

CONTENTS

Preface	xix
Dedication	xxiii
Contributors	xxv

1	Cell Wall Polysaccharide Composition and Covalent Crosslinking	1
	<i>Stephen C. Fry</i>	
1.1	Remit	2
1.1.1	Some definitions	2
1.1.1.1	Pectins	2
1.1.1.2	Hemicelluloses	3
1.1.1.3	Crosslinks	3
1.1.1.4	Non-polysaccharide components	3
1.1.1.5	Primary wall	3
1.1.1.6	Secondary wall	6
1.2	The classic primary cell walls of dicots	6
1.2.1	Pectins	6
1.2.1.1	Homogalacturonan domains	7
1.2.1.2	Rhamnogalacturonan-I domains	8
1.2.1.3	Rhamnogalacturonan-II domains	12
1.2.1.4	Xylogalacturonan domains	12
1.2.2	Hemicelluloses	12
1.2.2.1	Xyloglucans	13
1.2.2.2	Xylans	15
1.2.2.3	Mannans	15
1.2.2.4	Glucuronomannans	15
1.2.3	Cellulose	16
1.3	Secondary cell walls	16
1.4	Taxonomic consideration of primary cell walls	18
1.4.1	Poalean primary cell walls	18
1.4.1.1	Poalean xyloglucans	18
1.4.1.2	Poalean (feruloylated) xylans	19
1.4.1.3	Poalean mixed-linkage glucans	19
1.4.1.4	Other poalean polysaccharides	20
1.4.2	Taxonomically restricted features of non-poalean angiosperm walls	21
1.4.3	Cell walls of non-angiosperms	21
1.4.3.1	Charophytic algae	21
1.4.3.2	Bryophytes	22

	1.4.3.3	Lycopodiophytes	23
	1.4.3.4	Euphyllophytic pteridophytes	24
	1.4.3.5	Gymnosperms	24
1.5		Covalent bonds between wall polysaccharides	24
1.5.1		Glycosidic bonds joining polysaccharides into molecular 'trees'	25
1.5.2		Glycosidic bonds forming true ('lateral') crosslinks between polysaccharides?	26
1.5.3		Oxidative coupling products as crosslinks or intrapolymetric loops	27
1.5.4		Uronoyl esters and uronoyl amides	27
1.5.5		Borate diesters	28
1.6		Methodology	29
1.6.1		Specific and non-specific radiolabelling <i>in vivo</i>	29
1.6.2		Chemical and enzymic 'dissection' of wall polysaccharides	32
1.6.3		Fractionation and characterization of mono- and oligosaccharides	33
	1.6.3.1	Paper chromatography (PC)	33
	1.6.3.2	Thin-layer chromatography (TLC)	34
	1.6.3.3	Paper electrophoresis (PE)	34
	1.6.3.4	High-pressure liquid chromatography (HPLC)	35
	1.6.3.5	Methylation analysis and gas chromatography (GC)	35
	1.6.3.6	Mass spectrometry (MS)	35
1.7		Conclusions	36
		Acknowledgements	36
		References	36
2		Dissection of Plant Cell Walls by High-throughput Methods <i>Staffan Persson, Ibén Sørensen, Isabel Møller, William Willats and Markus Pauly</i>	43
2.1		Introduction	44
2.2		Enzyme fingerprinting	44
2.3		Structural determination of oligosaccharides	47
2.4		Fourier transform infrared spectroscopy (FTIR)	50
2.5		Microarray-based polymer profiling	52
2.6		Additional high-throughput methods	55
2.7		Future perspectives	57
		References	58
3		Approaches to Chemical Synthesis of Pectic Oligosaccharides <i>Sergey A. Nepogodiev, Robert A. Field and Ibén Damager</i>	65
3.1		Introduction	66

3.2	Pectic polysaccharides: structures and availability of fragments from natural sources	66
3.2.1	Structures	66
3.2.2	Preparation of pectic oligosaccharides by cleavage of polysaccharides	67
3.2.2.1	Homogalacturonan fragments	67
3.2.2.2	Rhamnogalacturonan-I fragments	68
3.3	Reported preparations of pectic oligosaccharides by chemical synthesis	69
3.4	Oligosaccharide synthesis – basic principles and key features	71
3.4.1	General points	71
3.4.2	Main considerations in chemical synthesis of complex oligosaccharides and polysaccharides	71
3.5	Synthesis of homogalacturonan fragments	74
3.5.1	Synthesis of oligogalacturonides by direct glycosylation with galacturonic acid derivatives	75
3.5.2	Synthesis of oligogalacturonides by a late stage oxidation approach	76
3.5.2.1	The convergent block synthesis approach	76
3.5.2.2	The reiterative synthesis strategy	78
3.5.2.3	Synthesis of selectively methyl-esterified oligogalacturonic acids	78
3.6	Rhamnogalacturonan-II fragments	81
3.7	Rhamnogalacturonan-I fragments	86
3.8	Future perspective	88
	References	89
4	Annotating Carbohydrate-active Enzymes in Plant Genomes: Present Challenges	93
	<i>Pedro M. Coutinho and Bernard Henrissat</i>	
4.1	Introduction	93
4.2	CAZy: what's behind the name?	96
4.3	Plant CAZymes: the quest for 'function'	97
4.4	Plant CAZymes: problems in functional annotation	103
	References	105
5	Biosynthesis of Plant Cell Wall and Related Polysaccharides by Enzymes of the GT2 and GT48 Families	109
	<i>Bruce A. Stone, Andrew K. Jacobs, Maria Hrmova, Rachel A. Burton and Geoffrey B. Fincher</i>	
5.1	Introduction	110
5.2	Structures and distribution of β -D-glucans synthesized by GT2 and GT48 enzymes	112

