosyl residues of the backbones of these 4-linked polysaccharides (Fig. 1). This has interesting consequences. For example, an endo-glycanase approaching such a chain interacts with different structures on alternating glycosyl residues. In other words, each of these polysaccharides presents a disaccharide repeating structure to endo-glycanases. Cellbiose is in fact the product of the endo-\( \beta-1,4 \)-glucanase (cellulase) breakdown of the \( \beta-1,4 \)-glucan chains of cellulose.

The disaccharide nature of the repeating units of galacturonan, glucan, and xylan chains also has consequences for the mechanism by which they are synthesized. Cellulose, for example, is believed to be synthesized by a large, plasma-membrane-located, multienzyme complex (rosette) that catalyzes the simultaneous synthesis of the numerous \( \beta-1,4 \)-glucan chains that associate to form a crystalline microfibril of cellulose (Giddings et al., 1980). If each glucan
chain synthesized by a rosette were elongated by a single UDP-Glc:glucosyltransferase that has only a single active site, the transferase would have to move from one side of the elongating glucan chain to the other to add the successive residues with orientations that are flipped in space. Alternatively, the transferase may have two active sites with the sugar in the second active site rotated 180° from the sugar in the first site. Since it is unlikely that a glycosyltransferase that is part of a larger multienzyme complex in a membrane can change its place within the complex during synthesis, we postulate that the transferases have two active sites or that assembly of these polysaccharides occurs by some other mechanism. For example, the diglycosyl feature of the backbones of wall polysaccharides could arise either by having two copies of the same glycosyltransferase (each with a single active site) positioned at opposite sides of each elongating chain, or by having two different glycosyltransferases (each with a single active site) located on the same side of the elongating chain. However, as illustrated in Figure 2, it would be more efficient if each of the glycosyltransferases possessed two active sites. This mode of chain elongation would allow the synthesis of disaccharide repeats without the need for the polysaccharide or protein to rotate. Saxena et al. (1995) have summarized the evidence for the existence of two active sites in glycosyltransferases of the type responsible for synthesis of plant cell wall polysaccharides and found no evidence that was incompatible with the involvement of disaccharide intermediates in the polymerization.

Extension of each polysaccharide chain by a single glycosyltransferase that adds disaccharide repeating units (such as cellulose in the case of cellulose, or rhamnosyl-galactosyluronic acid in the case of rhamnogalacturonan I) would simplify the packaging of the glycosyltransferases responsible for synthesis of the polysaccharide backbones. However, it also means that synthesis of the polysaccharides involves an activated diglycosyl intermediate as the donor. This may depend on the synthesis of a diglycosyl donor attached to the glycosyltransferase as shown in Figure 2, which is a mechanism similar to that depicted by the two-active-site model of Saxena et al. (1995). Alternatively, the synthesis could involve a diglycosyl donor activated by a nucleoside diphosphate or dolichol phosphate. Since experiments attempting to synthesize cellulose and the other wall polysaccharides with activated diglycosyl donors have not been reported in the literature, the lack of success in synthesizing wall polysaccharides in cell-free systems (Delmer et al., 1993; Brown et al., 1994) may be related to the use of ineffective donor molecules. This could be particularly important if an intermediate is passed, as it is constructed, from one polypeptide to another.

Figure 2. A model for the synthesis of wall polysaccharides with backbones constructed of a disaccharide repeat. The assembly process is subdivided into five steps defining the growth of the polysaccharide, from its nonreducing end, by the addition of pairs of glycosyl residues transferred from two molecules of nucleoside diphosphate sugar. The enzyme complex contains two sites capable of binding nucleoside diphosphate sugars. The sites are loaded by transfer of the glycosyl residues from the nucleoside diphosphate sugars to appropriate Ser or Thr hydroxyl groups on the glycosyltransferase. The model postulates that the two activated sugars are connected to form a dimer and then attached to the growing polysaccharide. The polysaccharide must translocate before two new activated glycosyl residues can initiate another elongation cycle. In this model, which is one of a variety that could be proposed, an activated monoglycosyl residue would be the appropriate donor.

The involvement of more than one catalytic polypeptide in the synthesis of the primary wall polysaccharides is particularly likely if the glycosyl residues of the backbone receive, during their polymerization, regularly spaced, covalently attached substituents, as appears to be true for several of the wall polysaccharides, particularly xyloglucan with
its regularly ordered side chains. The difficulty of reconstituting a biosynthetic system from solubilized membrane proteins increases as the number of different polypeptides required for the synthesis increases. This is likely to underlie some of the problems researchers have encountered in their attempts to synthesize wall polysaccharides in vitro. The number of different and differently linked glycosyl residues in all of the primary cell wall polysaccharides except homogalacturonan and cellulose makes it likely that synthesis of structurally defined primary cell wall polysaccharides will involve more than a single catalytic subunit.

The recent report (Pear et al., 1996) of the cloning of the first putative genes thought to encode a glycosyltransferase involved in cell wall synthesis, the cotton and rice CelA genes, has the potential of being a major step in elucidating the biosynthesis of primary cell wall polysaccharides. This discovery may also represent a first step in the testing of some of the ideas expressed in this article. Based on the nucleotide sequence, the CelA protein contains more than one binding domain for nucleoside diphosphate sugar, which is consistent with the model illustrated in Figure 2. However, just how these sites participate in polysaccharide synthesis remains to be determined.

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