PREFACE

This book introduces students and researchers to the polysaccharides and proteins that form the fundamental architecture of the plant cell wall, and the to the genes encoding the cellular machinery that synthesizes them. In assembling the chapters in the core of this book, we focus on the evolution of the many families of genes whose products are required to make a particular kind of polysaccharide, rather than describing all the gene products needed to make certain classes of polysaccharides. In so doing, we draw attention to the specific biochemical properties of the proteins, at the level of kinds of sugar linkages they make. Cell wall structure has, for the same reason, been split into chapters on *composition*, the building blocks to be synthesized, and *architecture* which relates to wall assembly.

Several gene products from different families are often needed to make a complex polysaccharide, and the protein elements of the synthase complex interact to produce the characteristic product. This collection of articles is designed to encourage thinking as to how this occurs in the dynamic context of cell growth and development.

As most of the cell wall is carbohydrate, most of the gene families annotated to possible function are involved in polysaccharide synthesis. Chapter 1 by Stephen Fry gives us an up-to-date comprehensive inventory of the classes of specific polysaccharides, drawing attention to the simple but effective methods to monitor their synthesis in vivo. The repertoire of covalent bonds in this inventory of cell wall structural components allows us to hypothesize about the kinds and minimum number of enzymes needed to make them. Persson and colleagues (Chapter 2) then provide a summary of numerous new tools to analyse these constituents, such as immunocytochemistry, oligosaccharide fingerprinting, and carbohydrate microarrays, which will fine-tune our capabilities to chemically phenotype subtle alterations in polysaccharide structures caused by mutation. Nepogodiev and colleagues (Chapter 3) describe the current knowledge in the chemical synthesis of pectic oligosaccharides, which will undoubtedly prove useful in the fingerprinting of mutant phenotypes and to provide novel substrates for biosynthesis.

One of the most valuable resources to cell wall researchers has been CAZy – the Carbohydrate-Active Enzymes database (http://www.cazy.org/) – which defines the known world of glycosyl transferases, hydrolases, lyases, and carbohydrate binding modules across the tree of life. Of 92 distinct gene families of glycosyl tranferases described in CAZy, 42 are represented in plants, although many of these are not involved directly in cell wall

biosynthesis. Coutinho and Henrissat (Chapter 4) describe their respective groups' continuing efforts to annotate the genes involved in encoding the polysaccharide synthesis machinery that is essential to cell wall biogenesis. Moreover, this chapter defines the genes of cell wall synthesis in their evolutionary context of enzymatic function, and in so doing they provide an inventory of proteins that constitute polysaccharide synthases essential for systems approaches to integrate Golgi and plasma membrane synthase mechanisms.

This book is not exhaustive of gene families, but several chapters cover the major families and the specific functions of their gene products in the synthesis of cell wall-related specific polysaccharides. The basic tenet is that no one family is responsible for the synthesis of a single class of complex polysaccharide, such as pectin or cross-linking glycan, but for the synthesis of a general class of anomeric linkages that are part of them. In the last article to be published by the esteemed Bruce Stone and his colleagues (Chapter 5), they describe the history of discovery of linkage structure, of biosynthesis, and of discovery of the genes encoding the catalytic components of the processive synthases of the (1→3)-β-D-glucan, callose, from family GT48, and the synthesis of (1→4)-β-D- and (1→3),(1→4)-β-D-glucans, in cellulose and the mixed-linkage glucans, from the cellulose- and cellulose-synthase-like superfamily within GT2. This chapter serves as a paradigm of discovery at all levels of biochemistry and molecular biology, and so it is fitting that we dedicate this book to Bruce, and also to Debby Delmer, a long-time biochemist who contributed a vast amount of knowledge on the biochemistry of cellulose synthesis and who discovered the first cellulose synthase gene in cotton (see dedication); they are among the leading pioneers who took polysaccharide chemistry and synthesis into the molecular genetics age.

Two of the major families of non-processive glycosyl transferases are GT8 and GT47. As Yin *et al.* (Chapter 6) explain, the GT8 family encodes enzymes of the **retaining** type, i.e. enzymes that retain the α-D- or β-L- linkage of the nucleotide sugar as an α-D- or β-L-glycosyl linkage. Thus, it is not surprising that this family encodes GAUT1, the enzyme that synthesizes the α-D-GalA linkage found in homogalacturonan (HG) from UDP-α-D-GalA. In contrast, as Geshi *et al.* Chapter 10) explain, the GT47 family encodes enzymes of the **inverting** type, which means that β-D- and α-L- linked sugars are made from their respective α-D- and β-L- linked nucleotide sugars. Both of these chapters illustrate how cell wall phenotypes in mutants knocked out in these GTs are now being used as clues to infer the biochemical activity of members of the GT family.