5.3	Early biochemical approaches to plant β -D-glucan synthases	119
5.4	Functional genomics and the identification of GT2 cellulose synthases	121
5.4.1	Cellulose synthesis by embryophytes	121
5.4.2	Cellulose synthesis by <i>Gluconoacetobacter xylinus</i> (formerly <i>Acetobacter xylinus</i>)	124
5.4.3	Cellulose synthesis by <i>Agrobacterium tumefaciens</i>	125
5.5	Identification of the functions of other GT2 enzymes from plants	125
5.6	Comparative genomics and the identification of GT2 (1,3;1,4)- β -D-glucan synthases	127
5.7	Genes for GT2 synthases for bacterial (1,3)- β -D-glucans and related polysaccharides	130
5.7.1	Curdlan	130
5.7.2	Cyclic (1,3;1,6)- β -D-glucan from <i>Bradyrhizobium japonicum</i>	131
5.7.3	Capsular polysaccharides from <i>Pasteurella multocida</i>	131
5.7.4	Chitin	131
5.8	Enzymic properties and catalytic mechanisms of the GT2 proteins	133
5.8.1	Topology	133
5.8.2	The catalytic region	133
5.8.3	3D structures	134
5.8.4	Catalytic mechanisms	137
5.8.5	Specification of linkage position in β -glycans	138
5.8.6	Bifunctional GT2 β -Glycan Synthases	139
5.8.7	Chain initiation, direction of chain elongation, and chain termination	140
5.9	Subcellular locations of GT2 enzymes in plants	142
5.10	Proteomics and biochemical approaches to the identification of GT48 (1,3)- β -D-glucan synthases from plants	142
5.11	Enzymic properties of the GT48 proteins	146
5.11.1	Topology	146
5.11.2	Catalytic mechanisms	147
5.12	Future role of biochemistry in the characterization of GT2 and GT48 enzymes	147
5.12.1	Biochemistry and the definition of gene function	148
5.12.2	Post-translational modifications of glycosyl transferases	149
5.12.3	Protein-protein interactions	150
5.13	Applications of modified levels of plant β -D-glucans	151
	Acknowledgements	153
	References	153

6	Glycosyltransferases of the GT8 Family	167
	<i>Yanbin Yin, Debra Mohnen, Ivana Gelineo-Albersheim, Ying Xu and Michael G. Hahn</i>	
6.1	Introduction	168
6.2	Phylogeny of family GT8	170
6.3	GT8 clades related to plant cell wall polysaccharide synthesis	188
6.3.1	Galacturonosyltransferase (GAUT) clade	188
6.3.1.1	Phylogeny of GAUT clade	189
6.3.1.2	Function of GAUT proteins	190
6.3.2	Galacturonosyl transferase-like (GATL) clade	192
6.3.2.1	Phylogeny of GATL clade	193
6.3.2.2	Function of GATL proteins	193
6.4	GT8 clades not related to cell wall synthesis	196
6.4.1	Plant glycogenin-like (PGSIP) clades	197
6.4.1.1	Phylogeny of PGSIP clades	198
6.4.1.2	Function of PGSIP proteins	200
6.4.2	Galactinol synthase (GolS) clade	201
6.4.2.1	Phylogeny of GolS clade	202
6.4.2.2	Function of GolS proteins	204
6.5	Conclusions	205
	Acknowledgements	205
	References	205
7	Genes and Enzymes of the GT31 Family: Towards Unravelling the Function(s) of the Plant Glycosyltransferase Family Members	213
	<i>Jack Egelund, Miriam Ellis, Monika Doblin, Yongmei Qu and Antony Bacic</i>	
7.1	Introduction	214
7.2	Identification and characterization of the first β -(1,3)-GalTs	215
7.2.1	The first β -(1,3)-GT family members of non-plant origin	215
7.2.2	The first β -(1,3)-GalTs of plant origin	216
7.3	Grouping of accessions based on their phylogenetic relationship	216
7.4	Conserved motifs and implications for catalysis	221
7.4.1	Motif of the catalytic domain belongs to the GT-A fold superfamily	221
7.4.2	Sequence analysis and functional assignment of conserved motifs	222
7.4.3	Implication of different substrate specificity for β -(1,3)-GTs	223
7.5	Domains conserved within the plant-specific clades	223
7.5.1	Galactosyltransferase domain-containing clades 7 and 10	225

