Cellulose Biosynthesis: Counting the Chains

At the heart of the process by which plants take shape are cellulose microfibrils, wrapped around each cell in a pattern that constrains the direction in which the cell can grow. Although much of the biosphere’s carbon passes through the process of cellulose biosynthesis, our understanding of this process has been slow to develop and is based on an intricate mixture of experimental observations with hypotheses and models (Guerriero et al., 2010). It has been observed that each cellulose microfibril is laid down outside the cell membrane by a traveling cellulose synthesis complex (CSC), which is shaped as a six-lobed rosette and contains multiple cellulose synthase catalytic subunits (CESAs; Kimura et al., 1999; Guerriero et al., 2010). Assuming that all the CESAs are active, there should be one for each cellulose chain being synthesized. It has been felt for some years that we might eventually come to understand the structure of the microfibrils, and the structure and function of the rosette CSCs, together. In this issue, Newman et al. (2013) bring that convergence of ideas a step closer.

We now know a good deal about the individual CESAs, due to their homology with bacterial cellulose synthases. The *Rhodobacter sphaeroides* cellulose synthase structure was solved by Morgan et al. (2013), showing that multiple transmembrane helices surround a channel through which the newly synthesized cellulose chain emerges from the cell. Cotton (*Gossypium hirsutum*) GhCESA1 seems functionally similar but contains additional plant-specific, peripheral domains that may help the catalytic subunits to self-assemble into the rosette CSC (Sethaphong et al., 2013; Slabaugh et al., 2013). The question is, how many of these catalytic subunits are there, and how many chains are in each microfibril?

It was long assumed that a microfibril contained 36 chains and that there were 36 CESAs in a rosette CSC. Then, each of the six lobes of the rosette CSC would contain six CESAs. Sethaphong et al. (2013) illustrated how a CESA hexamer might look, glued together by the plant-specific subdomains, but they were careful to show dimeric, trimeric, and tetrameric structures also.

A 36-chain microfibril would have a cross-sectional area of 11.6 nm$^2$, using chain dimensions appropriate for primary wall cellulose (Thomas et al., 2013), but most current estimates of primary wall microfibril diameter, from a range of spectroscopic and diffraction techniques, are about 3 nm (Fernandes et al., 2011; Thomas et al., 2013, and refs. therein), giving a cross-sectional area of only 7 nm$^2$. So the microfibrils are too small to contain 36 chains. Fernandes et al. (2011) and Thomas et al. (2013) described 24-chain models, with surface chains differing in conformation but not in packing, and showed that the microfibrils were loosely aggregated. They noted, however, that 18-chain models also fitted the diffraction data if some pairs of microfibrils coalesced along part of their length, thus slightly increasing the overall average width.

Uncertainties about the nature and location of the disorder have prevented more precise calculation of dimensions from the x-ray diffraction data, because disorder and small dimensions have similar effects on the diffraction patterns. However, Newman (2008) devised an elegant way to predict, from first principles, the x-ray diffraction patterns from microfibrils of any size and structure, regular or not. Applying this method to cellulose from mung bean (*Vigna radiata*) hypocotyls, Newman et al. (2013) have now reconstructed the average diffraction patterns from ensembles of microfibril structures of various sizes and degrees of disorder and matched these against the observed diffraction patterns. The 36-chain models are clearly ruled out, and the best fit is with a mix of irregularly shaped 18-chain microfibrils, a few of them coalescent or “twinned” in pairs as suggested by Thomas et al. (2013; see figure 6 in Newman et al., 2013). This model is also consistent with surface-volume ratios calculated, with some assumptions, from the authors’ NMR spectra of primary wall cellulose from mung bean and a range of other species, including Arabidopsis (*Arabidopsis thaliana*).

Microfibrils with 18 chains would suggest rosette CSCs with 18 CESAs in six groups of three, presumably the three distinct CESAs that appear to cooperate in synthesizing cellulose in any one cell type (Taylor et al., 2003). Newman et al. (2013) go on to speculate on how these 18 CESA subunits might self-assemble into a rosette CSC. They assume that the structure...
would have 6-fold symmetry. That is, the six CESAs shown red in Figure 1 would all face inward. If so, the ribbon-like emerging chains would need to twist in one direction or the other to become aligned into sheets within the nascent microfibril. Also, it has been suggested that the rosette CSCs rotate as they move, so that the whole microfibril is laid down with a slow twist. How these geometric constraints would be accommodated is unclear, but it can be seen how a diversity of cross-sectional structures might result and how the structure might change along the length of the newly deposited microfibril.

These findings encourage exploration of how 18-CESA rosette CSCs might structure themselves and function, provoking questions about the locations of other proteins such as CESA INTERACTIVE1 (CSI1). CSI1 directs the trajectories of rosette CSCs, and thus the alignment of the microfibrils that they leave behind, by linking to the underlying cortical microtubules (Li et al., 2012) and therefore is a factor in the development of cell shape. Newman et al. (2013) also provide tools to look more closely at the structure of cellulose in certain key situations, such as where its recalcitrance limits conversion to liquid biofuels (Harris et al., 2012) and where its diffraction patterns suggest altered crystallization in the phenotypes of diagnostic CesA mutants (Harris et al., 2012; Slabaugh et al., 2013). So research on cellulose synthesis and microfibril structure now have a chance to move forward together, and in consequence, we can expect rapid developments in both of these fields.

Michael C. Jarvis*
School of Chemistry, Glasgow University, Glasgow G12 8QQ, United Kingdom

ACKNOWLEDGMENTS
I thank Candace Haigler for critical comments.

LITERATURE CITED