We are beginning to achieve success in defining the function of a few members of many other GT families as well, as illustrated in the chapters by Egelund and colleagues on GT31 (Chapter 7), whose members are thought to function in *O*-glycosylation of proteins, such as the arabinogalactan proteins, and by Keegstra and Cavalier (Chapter 8) on GT34, whose members include retaining xylosyl and galactosyltransferase involved in xyloglucan

and galactomannan synthesis, respectively, and GT37, whose members include a fucosyl transferase responsible for completing the characteristic trisaccharide sidegroup of xyloglucan but is otherwise largely unknown. Anders and Dupree (Chapter 9) describe recent knowledge of family GT43 and its potential role of at least some of its members in xylan biosynthesis, and Peterson and colleagues (Chapter 12) describe an ancient family, GT77, whose members extend from microalgae to flowering plants, possibly encoding xylosyl and arabinosyl transferases to produce complex pectins and *O*-linked structural glycoproteins. Edvardsson and colleagues report on family GT64 (Chapter 11), whose members, based on animal models, are transferases of aminosugars and their *N*-acetylated derivatives, but whose specific functions in plants are still to be resolved.

The later chapters begin to bring together the polysaccharide synthases and glycosyl transferases into the big picture of how plant cell walls are made. Kieliszewski and colleagues (Chapter 13) focus on the structural proteins that function to interlock the cell wall through interaction with the carbohydrate components, and hence bring together the gene families hypothesized to be involved in protein *O*-glycosylation, GT31 and GT77. A group of proteins that carry the DUF266 domain are also hypothesized to be involved in *O*-glycosylation. No chapter is devoted to these, as they have not yet been included in the CAZy classification.

With biosynthesis thus covered, McCann and Knox (Chapter 14) assemble a wall from the elements and review how these elements are dynamic during development, and they provide excellent insight into how cytochemical tools are used to define regional domain-specific walls, even in the wall surrounding a single cell.

The book concludes with a short bioegineering section. Øbro and colleagues (Chapter 15) and Huang *et al.* (Chapter 16) provide the history of engineering cell wall polysaccharides and storage polysaccharides *in planta* by plant or microbial enzymes, as well as giving insights into the next generation of products that are possible.

Researchers wanting to characterize an unannotated GT often cannot know whether they will end up working on polysaccharide biosynthesis, protein *O*-glycosylation or even on protein *N*-glycosylation — latter will take the researcher out of the cell wall area. Chapter 17 by Bardor and colleagues deals with *N*-glycosylation but does so from a bioengineering perspective, thus demonstrating that knowledge of plant GTs may allow us to engineer plant cells to produce protein-based pharmaceuticals with human-style glycosylation. Bioengineering of cell wall polysaccharides to target medical applications is an area in its infancy. Morra and his colleagues are pioneers in this field and in Chapter 18 they discuss the possibilities of using rhamnogalacturonan-I as a versatile starting material for engineering biocompatible coating materials for implants and medical devices; in doing so, they also touch upon analytical methods that could find broader uses in cell wall biology.

Until recently, plant cell wall biologists regarded their work as a means to understand growth and development and gain useful information about the polysaccharides themselves. This information is integral to our understanding of plant stature and form, but advances in this knowledge are now part of grander, more immediate challenges to meet not only the world's needs for sustainable food and fibre, but also to provide new crops whose cell walls are the feedstock for efficient production of alternative carbon-neutral fuels. Understanding the genes of cell wall formation is only the initial knowledge base. The next step will be to understand transcript and protein networks and learn how the protein products of these genes form functional complexes. Extending a systems-level understanding to cell wall formation will identify the bottlenecks to productivity at the metabolic and cellular level. Only one-half of the genes of cell wall biogenesis are even hazily understood, and new high-throughput tools in phenotyping, from mass spectrometry of protein complexes to molecular imaging, will be needed to define the complete set. We have a long way to go, and the challenge to researchers new to this field is to use this knowledge to move forward more briskly.

Nicholas Carpita & Peter Ulvskov