7.5.2	Galactoside-binding lectin (galectin) domain specific to clade 7	225
7.5.3	Domain of unknown function (DUF604) in clade 1	227
7.5.4	No identifiable domains in accessions of clade 11	228
7.6	Conclusions	228
	Acknowledgements	229
	References	229
8	Glycosyltransferases of the GT34 and GT37 Families <i>Kenneth Keegstra and David Cavalier</i>	235
8.1	Introduction	235
8.2	Family GT37 enzymes	236
8.2.1	Xyloglucan fucosyltransferase (FUT1)	236
8.2.2	Other FUT genes	240
8.3	Family GT34 enzymes	241
8.3.1	Galactomannan galactosyltransferase	241
8.3.2	Xyloglucan xylosyltransferase	243
8.4	Concluding comments	246
	References	247
9	Glycosyltransferases of the GT43 Family <i>Nadine Anders and Paul Dupree</i>	251
9.1	Introduction	251
9.2	GT43 glycosyltransferases in plants – putative β -1,4-xylosyltransferases	255
9.3	GT43 glycosyltransferases in animals – β -1,3-glucuronosyltransferases	256
9.4	Structural characteristics of GT43 proteins	257
9.5	Concluding remarks	259
	References	260
10	Glycosyltransferases of the GT47 Family <i>Naomi Geshi, Jesper Harholt, Yumiko Sakuragi, Jacob Krüger Jensen and Henrik Vibe Scheller</i>	265
10.1	Introduction	266
10.2	Phylogenetic analysis of CAZy GT47	266
10.3	Group A	269
10.4	Group D	272
10.5	Group B	275
10.6	Group C	277
10.7	Subcellular localization and protein–protein interactions	278
10.8	Conclusion	280
	References	280

11	The Plant Glycosyltransferase Family GT64: in Search of a Function	285
	<i>Ellinor Edvardsson, Sunil Kumar Singh, Min-Soo Yun, Agata Mansfeld, Marie-Theres Hauser and Alan Marchant</i>	
11.1	Introduction	286
11.2	GT64 family members are found in a diverse range of species	286
11.3	The Arabidopsis GT64 family	287
11.3.1	Structure of the GT64 genes and proteins	287
11.3.2	Function of Arabidopsis GT64s proteins	290
11.3.2.1	At3g55830 EPC1	290
11.3.2.2	At1g80290 EPC-L1	295
11.3.2.3	At5g04500 EPC-L2	295
11.4	Possible activities of the plant GT64 enzymes	296
11.4.1	Could EPC1 function in AGP synthesis?	297
11.4.2	Could EPC1 play a role in FLA biosynthesis?	298
11.4.3	EPC1 as a negative regulator of ABA signalling	298
11.5	Concluding remarks	299
	References	299
12	Glycosyltransferases of the GT77 Family	305
	<i>Bent Larsen Petersen, Kirsten Faber and Peter Ulvskov</i>	
12.1	Introduction	305
12.2	The oldest cell wall	306
12.3	Pfam and fold prediction	307
12.4	Establishing GT77	311
12.4.1	The <i>Dictyostelium discoideum</i> (1,3)- α -D-galactosyltransferase	312
12.4.2	Clade B – rhamnogalacturonan-II biosynthesis	312
12.4.3	Clade A – the mixed algal and higher plant clade	315
12.4.4	Clade C and PfamB 13934	317
12.4.5	Clade D	317
12.4.6	Clade E – the youngest clade	317
12.5	Discussion	318
	Acknowledgments	318
	References	319
13	Hydroxyproline-rich Glycoproteins: Form and Function	321
	<i>Marcia J. Kieliszewski, Derek T.A. Lamport, Li Tan and Maura C. Cannon</i>	
13.1	Introduction	322
13.1.1	Background	322
13.1.2	Definitions	322
13.2	Post-translational modifications	323
13.2.1	Rules for proline hydroxylation	323

13.2.2	O-Hyp-glycosylation codes – the Hyp contiguity hypothesis	324
13.2.3	Glycosylation enhances secretion of designer glycoproteins	327
13.2.4	Glycosylation anomalies	328
13.2.5	Crosslinking code involving tyrosine motifs	329
13.3	Molecular function, biological role	329
13.3.1	Arabinogalactan proteins (AGPs)	329
13.3.2	Extensins as self-assembling amphiphiles	330
13.3.3	Role of multiple extensins	332
13.4	Evolution	334
13.4.1	Conserved motifs	334
13.4.2	Evolution of the cell plate – nascent cell wall	335
13.5	Epilogue	336
	Acknowledgments	336
	References	336
14	Plant Cell Wall Biology: Polysaccharides in Architectural and Developmental Contexts	343
	<i>Maureen C. McCann and J. Paul Knox</i>	
14.1	Introduction	344
14.2	Plant cell wall biology basics	344
14.3	Analytical tools to study cell wall microstructures and the diversity of cell wall architectures	346
14.3.1	Molecular probes	346
14.3.2	Advances in microscopies	349
14.3.3	Spectroscopic and spectrometric technologies	350
14.4	Cell wall architectures: primary cell walls	350
14.4.1	Modulation of cellulose–hemicellulose networks and cell enlargement	352
14.4.2	Pectins: modular multifunctional polymers of the primary cell wall matrix	353
14.5	<i>In vitro</i> polysaccharide composites	355
14.6	Cell wall diversity	355
14.7	Cell wall architectures: secondary cell walls	358
14.8	Prospects for plant cell wall biology	360
	Acknowledgements	360
	References	360
15	Enzymatic Modification of Plant Cell Wall Polysaccharides	367
	<i>Jens Øbro, Takahisa Hayashi and Jørn Dalgaard Mikkelsen</i>	
15.1	Introduction	368
15.2	<i>In vivo</i> modifications	369
15.3	Post-harvest modifications	376
15.4	Perspectives	380

Acknowledgments	381
References	382
16 Production of Heterologous Storage Polysaccharides in Potato Plants	389
<i>Xing-Feng Huang, Jean-Paul Vincken, Richard G.F. Visser and Luisa M. Trindade</i>	
16.1 Introduction	390
16.2 Starch: native and modified starch, consequences for its properties	390
16.2.1 Starch biosynthesis in storage organs	391
16.2.2 Alteration of starch composition through the modeling of plant genes	391
16.2.2.1 Amylose-free starch	391
16.2.2.2 High-amylose starch	393
16.2.3 Modification of starch properties by expression of bacterial proteins in plants	393
16.3 Production of novel storage polysaccharides in plants	394
16.3.1 Glucans synthesized in transgenic plants	394
16.3.1.1 Alternan	395
16.3.1.2 Dextran	396
16.3.1.3 Mutan	396
16.3.1.4 Other novel carbohydrates	398
16.3.2 Fructans synthesized heterologously in transgenic potato plants	398
16.3.2.1 Enzymes of fructan biosynthesis	399
16.3.2.2 Industrial applications of fructans	400
16.3.2.3 Fructans in transgenic plants	400
16.4 Final remarks	402
References	403
17 Glycan Engineering in Transgenic Plants	409
<i>Muriel Bardor, José A. Cremata and Patrice Lerouge</i>	
17.1 Introduction	409
17.2 N-glycosylation: a major post-translational modification of secreted proteins	410
17.2.1 N-Glycosylation of plant-derived pharmaceuticals: antibodies as a glycoprotein model	412
17.2.2 Differences in N-glycan structure may compromise <i>in vivo</i> use of plant-derived therapeutic proteins	413
17.3 Strategies for glycan engineering in transgenic plants	415
17.3.1 Retention in the ER	415
17.3.2 <i>In planta</i> reconstruction of human-like N-glycans by knock-in strategies	415

17.3.3	Removal of immunogenic plant epitopes by knock-out strategies	417
17.3.4	<i>In planta</i> sialylation of recombinant proteins	418
17.4	Conclusions	419
	Acknowledgements	420
	References	421
18	Polysaccharide Nanobiotechnology: A Case Study of Dental Implant Coating	425
	<i>Marco Morra, Clara Cassinelli, Giovanna Cascardo, Hanna Kokkonen, Juha Tuukkanen, Claudio Della Volpe, Stefano Siboni, Giordano Segatta, Marco Brugnara and Giacomo Ceccone</i>	
18.1	Introduction: titanium dental implants and surface modifications	426
18.2	Rationale for the surface modification of titanium dental implants by nanolayers of MHRs	428
18.2.1	Background	428
18.2.2	Modification of solid surfaces by MHRs: general principles	429
18.2.3	Experiments on osteogenic cell adhesion to MHR-coated surfaces	431
18.2.4	Wettability of MHR-coated surfaces	434
18.3	Surface modification of titanium dental implants by MHRs	438
18.3.1	Surface modification of titanium by MHR: surface chemical analysis	438
18.3.2	Surface modification of titanium by MHRs: wettability measurements on machined and rough surfaces	443
18.4	Reflections and conclusions	444
	Acknowledgments	446
	References	446
	Index	451